

01 CPA-USP81 Cloxacillin

Document-Id: Document-1994 (Revision: 3)
Document type: Cylinder-plate assay (USP <81>)/1
Last modified: 06.09.2021 11:03:33
Database: O130_Permanent_PLA_305
Database GUID: 996faea8-9ec8-4fb7-8524-ea8686ffdf5d

Calculation information

Calculation performed: 06.09.2021 11:03:32, Benjamin Schneider (PLA 3.0.5 Build 816, NBARW01, 10014)
Report generated: 06.09.2021 11:12:20, Benjamin Schneider (PLA 3.0.5 Build 816, NBARW01, 10014)

Signatures

Responsibility

Review

Approval

Comments

Comment by Benjamin Schneider: (11.08.2021 14:14:21)

Example cylinder-plate assay based on USP <81>: The standard consists of five concentration steps (S1 to S5) with S3 being used as reference. The relative potency of the unknown sample U3 is estimated based on the standard. In accordance with USP <81>, the test system includes tests on the relative standard deviation CV (%), the coefficient of determination (r^2), and the relative potency. For a direct comparison, the response values are taken from USP <81>. Moreover, the concentration is log10-transformed as recommended by USP <81>.



DOCUMENT-1994



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Documentation

Date	10.06.2021 10:13:50
Substance	Cloxacillin, Penicillin, Cloxacillin is an antibiotic agent used for the treatment of beta-hemolytic streptococcal and pneumococcal infections as well as staphylococcal infections.
Inoculum	
Test organism	
Species	Staphylococcus aureus
Incubation	24h, 35° C
Incubation medium	
Ingredient	Peptone, 6g
Ingredient	Pancreatic digest of casein, 4g
Ingredient	Yeast extract, 3g
Ingredient	Beef extract, 1.5g
Ingredient	Dextrose, 1g
Ingredient	Agar, 15g
Ingredient	Water, 1000ml
pH	6.6
Inoculum medium	
Ingredient	Peptone, 6g
Ingredient	Pancreatic digest of casein, 4g
Ingredient	Yeast extract, 3g
Ingredient	Beef extract, 1.5g
Ingredient	Dextrose, 1g
Ingredient	Agar, 15g
Ingredient	Water, 1000ml
pH	6.6
Inoculum concentration	0.1ml / 100ml
Inoculum volume per plate	4ml
Base layer	
Medium	
Ingredient	Peptone, 6g
Ingredient	Yeast extract, 3g
Ingredient	Beef extract, 1.5g
Ingredient	Agar, 15g
Ingredient	Water, 1000ml
pH	6.6
Volume per plate	21ml

Assay overview

Property	Value
Cylinders per plate	6
Reference measurements per plate	3
Plate replicate	3
Total number of plates	15
Concentration unit	µg/ml

Assay elements	Summary
<ul style="list-style-type: none"> ■ STD ■ U3 	Standard sample, Sequence: 3.2, 4, 5, 6.25, 7.8125 Test sample, Predilution: 1

Property	Value
<i>Response data processing*</i>	
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated
Dose transformation	Logarithmic: Decimal logarithm (base 10)
Sample correction method	Correction point (mean reference step response)
Response unit	(not available)

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

Plates

	Zone 1		Zone 2		Zone 3		Zone 4		Zone 5		Zone 6	
1	■ [1]	14.60000	■ [1]	14.10000	■ [1]	13.50000	■ [3]	16.10000	■ [3]	15.60000	■ [3]	15.80000
2	■ [1]	14.50000	■ [1]	14.10000	■ [1]	14.40000	■ [3]	16.00000	■ [3]	15.90000	■ [3]	16.20000
3	■ [1]	14.00000	■ [1]	14.20000	■ [1]	14.10000	■ [3]	15.70000	■ [3]	15.70000	■ [3]	15.80000
4	■ [2]	14.70000	■ [2]	15.10000	■ [2]	14.80000	■ [3]	15.80000	■ [3]	15.60000	■ [3]	15.50000
5	■ [2]	14.70000	■ [2]	14.90000	■ [2]	15.20000	■ [3]	15.70000	■ [3]	15.50000	■ [3]	15.60000
6	■ [2]	14.80000	■ [2]	15.00000	■ [2]	14.30000	■ [3]	15.70000	■ [3]	15.40000	■ [3]	15.30000
7	■ [4]	16.60000	■ [4]	16.80000	■ [4]	16.30000	■ [3]	15.60000	■ [3]	15.80000	■ [3]	16.00000
8	■ [4]	16.60000	■ [4]	16.50000	■ [4]	16.20000	■ [3]	15.80000	■ [3]	15.60000	■ [3]	15.70000
9	■ [4]	16.90000	■ [4]	16.50000	■ [4]	16.80000	■ [3]	16.10000	■ [3]	15.70000	■ [3]	15.80000
10	■ [5]	17.30000	■ [5]	17.00000	■ [5]	17.00000	■ [3]	15.60000	■ [3]	15.60000	■ [3]	15.50000
11	■ [5]	17.30000	■ [5]	17.40000	■ [5]	17.20000	■ [3]	15.60000	■ [3]	15.70000	■ [3]	15.50000
12	■ [5]	17.30000	■ [5]	17.30000	■ [5]	16.70000	■ [3]	15.90000	■ [3]	15.80000	■ [3]	15.80000
13	■	15.30000	■	15.80000	■	15.70000	■ [3]	15.70000	■ [3]	15.80000	■ [3]	15.70000
14	■	15.80000	■	15.80000	■	15.50000	■ [3]	15.90000	■ [3]	15.70000	■ [3]	15.70000
15	■	15.20000	■	15.10000	■	15.10000	■ [3]	15.50000	■ [3]	15.80000	■ [3]	15.30000

■ STD ■ U3

Assay suitability tests

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

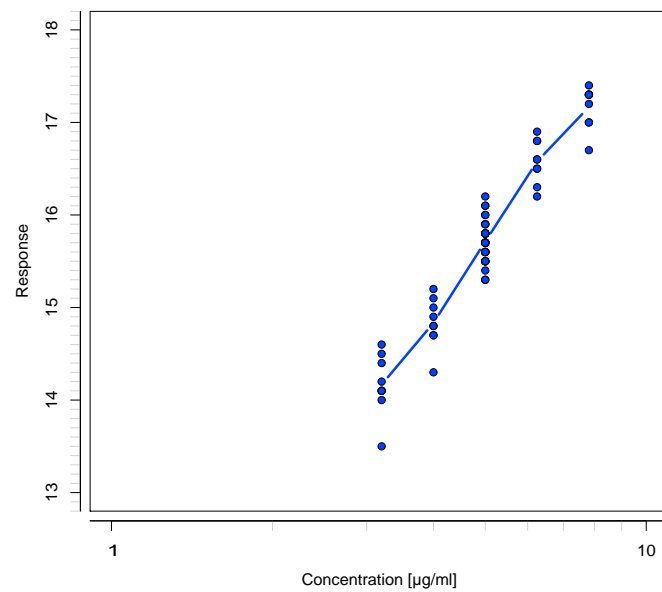
<ul style="list-style-type: none"> ■ STD Test: Step - Relative standard deviation CV (%) Scope: Standard only; Severity level: Warning 		Passed
Calculated value	0.90269 ; 2.28732	
Upper margin	10.00000	
<ul style="list-style-type: none"> ■ STD Test: Coefficient of determination (R²), regular Scope: Standard only; Severity level: Warning 		Passed
Calculated value	0.99686	
Lower margin	0.95000	

■ STD: Setup

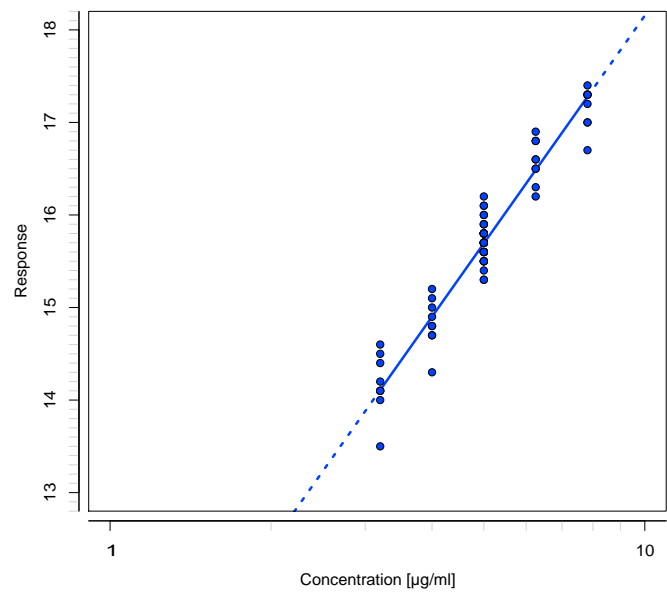
Property	Value
Concentration unit	µg/ml
Stepwise concentration sequence	3.2, 4, 5, 6.25, 7.8125
Reference step	S3
Position	3
Concentration	5

■ STD: Result

Data plot



Regression plot



Response data

Dilution step	S1 (1)	S3 (3)	S2 (2)	S3 (3)	S4 (4)	S3 (3)	S5 (5)	S3 (3)
Working concentration [µg/ml]	3.20000	5.00000	4.00000	5.00000	6.25000	5.00000	7.81250	5.00000
Plate replicate 1	14.60000 14.10000 13.50000	16.10000 15.60000 15.80000	14.70000 15.10000 14.80000	15.80000 15.60000 15.50000	16.60000 16.80000 16.30000	15.60000 15.80000 16.00000	17.30000 17.00000 17.00000	15.60000 15.60000 15.50000
Plate replicate 2	14.50000 14.10000 14.40000	16.00000 15.90000 16.20000	14.70000 14.90000 15.20000	15.70000 15.50000 15.60000	16.60000 16.50000 16.20000	15.80000 15.60000 15.70000	17.30000 17.40000 17.20000	15.60000 15.70000 15.50000
Plate replicate 3	14.00000 14.20000 14.10000	15.70000 15.70000 15.80000	14.80000 15.00000 14.30000	15.70000 15.40000 15.30000	16.90000 16.50000 16.80000	16.10000 15.70000 15.80000	17.30000 17.30000 16.70000	15.90000 15.80000 15.80000
Mean	14.16667	15.86667	14.83333	15.56667	16.57778	15.78889	17.16667	15.66667
SD	0.32404	0.20000	0.26458	0.15811	0.23333	0.16915	0.22361	0.14142
CV	2.29%	1.26%	1.78%	1.02%	1.41%	1.07%	1.30%	0.90%
Mean after RDP	14.16667	15.86667	14.83333	15.56667	16.57778	15.78889	17.16667	15.66667
Correction point		15.72222		15.72222		15.72222		15.72222
Correction value	0.14444	0.14444	-0.15556	-0.15556	0.06667	0.06667	-0.05556	-0.05556
Corrected mean	14.02222		14.98889		16.51111		17.22222	

Regression

Parameter	Estimate	Error
Intercept	9.97938	0.18878
Slope	8.17482	0.26503

Quality of regression	
r^2	0.99686
r^2 adjusted	0.99581

Sample suitability tests

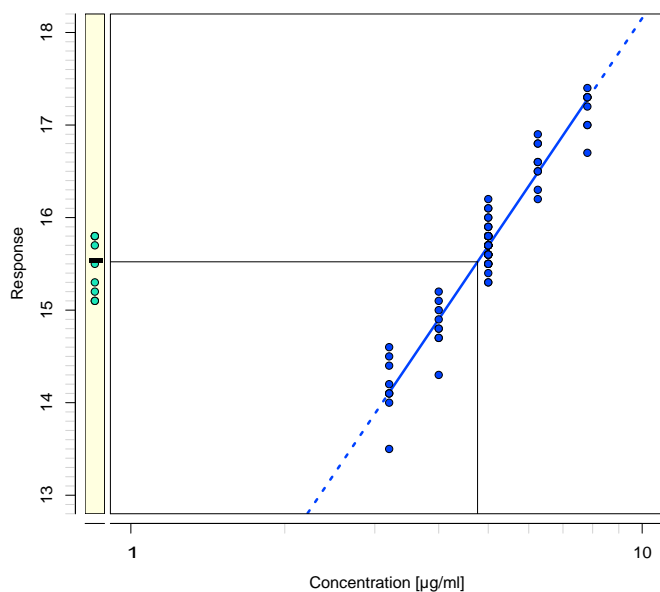
	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	0	0	0	0
Overall test result	Passed			

U3: Setup

Property	Value
Concentration unit	µg/ml
Predilution	1

U3: Result

Interpolation plot



Response data

Dilution step	Test	S3 (3)
Working concentration [µg/ml]	N/A	5.00000
Plate replicate 1	15.30000 15.80000 15.70000	15.70000 15.80000 15.70000
Plate replicate 2	15.80000 15.80000 15.50000	15.90000 15.70000 15.70000
Plate replicate 3	15.20000 15.10000 15.10000	15.50000 15.80000 15.30000
Mean	15.47778	15.67778
SD	0.30732	0.17873
CV	1.99%	1.14%
Mean after RDP	15.47778	15.67778
Comparison value*		15.72222
Correction value	-0.04444	-0.04444
Corrected mean	15.52222	

*Mean reference step response (correction point)

Interpolation

Property	Value
Absolute potency [µg/ml]	4.76473
Potency of the working sample [µg/ml]	4.76473
Relative potency (percentage of reference concentration)	95.29%

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			

U3		Passed
Test: Relative potency (percentage of reference concentration) Scope: Test samples only; Severity level: Warning		
Calculated value	95.29465	
Margins	80.00000 to 125.00000	