

**TECHNOLOGY PREVIEW 03/2020** – This report has been generated with the upcoming Dose-Response-Analysis Package for PLA 3.0.5 (or higher). The package has not been released and has not been finally verified for production use. Contact Stegmann Systems (<http://support.bioassay.de>) to report issues or suggest additional features.

# Linear Fit - Interpolation

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## Signatures

.....  
Responsibility

.....  
Review

.....  
Approval

## Table of contents

STD.....	5
TST1 - Source Material.....	8
TST2 - Stock Solution with Predilution.....	11
TST3 - Mixed Response Data Handling.....	14
TST4 - Adjustment.....	17
TST5 - Normalization.....	20
TST6 - Transformation.....	23
Group-RDH.....	26
Group-Others.....	27

## Documentation

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DOCUMENT-28



741AC16E-3F79-46E6-A8A7-5CB4AD388293

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## Assay overview

Assay elements	Summary
<ul style="list-style-type: none"> <li><span style="color: blue;">■</span> STD</li> <li><span style="color: cyan;">■</span> TST1 - Source Material</li> <li><span style="color: purple;">■</span> TST2 - Stock Solution with Pre...</li> <li><span style="color: yellow;">■</span> TST3 - Mixed Response Data Han...</li> <li><span style="color: orange;">■</span> TST4 - Adjustment</li> <li><span style="color: green;">■</span> TST5 - Normalization</li> <li><span style="color: pink;">■</span> TST6 - Transformation</li> </ul>	<p>Reference standard, multi-dose (3 dilution steps, 6 replicates)</p> <p>Test sample, single-dose (3 replicates), defined concentration: 45.0 IU/L</p> <p>Test sample, single-dose (3 replicates), defined concentration: 75.0 IU/L</p> <p>Test sample, single-dose (3 replicates)</p> <p>Test sample, single-dose (3 replicates)</p> <p>Test sample, single-dose (3 replicates)</p> <p>Test sample, single-dose (3 replicates)</p>

Property	Value
<i>Response data processing*</i>	
Response adjustment	Adjustment by fixed value: 1 (TST4 - Adjustme...)
Response adjustment	Adjustment by fixed value: 1 (TST3 - Mixed Re...)
Response normalization	Normalization by fixed value: 2 (TST3 - Mixed Re...)
Response adjustment	Adjustment by fixed value: -0.5 (TST3 - Mixed Re...)
Response normalization	Normalization by fixed value: 0.5 (TST3 - Mixed Re...)
Response normalization	Normalization by fixed value: 2 (TST5 - Normaliz...)
Response transformation	Square root response transformation (TST6 - Transfor...)
<i>Analytical model</i>	
Model	Linear model
Dose transformation	Logarithmic: binary logarithm (base 2)

\* The response data processing steps are executed in the reported order.

## Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		[1] 1.920	[1] 1.900	[1] 1.840	[1] 1.940	[1] 2.060	[1] 1.840		[1] 2.200	[1] 1.950	[1] 2.000	
C		[2] 2.120	[2] 2.140	[2] 2.100	[2] 2.020	[2] 2.120	[2] 2.040		[1] 2.190	[1] 2.150	[1] 2.260	
D		[3] 2.340	[3] 2.280	[3] 2.280	[3] 2.260	[3] 2.260	[3] 2.300		[1] 2.120	[1] 2.080	[1] 2.040	
E									[1] 3.120	[1] 3.080	[1] 3.040	
F									[1] 4.240	[1] 4.160	[1] 4.080	
G									[1] 4.494	[1] 4.326	[1] 4.162	
H												

## Dataset

	Assay Element	Group ID	Dilution step	Response	Technical Outlier	Row
1	STD	1	1	1.92000	false	2
2	STD	1	1	1.90000	false	2
3	STD	1	1	1.84000	false	2
4	STD	1	1	1.94000	false	2
5	STD	1	1	2.06000	false	2
6	STD	1	1	1.84000	false	2
7	TST1 - Source M...	2	1	2.20000	false	2
8	TST1 - Source M...	2	1	1.95000	false	2
9	TST1 - Source M...	2	1	2.00000	false	2
10	STD	1	2	2.12000	false	3
11	STD	1	2	2.14000	false	3
12	STD	1	2	2.10000	false	3
13	STD	1	2	2.02000	false	3
14	STD	1	2	2.12000	false	3
15	STD	1	2	2.04000	false	3
16	TST2 - Stock So...	3	1	2.19000	false	3
17	TST2 - Stock So...	3	1	2.15000	false	3
18	TST2 - Stock So...	3	1	2.26000	false	3
19	STD	1	3	2.34000	false	4
20	STD	1	3	2.28000	false	4

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	Assay Element	Group ID	Dilution step	Response	Technical Outlier	Row
21	STD	1	3	2.28000	false	4
22	STD	1	3	2.26000	false	4
23	STD	1	3	2.26000	false	4
24	STD	1	3	2.30000	false	4
25	TST3 - Mixed Re...	4	1	2.12000	false	4
26	TST3 - Mixed Re...	4	1	2.08000	false	4
27	TST3 - Mixed Re...	4	1	2.04000	false	4
28	TST4 - Adjustme...	5	1	3.12000	false	5
29	TST4 - Adjustme...	5	1	3.08000	false	5
30	TST4 - Adjustme...	5	1	3.04000	false	5
31	TST5 - Normaliz...	6	1	4.24000	false	6
32	TST5 - Normaliz...	6	1	4.16000	false	6
33	TST5 - Normaliz...	6	1	4.08000	false	6
34	TST6 - Transfor...	7	1	4.49440	false	7
35	TST6 - Transfor...	7	1	4.32640	false	7
36	TST6 - Transfor...	7	1	4.16160	false	7

	Column	Plate
1	2	1
2	3	1
3	4	1
4	5	1
5	6	1
6	7	1
7	9	1
8	10	1
9	11	1
10	2	1
11	3	1
12	4	1
13	5	1
14	6	1
15	7	1
16	9	1
17	10	1
18	11	1
19	2	1
20	3	1
21	4	1
22	5	1
23	6	1
24	7	1
25	9	1
26	10	1
27	11	1
28	9	1
29	10	1
30	11	1
31	9	1
32	10	1
33	11	1
34	9	1
35	10	1
36	11	1

**Assay suitability tests**

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Overall test result	<b>Passed</b>			

<p><b>STD</b>                  F-Test (hypothesis test): Model                  Scope: Standard only; Severity level: rejection</p>		<b>Passed</b>
<p>Calculated F-value  <math>F_{critical}(95\%)</math></p>	<p>131.39480                  0.00406</p>	

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<b>■ STD</b> Equivalence test (parameter estimate), Parameter: Slope (nonlinear models) Scope: Standard only; Severity level: warning		Passed
Calculated value	-0.11672	
Limits	-0.13831 to -0.09514	
Margins	-6.00000 to 0	

**Interpolation results overview**

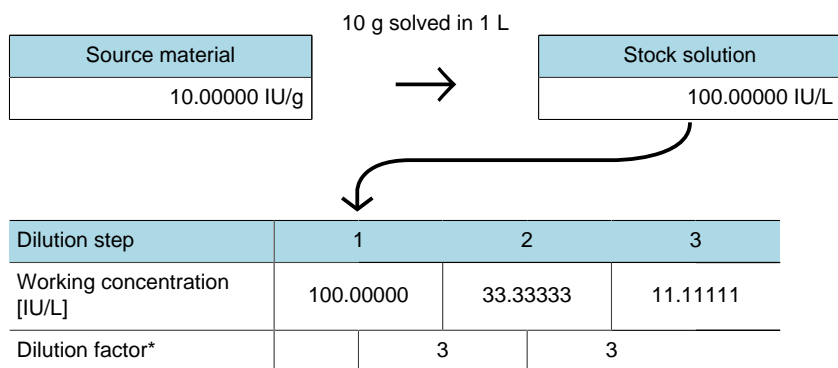
Assay elements	Calculated working concentration	Calculated absolute concentration	Recovery	Suitability test status
TST1 - Source Material	44.26895 [IU/L]	8.85379 [IU/g]	98.38%	Failed (rejected)
TST2 - Stock Solution with Predilution	18.16537 [IU/L]	72.66147 [IU/L]	96.88%	Failed (warning)
TST3 - Mixed Response Data Handling	37.04488 [IU/L]	37.04488 [IU/L]	N/A	Passed
TST4 - Adjustment	37.04488 [IU/L]	37.04488 [IU/L]	N/A	Passed
TST5 - Normalization	37.04488 [IU/L]	37.04488 [IU/L]	N/A	Passed
TST6 - Transformation	37.04488 [IU/L]	37.04488 [IU/L]	N/A	Passed

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**STD: Setup**

Property	Value
Concentration: Defined by source material	10.00000 IU/g
Preparation scheme	MultiDoseScheme-1
Number of replicates	6
Dilution scale	Stepwise dilution sequence
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

**Working concentration**

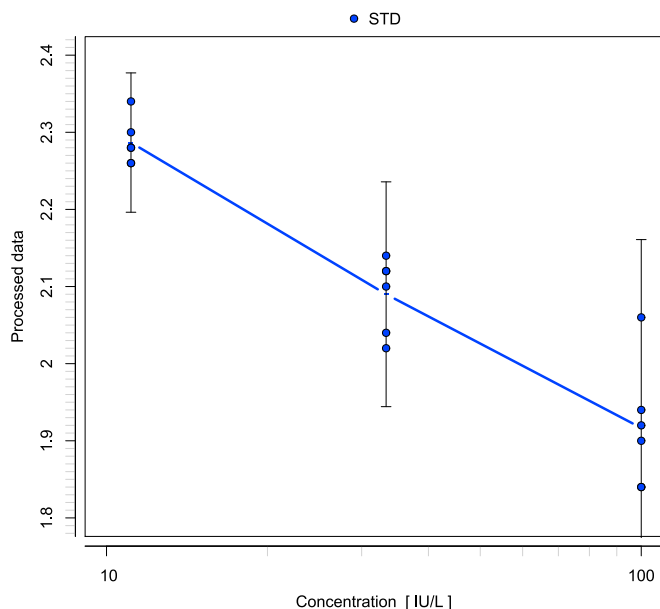


\* Dilution factors are defined by the user.

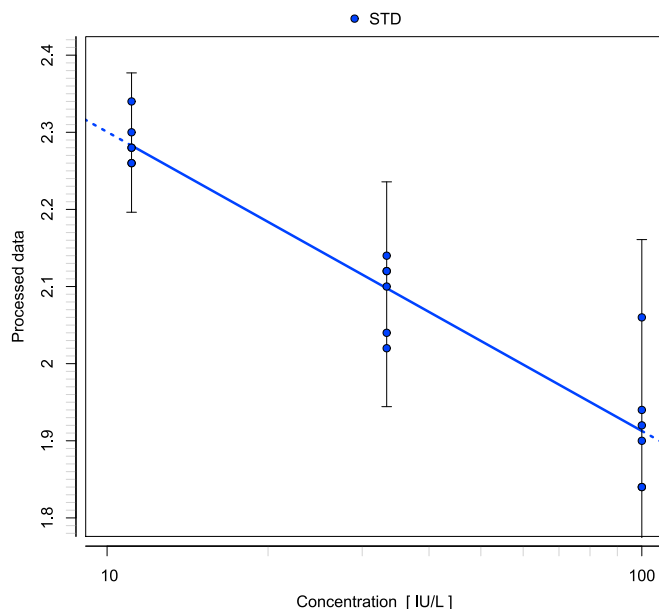
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## STD: Result

**Data plot**



**Regression plot**



### Response data

Dilution step	1	2	3
Working concentration [IU/L]	100.00000	33.33333	11.11111
Response	1.92000 1.90000 1.84000 1.94000 2.06000 1.84000	2.12000 2.14000 2.10000 2.02000 2.12000 2.04000	2.34000 2.28000 2.28000 2.26000 2.26000 2.30000
Mean response	1.91667	2.09000	2.28667
SD	0.08140	0.04858	0.03011
CV	4.25%	2.32%	1.32%

### Response data (processed)

Dilution step	1	2	3
Working concentration [IU/L]	100.00000	33.33333	11.11111
Response	1.92000 1.90000 1.84000 1.94000 2.06000 1.84000	2.12000 2.14000 2.10000 2.02000 2.12000 2.04000	2.34000 2.28000 2.28000 2.26000 2.26000 2.30000
Mean response	1.91667	2.09000	2.28667
SD	0.08140	0.04858	0.03011
CV	4.25%	2.32%	1.32%

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**Analysis of variance (ANOVA)**

Source of variation	d.f.	Sum of squares	Mean squares	F-ratio	Probability
Model	1	0.41070	0.41070	131.39480	3.983 E-09
Residual error	16	0.05001	0.00313		
Lack of fit	1	5.444 E-04	5.444 E-04	0.16509	0.69025
Pure error	15	0.04947	0.00330		
Total	17	0.46071	0.02710		

**Regression results**

Parameter	Estimate	Standard error	Confidence interval (95%)	Width of CI
Intercept	2.68826	0.05317	2.57554 to 2.80098	8.39%
Slope (nonlinear models)	-0.11672	0.01018	-0.13831 to -0.09514	36.99%

Quality of regression	
r <sup>2</sup>	0.89145
r <sup>2</sup> adjusted	0.88466

**Covariance**

	Intercept	Slope
Intercept	0.00283	-5.245 E-04
Slope	-5.245 E-04	1.037 E-04

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## ■ TST1 - Source Material: Setup

The assay element "TST1 - Source Material" is included in the group "Group-Others". The aggregated evaluation for this group is displayed on page 27 of this report.

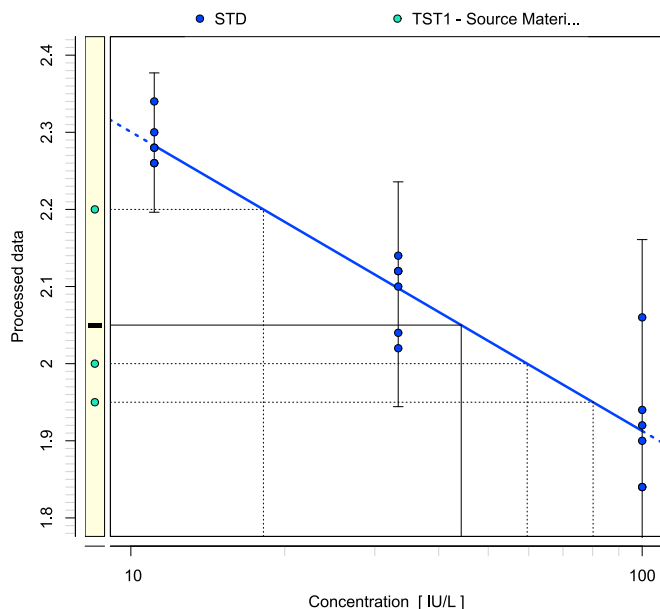
Property	Value
Concentration: Defined by source material	9.00000 IU/g
Preparation scheme	SingleDoseScheme-1
Number of replicates	3
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted



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## TST1 - Source Material: Result

Interpolation plot



### Response data

	Raw Data	Processed Data	Interpolated concentration [IU/L]
Response(s)	2.20000 1.95000 2.00000	2.20000 1.95000 2.00000	18.16537 80.16829 59.57320
Mean response	2.05000	2.05000	44.26895
SD	0.13229	0.13229	
CV	6.45%	6.45%	

### Interpolation results

Property	Value
Mean response	2.05000
Calculated concentration of the working sample [IU/L]	44.26895
Calculated concentration of the sample stock solution [IU/L]	44.26895
Calculated concentration of the sample source material [IU/g]	8.85379
Defined concentration of the sample source material [IU/g]	9.00000
Recovery	98.38%

### Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	1	1	0	1
Overall test result	<b>Failed (rejected)</b>			

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<p><b>■ TST1 - Source Material</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: warning</p>		Passed
Calculated value	18.16537 ; 80.16829	
Margins	15.00000 to 85.00000	Failed
<p><b>■ TST1 - Source Material</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: information</p>		
Calculated value	18.16537 ; 80.16829	Failed
Margins	25.00000 to 75.00000	
<p><b>■ TST1 - Source Material</b>                      Test: Width of interpolation interval                      Scope: all assay elements; Severity level: rejection</p>		Failed
Calculated value	1.40060	
Margins	0 to 0.70000	

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## ■ TST2 - Stock Solution with Predilution: Setup

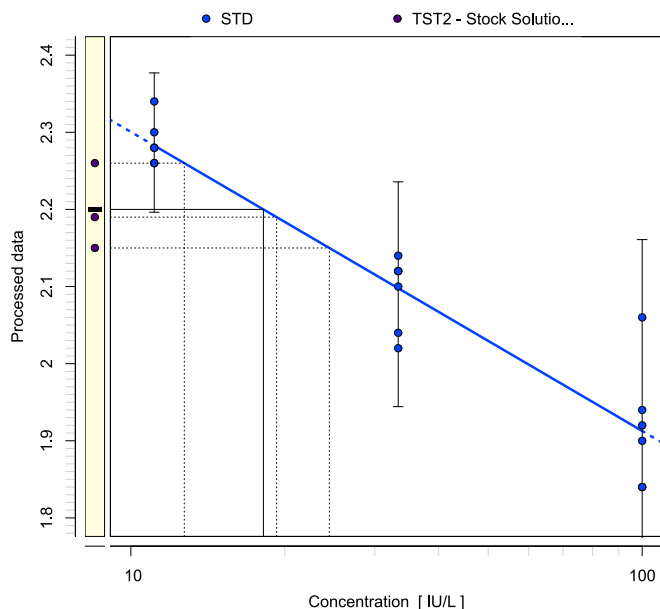
The assay element "TST2 - Stock Solution with Predilution" is included in the group "Group-Others". The aggregated evaluation for this group is displayed on page 27 of this report.

Property	Value
Concentration: Defined by stock solution	75.00000 IU/L
Preparation scheme	SingleDoseScheme-2
Number of replicates	3
Predilution factors	4
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

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## TST2 - Stock Solution with Predilution: Result

Interpolation plot



### Response data

	Raw Data	Processed Data	Interpolated concentration [IU/L]
Response(s)	2.19000 2.15000 2.26000	2.19000 2.15000 2.26000	19.27678 24.44533 12.72044
Mean response	2.20000	2.20000	18.16537
SD	0.05568	0.05568	
CV	2.53%	2.53%	

### Interpolation results

Property	Value
Mean response	2.20000
Calculated concentration of the working sample [IU/L]	18.16537
Calculated concentration of the sample stock solution [IU/L]	72.66147
Defined concentration of the sample stock solution [IU/L]	75.00000
Recovery	96.88%

### Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	1	0	1	1
Overall test result	<b>Failed (warning)</b>			

TST2 - Stock Solution with Predilution		Failed
Test: Interpolation interval: Working concentration Scope: Test samples only; Severity level: warning		
Calculated value	12.72044 ; 24.44533	
Margins	15.00000 to 85.00000	

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<b>■ TST2 - Stock Solution with Predilution</b> Test: Interpolation interval: Working concentration Scope: Test samples only; Severity level: information		Failed
Calculated value	12.72044 ; 24.44533	
Margins	25.00000 to 75.00000	
<b>■ TST2 - Stock Solution with Predilution</b> Test: Width of interpolation interval Scope: all assay elements; Severity level: rejection		Passed
Calculated value	0.64545	
Margins	0 to 0.70000	

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## **TST3 - Mixed Response Data Handling: Setup**

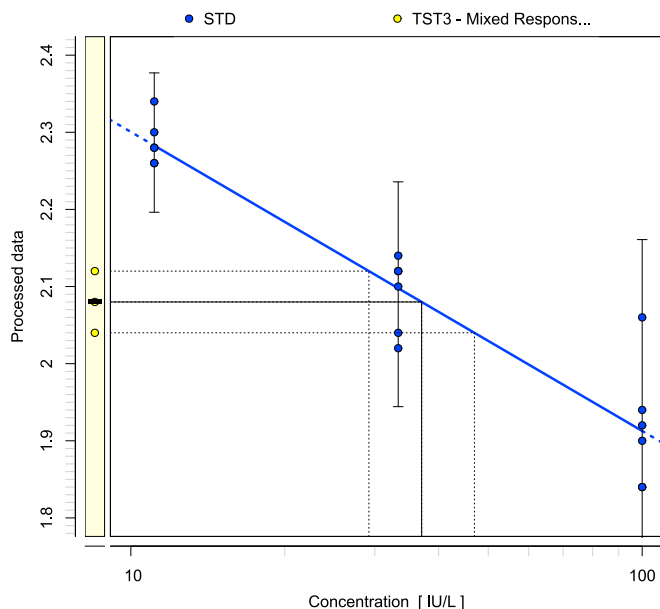
The assay element "TST3 - Mixed Response Data Handling" is included in the group "Group-RDH". The aggregated evaluation for this group is displayed on page 26 of this report.

Property	Value
Preparation scheme	SingleDoseScheme-1
Number of replicates	3
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

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**TST3 - Mixed Response Data Handling: Result**

**Interpolation plot**



**Response data processing**

**Processing instructions**

*Adjustment → Normalization → Adjustment → Normalization*

Step	Details
Response adjustment	Adjustment by fixed value: 1
Response normalization	Normalization by fixed value: 2
Response adjustment	Adjustment by fixed value: -0.5
Response normalization	Normalization by fixed value: 0.5

**Response data**

	Raw Data	Processed Data	Interpolated concentration [IU/L]
Response(s)	2.12000 2.08000 2.04000	2.12000 2.08000 2.04000	29.21238 37.04488 46.97747
Mean response	2.08000	2.08000	37.04488
SD	0.04000	0.04000	
CV	1.92%	1.92%	

**Interpolation results**

Property	Value
Mean response (processed data)	2.08000
Calculated concentration of the working sample [IU/L]	37.04488
Calculated concentration of the sample stock solution [IU/L]	37.04488

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**Sample suitability tests**

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	3	0	0	0
Overall test result	<b>Passed</b>			

<p><b>■ TST3 - Mixed Response Data Handling</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: warning</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	15.00000 to 85.00000	
<p><b>■ TST3 - Mixed Response Data Handling</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: information</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	25.00000 to 75.00000	
<p><b>■ TST3 - Mixed Response Data Handling</b>                      Test: Width of interpolation interval                      Scope: all assay elements; Severity level: rejection</p>		<b>Passed</b>
Calculated value	0.47956	
Margins	0 to 0.70000	



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## ■ TST4 - Adjustment: Setup

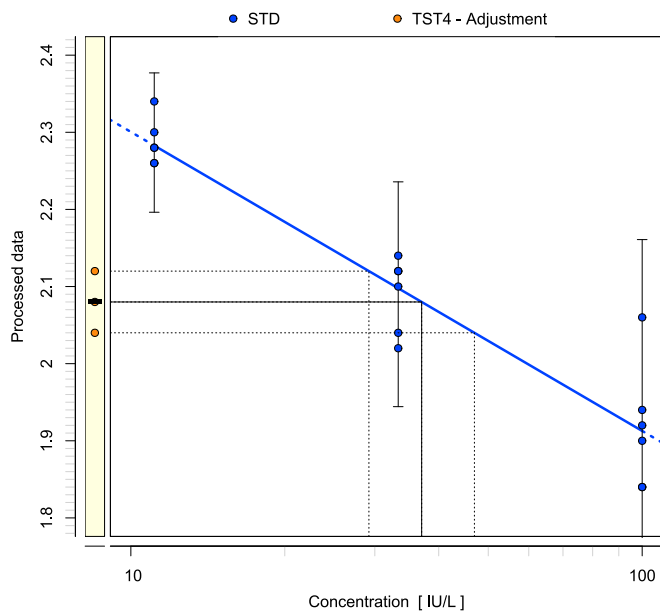
The assay element "TST4 - Adjustment" is included in the group "Group-RDH". The aggregated evaluation for this group is displayed on page 26 of this report.

Property	Value
Preparation scheme	SingleDoseScheme-1
Number of replicates	3
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

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## ■ TST4 - Adjustment: Result

**Interpolation plot**



### Response data processing

#### Processing instructions

Adjustment

Step	Details
Response adjustment	Adjustment by fixed value: 1

#### Response data

	Raw Data	Processed Data	Interpolated concentration [IU/L]
Response(s)	3.12000 3.08000 3.04000	2.12000 2.08000 2.04000	29.21238 37.04488 46.97747
Mean response	3.08000	2.08000	37.04488
SD	0.04000	0.04000	
CV	1.30%	1.92%	

#### Interpolation results

Property	Value
Mean response (processed data)	2.08000
Calculated concentration of the working sample [IU/L]	37.04488
Calculated concentration of the sample stock solution [IU/L]	37.04488

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**Sample suitability tests**

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	3	0	0	0
Overall test result	<b>Passed</b>			

<p><b>■ TST4 - Adjustment</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: warning</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	15.00000 to 85.00000	
<p><b>■ TST4 - Adjustment</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: information</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	25.00000 to 75.00000	
<p><b>■ TST4 - Adjustment</b>                      Test: Width of interpolation interval                      Scope: all assay elements; Severity level: rejection</p>		<b>Passed</b>
Calculated value	0.47956	
Margins	0 to 0.70000	

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## ■ TST5 - Normalization: Setup

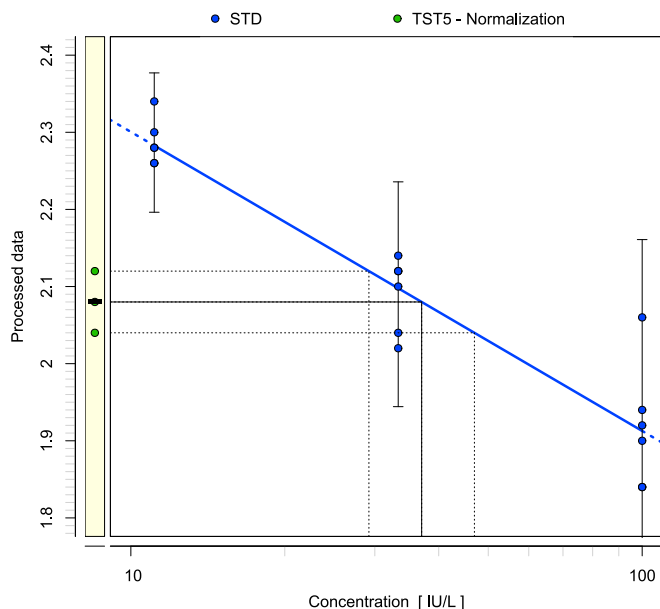
The assay element "TST5 - Normalization" is included in the group "Group-RDH". The aggregated evaluation for this group is displayed on page 26 of this report.

Property	Value
Preparation scheme	SingleDoseScheme-1
Number of replicates	3
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

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**TST5 - Normalization: Result**

**Interpolation plot**



**Response data processing**

**Processing instructions**

Normalization

Step	Details
Response normalization	Normalization by fixed value: 2

**Response data**

	Raw Data	Processed Data	Interpolated concentration [IU/L]
Response(s)	4.24000 4.16000 4.08000	2.12000 2.08000 2.04000	29.21238 37.04488 46.97747
Mean response	4.16000	2.08000	37.04488
SD	0.08000	0.04000	
CV	1.92%	1.92%	

**Interpolation results**

Property	Value
Mean response (processed data)	2.08000
Calculated concentration of the working sample [IU/L]	37.04488
Calculated concentration of the sample stock solution [IU/L]	37.04488

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**Sample suitability tests**

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	3	0	0	0
Overall test result	<b>Passed</b>			

<p><b>■ TST5 - Normalization</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: warning</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	15.00000 to 85.00000	
<p><b>■ TST5 - Normalization</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: information</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	25.00000 to 75.00000	
<p><b>■ TST5 - Normalization</b>                      Test: Width of interpolation interval                      Scope: all assay elements; Severity level: rejection</p>		<b>Passed</b>
Calculated value	0.47956	
Margins	0 to 0.70000	

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## ■ TST6 - Transformation: Setup

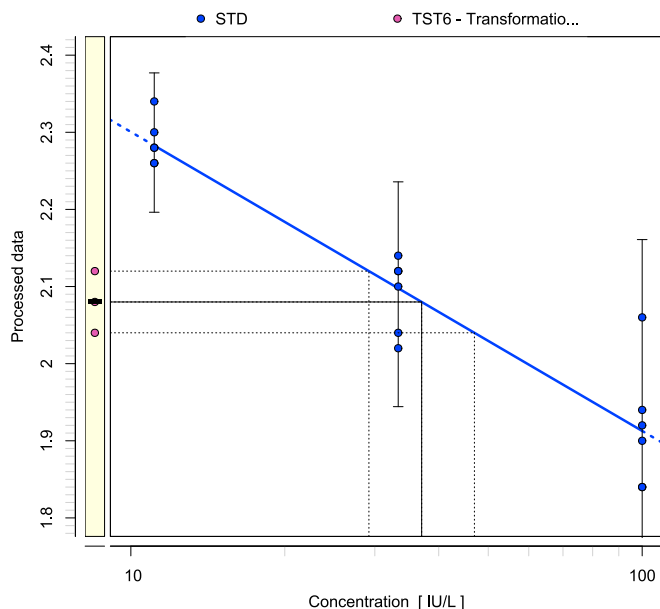
The assay element "TST6 - Transformation" is included in the group "Group-RDH". The aggregated evaluation for this group is displayed on page 26 of this report.

Property	Value
Preparation scheme	SingleDoseScheme-1
Number of replicates	3
Data selection scheme	FullSequenceScheme
Outlier detection	No outlier detection
Response range	Not restricted

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## TST6 - Transformation: Result

Interpolation plot



### Response data processing

#### Processing instructions

Transformation

Step	Details
Response transformation	Square root response transformation

#### Response data

	Raw Data	Processed Data	Interpolated concentration [IU/L]
Response(s)	4.49440 4.32640 4.16160	2.12000 2.08000 2.04000	29.21238 37.04488 46.97747
Mean response	4.32747	2.08000	37.04488
SD	0.16640	0.04000	
CV	3.85%	1.92%	

#### Interpolation results

Property	Value
Mean response (processed data)	2.08000
Calculated concentration of the working sample [IU/L]	37.04488
Calculated concentration of the sample stock solution [IU/L]	37.04488



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**Sample suitability tests**

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	3	0	0	0
Overall test result	<b>Passed</b>			

<p><b>■ TST6 - Transformation</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: warning</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	15.00000 to 85.00000	
<p><b>■ TST6 - Transformation</b>                      Test: Interpolation interval: Working concentration                      Scope: Test samples only; Severity level: information</p>		<b>Passed</b>
Calculated value	29.21238 ; 46.97747	
Margins	25.00000 to 75.00000	
<p><b>■ TST6 - Transformation</b>                      Test: Width of interpolation interval                      Scope: all assay elements; Severity level: rejection</p>		<b>Passed</b>
Calculated value	0.47956	
Margins	0 to 0.70000	

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**Group-RDH: Setup**

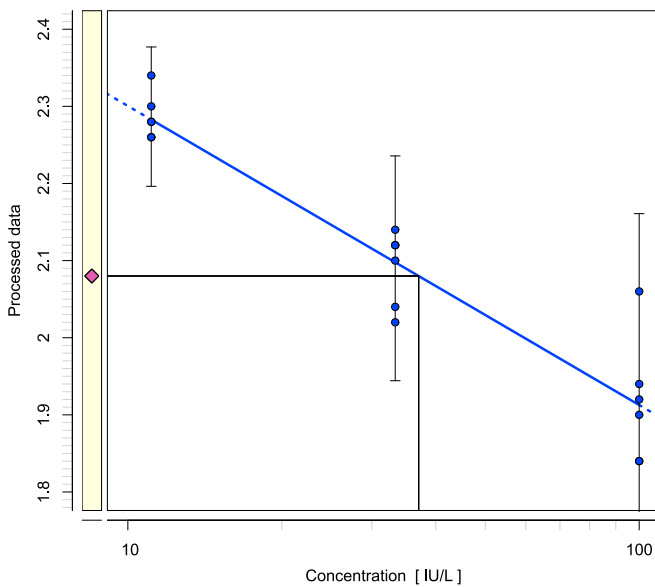
Assay elements	Summary
TST3 - Mixed Response Data Han...	Test sample, single-dose (3 replicates)
TST4 - Adjustment	Test sample, single-dose (3 replicates)
TST5 - Normalization	Test sample, single-dose (3 replicates)
TST6 - Transformation	Test sample, single-dose (3 replicates)

**Information**

All samples have the same result, but start different response values and response data handling.

**Group-RDH: Result**

Interpolation plot



Assay elements	Suitability test status
TST3 - Mixed Respons...	Passed
TST4 - Adjustment	Passed
TST5 - Normalization	Passed
TST6 - Transformatio...	Passed

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## Group-Others: Setup

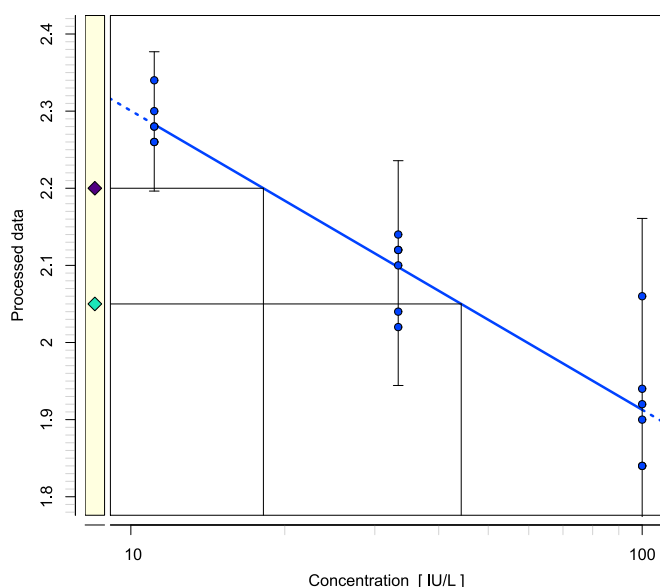
Assay elements	Summary
■ TST1 - Source Material	Test sample, single-dose (3 replicates), defined concentration: 45.0 IU/L
■ TST2 - Stock Solution with Pre...	Test sample, single-dose (3 replicates), defined concentration: 75.0 IU/L

### Information

A group with some samples in it.

## Group-Others: Result

Interpolation plot



Assay elements	Suitability test status
■ TST1 - Source Materi...	Failed (rejected)
■ TST2 - Stock Solutio...	Failed (warning)

### Interpolation results

Assay elements	Calculated working concentration	Calculated absolute concentration	Recovery
TST1 - Source Material	44.26895 [IU/L]	8.85379 [IU/g]	98.38%
TST2 - Stock Solution with Predilution	18.16537 [IU/L]	72.66147 [IU/L]	96.88%