04 TdA-JP4.02 Gramicidin

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Calculation information

Calculation performed:	06.09.2021 11:02:31, Benjamin Schneider (PLA 3.0.5 Build 816, NBARW01, 10014)
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Signatures

Responsibility	Review	Approval

Comments

Comment by Benjamin Schneider: (11.08.2021 16:43:04)

Example turbidimetric assay based on JP 4.02: The standard consists of five concentration steps (S1 to S5) with S3 being used as reference. The relative potency of the unkown sample TST1 is estimated based on the standard. The test system includes tests on the combined standard deviation of the standard, the coefficient of determination (r^2), and the relative potency.

DOCUMENT-1997

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Documentation

Date	11.06.2021 15:44:17
Substance	Gramicidin, Polypeptide, Gramicidin is a mixture of peptide substances having antibacterial activity produced by the growth of Bacillus brevis Dubos. Gramicidin occurs as a white to light yellowish white crystalline powder.
Inoculum	
Test organism	
Species	Enterococcus hirae
Incubation	22h, 36.5° C
Incubation medium	
Ingredient	Glucose, 10g
Ingredient	Casein peptone, 5g
Ingredient	Yeast extract, 20g
Ingredient	Potassium dihydrogen phosphate, 2g
Ingredient	Polysorbate 80, 0.1g
Ingredient	Agar, 15g
Ingredient	Water, 1000ml
pH	6.7
Inoculum medium	
Ingredient	Glucose, 1g
Ingredient	Casein peptone, 5g
Ingredient	Meat extract, 1.5g
Ingredient	Yeast extract, 1.5g
Ingredient	Sodium chloride, 3.5g
Ingredient	Potassium dihydrogen phosphate, 1.32g
Ingredient	Disodium hydrogen phosphate, 3g
Ingredient	Water, 1000ml
pH	7
Inoculum concentration	0.02ug/ml
Inoculum volume per tube	10ml
Incubation	250min, 37°C

Assay overview

Assay elements	Summary
■ STD	Standard sample, 3 replicates, Sequence: 0.065, 0.08, 0.1, 0.125, 0.155
■ TST1	Test sample, 3 replicates, Predilution: 1

Property	Value
*Response data processing**	
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated
Dose transformation	No dose transformation
Response unit	(not available)

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

Rack layout

	1	2	3
1	[1] 0.855	[2] 0.814	[3] 0.628
2	[4] 0.693	[5] 0.530	[1] 0.713

	1	2	3
1	[1] 0.842	[2] 0.827	[3] 0.695
2	[4] 0.685	[5] 0.578	[1] 0.796

	1	2	3
1	[1] 0.850	[2] 0.839	[3] 0.756
2	[4] 0.670	[5] 0.532	[1] 0.720

Assay suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Overall test result	**Passed**			

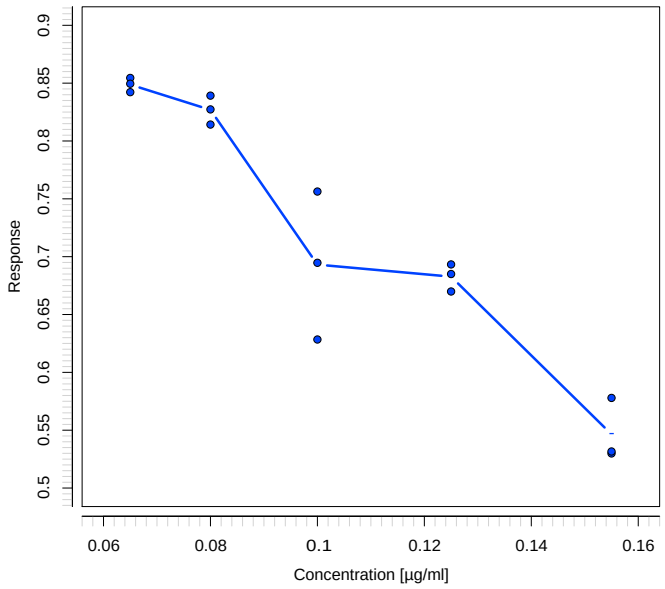
■ STD Test: Combined standard deviation of the standard Scope: Standard only; Severity level: Warning		Passed
Calculated value Upper margin	0.03215 0.10000	
■ STD Test: Coefficient of determination (R^2), regular Scope: Standard only; Severity level: Warning		Passed
Calculated value Lower margin	0.94385 0.90000	

STD: Setup

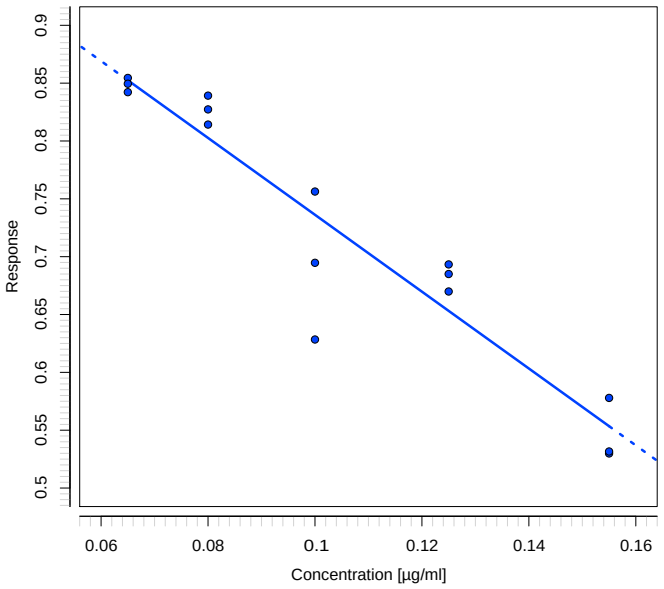
Property	Value
Concentration unit	µg/ml
Number of replicates	3
Stepwise concentration sequence	0.065, 0.08, 0.1, 0.125, 0.155
Reference step	
Position	3
Concentration	0.1

STD: Result

Data plot



Regression plot



Response data

Dilution step	1	2	3	4	5
Working concentration [µg/ml]	0.06500	0.08000	0.10000	0.12500	0.15500
Response	0.85450	0.81420	0.62840	0.69330	0.52990
	0.84220	0.82730	0.69470	0.68500	0.57790
	0.84950	0.83920	0.75630	0.66990	0.53160
Mean	0.84873	0.82690	0.69313	0.68273	0.54647
SD	0.00619	0.01250	0.06396	0.01186	0.02724
CV	0.73%	1.51%	9.23%	1.74%	4.98%

Response data processing

Processing instructions

Adjustment → Normalization → Transformation → Averaging

Step	Details
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated

Regression

Parameter	Estimate	Error
Intercept	1.06843	0.05137
Slope	-3.32223	0.46785

	Quality of regression
r^2	0.94385
r^2 adjusted	0.92513

Sample suitability tests

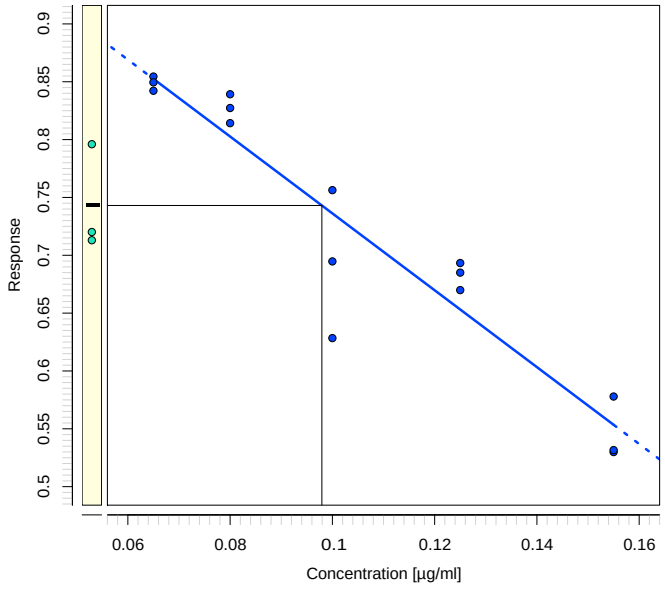
	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	0	0	0	0
Overall test result	**Passed**			

TST1: Setup

Property	Value
Concentration unit	µg/ml
Number of replicates	3
Predilution	1

TST1: Result

Interpolation plot



Response data

	Raw data
Response	0.71300 0.79600 0.72010
Mean	0.74303
SD	0.04601
CV	6.19%

Response data processing

Processing instructions

Adjustment → Normalization → Transformation → Averaging

Step	Details
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated

Interpolation

Property	Value
Absolute potency [µg/ml]	0.09794
Potency of the working sample [µg/ml]	0.09794
Relative potency (percentage of reference concentration)	97.94%

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	1	0	0	0
Overall test result	**Passed**			

TST1 Test: Relative potency (percentage of reference concentration) Scope: Test samples only; Severity level: Warning		Passed
Calculated value	97.94450	
Margins	80.00000 to 125.00000	