

## 02 CPA-JP4.02 Erythromycin

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### Calculation information

Calculation performed: 06.09.2021 11:03:17, 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 15:59:45)

Example cylinder-plate assay based on JP 4.02: The standard and the test sample consist of a high and a low concentration step. The standard sample is used as reference to determine the relative potency of the test sample TST1. The test system includes tests on the relative response standard deviation (high and low concentration) and the potency factor A.



DOCUMENT-1993



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## Documentation

|                   |   |
|-------------------|---|
| Date              | 11.06.2021 09:08:55   |
| Substance         | Erythromycin, Macrolide, Erythromycin is a macrolide substance having antibacterial activity produced by the growth of <i>Saccharopolyspora erythraea</i> . Erythromycin occurs as a white to light yellowish white powder. |
| Inoculum          |   |
| Test organism     |   |
| Species           | <i>Staphylococcus aureus</i>  |
| Incubation        | 24h, 35° C  |
| Incubation medium |   |
| Ingredient        | Glucose, 1g   |
| Ingredient        | Peptone, 6g   |
| Ingredient        | Meat extract, 1.5g  |
| Ingredient        | Yeast extract, 3g   |
| Ingredient        | Agar, 15g   |
| Ingredient        | Water, 1000ml   |
| pH                | 7.8   |
| Seed layer        |   |
| Medium            |   |
| Ingredient        | Glucose, 1g   |
| Ingredient        | Peptone, 6g   |
| Ingredient        | Meat extract, 1.5g  |
| Ingredient        | Yeast extract, 3g   |
| Ingredient        | Agar, 15g   |
| Ingredient        | Water, 1000ml   |
| pH                | 7.8   |
| Volume per plate  | 5ml   |
| Inoculum vol%     | 1   |
| Base layer        |   |
| Medium            |   |
| Ingredient        | Glucose, 1g   |
| Ingredient        | Peptone, 6g   |
| Ingredient        | Meat extract, 1.5g  |
| Ingredient        | Yeast extract, 3g   |
| Ingredient        | Agar, 15g   |
| Ingredient        | Water, 1000ml   |
| pH                | 7.8   |
| Volume per plate  | 20ml  |

## Assay overview

| Property                               | Value |
|--|-------|
| Sets per plate                         | 1     |
| Number of plates                       | 5     |
| <i>Standard solutions</i>              |       |
| Potency of high concentration solution | 20    |
| Potency of low concentration solution  | 5     |
| Potency unit                           | µg/ml |

| Assay elements   | Summary  |
|--|--|
| <ul style="list-style-type: none"> <li><span style="color: blue;">■</span> STD</li> <li><span style="color: green;">■</span> TST1</li> </ul> | Standard sample, Sequence: 5, 20<br>Test sample, Sequence: 5, 20, Predilution: 1 |

| Property                         | Value            |
|----------------------------------|------------------|
| <i>Response data processing*</i> |                  |
| Response adjustment              | No adjustment    |
| Response normalization           | No normalization |
| Response unit                    | (not available)  |

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

## Plates

|   | Position 1                              |          | Position 2                              |          | Position 3                           |          | Position 4                           |          |
|---|---|----------|---|----------|--------------------------------------|----------|--------------------------------------|----------|
| 1 | <span style="color: blue;">■</span> [1] | 18.56420 | <span style="color: blue;">■</span> [2] | 14.75640 | <span style="color: green;">■</span> | 18.79620 | <span style="color: green;">■</span> | 14.49470 |
| 2 | <span style="color: blue;">■</span> [1] | 18.45540 | <span style="color: blue;">■</span> [2] | 14.30080 | <span style="color: green;">■</span> | 18.40070 | <span style="color: green;">■</span> | 14.48520 |
| 3 | <span style="color: blue;">■</span> [1] | 18.80580 | <span style="color: blue;">■</span> [2] | 14.51950 | <span style="color: green;">■</span> | 18.48020 | <span style="color: green;">■</span> | 14.32250 |
| 4 | <span style="color: blue;">■</span> [1] | 18.82800 | <span style="color: blue;">■</span> [2] | 15.02090 | <span style="color: green;">■</span> | 18.60660 | <span style="color: green;">■</span> | 14.28240 |
| 5 | <span style="color: blue;">■</span> [1] | 18.14760 | <span style="color: blue;">■</span> [2] | 14.32310 | <span style="color: green;">■</span> | 18.78090 | <span style="color: green;">■</span> | 14.29420 |

■ STD ■ TST1

## Assay suitability tests

|                         | Passed        | Failed (rejected) | Failed (warning) | Info |
|-------------------------|---------------|-------------------|------------------|------|
| Assay suitability tests | 4             | 0                 | 0                | 0    |
| Overall test result     | <b>Passed</b> |                   |                  |      |

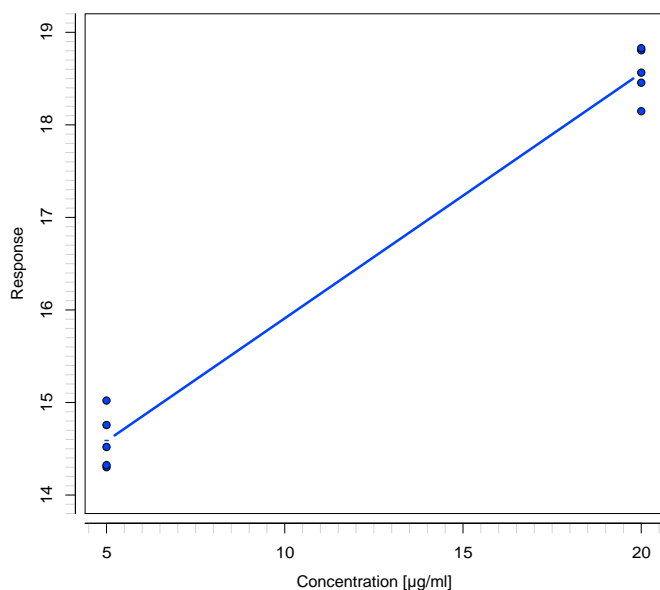
|  |          |        |
|--|----------|--------|
| <b>■ STD</b><br>Test: Sample relative response standard deviation<br>Scope: All assay elements; Severity level: Warning  |          | Passed |
| Calculated value   | 1.50782  |        |
| Upper margin   | 10.00000 |        |
| <b>■ TST1</b><br>Test: Sample relative response standard deviation<br>Scope: All assay elements; Severity level: Warning |          | Passed |
| Calculated value   | 0.94785  |        |
| Upper margin   | 10.00000 |        |
| <b>■ STD</b><br>Test: Sample relative response standard deviation<br>Scope: All assay elements; Severity level: Warning  |          | Passed |
| Calculated value   | 2.09394  |        |
| Upper margin   | 10.00000 |        |
| <b>■ TST1</b><br>Test: Sample relative response standard deviation<br>Scope: All assay elements; Severity level: Warning |          | Passed |
| Calculated value   | 0.73228  |        |
| Upper margin   | 10.00000 |        |

## ■ STD: Setup

| Property           | Value |
|--------------------|-------|
| Concentration unit | µg/ml |
| Sequence           | 20, 5 |

## ■ STD: Result

Data plot



Response data

| Dilution step   | 1  | 2  |
|-----------------|--|--|
| Potency [µg/ml] | 20.00000   | 5.00000  |
| Response        | 18.56420<br>18.45540<br>18.80580<br>18.82800<br>18.14760 | 14.75640<br>14.30080<br>14.51950<br>15.02090<br>14.32310 |
| Mean            | 18.56020   | 14.58414   |
| SD              | 0.27985  | 0.30538  |
| CV              | 1.51%  | 2.09%  |

## Sample suitability tests

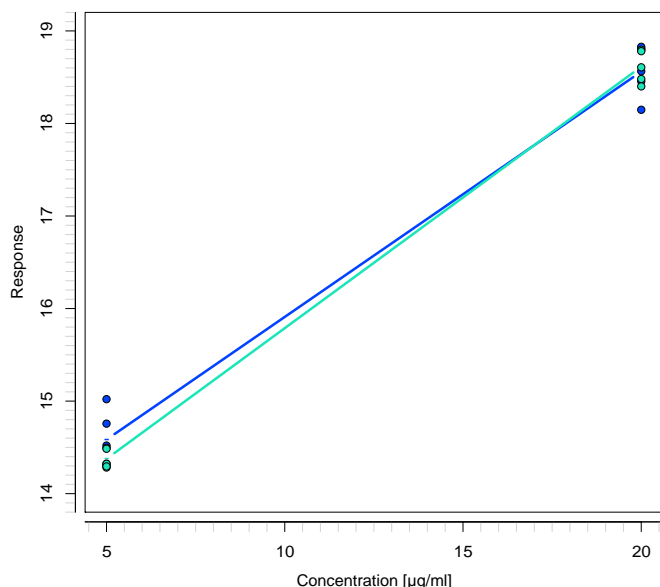
|                          | Passed        | Failed (rejected) | Failed (warning) | Info |
|--------------------------|---------------|-------------------|------------------|------|
| Assay suitability tests  | 4             | 0                 | 0                | 0    |
| Sample suitability tests | 0             | 0                 | 0                | 0    |
| Overall test result      | <b>Passed</b> |                   |                  |      |

### TST1: Setup

| Property           | Value |
|--------------------|-------|
| Concentration unit | µg/ml |
| Sequence           | 20, 5 |
| Predilution        | 1     |

### TST1: Result

Data plot



Response data

| Dilution step           | 1  | 2  |
|-------------------------|--|--|
| Assumed potency [µg/ml] | 20.00000   | 5.00000  |
| Response                | 18.79620<br>18.40070<br>18.48020<br>18.60660<br>18.78090 | 14.49470<br>14.48520<br>14.32250<br>14.28240<br>14.29420 |
| Mean                    | 18.61292   | 14.37580   |
| SD                      | 0.17642  | 0.10527  |
| CV                      | 0.95%  | 0.73%  |

### Calculation result

| Property                              | Value    |
|---------------------------------------|----------|
| Potency factor A                      | 0.97408  |
| Potency of the working sample [µg/ml] | 19.48150 |
| Absolute potency [µg/ml]              | 19.48150 |

### Sample suitability tests

|                          | Passed        | Failed (rejected) | Failed (warning) | Info |
|--------------------------|---------------|-------------------|------------------|------|
| Assay suitability tests  | 4             | 0                 | 0                | 0    |
| Sample suitability tests | 1             | 0                 | 0                | 0    |
| Overall test result      | <b>Passed</b> |                   |                  |      |

| TST1  |                    | Passed |
|---|--------------------|--------|
| Test: Potency factor A<br>Scope: Test samples only; Severity level: Warning |                    |        |
| Calculated value  | 0.97408            |        |
| Margins   | 0.90000 to 1.10000 |        |