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PETIA *Polymyxin-B Assay*

(Particle-enhanced immunoturbidimetric inhibition assay for plasma polymyxin-B)

This package insert for the PETIA Polymyxin-B Assay must be read carefully prior to use, and the package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1 NAME

PETIA Polymyxin-B Assay

2 PACKAGING SPECIFICATIONS

Reagents: R1: 33mL, R2: 11mL

Calibrator-A：3mL；Calibrator-B：1mL； Calibrator-C：1mL；Calibrator-D：1mL； Calibrator-E：1mL； Calibrator-F：1mL

3 INTENDED USE

The PETIA Polymyxin-B Assay is intended for the quantitative determination of Polymyxin-B in human plasma on automated clinical chemistry analyzers.

Polymyxin-B is a polypeptide antibiotic against multidrug-resistant Gram-negative bacteria, commonly used to treat severe infections caused by Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, etc. Polymyxin-B has a narrow therapeutic window. Low blood concentration cannot effectively kill bacteria, while excessively high concentration may easily cause severe adverse reactions such as nephrotoxicity, neurotoxicity, and respiratory depression. The toxic manifestations of Polymyxin-B often overlap with symptoms of severe infection. Therefore, blood concentration monitoring is helpful for optimizing dosage, improving efficacy and reducing toxicity. The assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

4 PRINCIPLES OF THE PROCEDURE

The PETIA Polymyxin-B Assay is based on the principle of latex-enhanced competitive immunoturbidimetry. During the assay, Polymyxin-B in the sample competes with Polymyxin-B conjugated to BSA for binding to anti-Polymyxin-B monoclonal antibodies coated on the surface of latex particles. When the antibody-coated latex is added in the reaction vessels, Polymyxin-B-BSA conjugate reacts with the latex surface antibodies and forms aggregates. Since the turbidity of the reaction solution caused by latex aggregation is negatively correlated with the concentration of Polymyxin-B in the sample, the Polymyxin-B concentration can be determined by measuring the change of absorbance at 600nm.

5 REAGENTS

Reagent-1 (R1): Polymyxin-B-protein conjugate, stored in MES buffer; Preservative: Sodium azide.

Reagent-2 (R2): Latex particles sensitized with anti-Polymyxin-B monoclonal antibody, stored in buffer containing stabilizer; Preservative: Sodium azide.

Calibrators: Solutions with different concentrations of Polymyxin-B; Preservative: Sodium azide.

6 STORAGE AND SHELF LIFE

The PETIA Polymyxin-B Assay reagents are provided in liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Improper storage of reagents can affect the assay performance.

Reagents were stable up to 30 days when stored on-board the instrument based on supporting data.

The PETIA Polymyxin-B Assay reagents contain ⩽ 0.05% sodium azide. The affected plumbing and instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components

7 SAMPLE REQUIREMENTS

- EDTA-anticoagulated plasma is required. Please use blood collection tubes following the manufacturer's instructions. Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.
- Do not induce foaming and avoid repeated freezing/thawing to preserve the integrity of the specimen before it is assayed.
- Clarified specimens may be stored up to one week at 2 to 8°C. If testing will be delayed more than 6 days, specimens should be stored frozen (≤ -20°C) up to four weeks prior to being tested.
- Handle all patient specimens as if they were potentially infectious.

8 INSTRUMENTS

This product is compatible with various types of automated analyzer. Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Hitachi 7180 Biochemical analyzer.

9 ASSAY PROCEDURE

- Confirm sufficient sample volume in the sample cup before detection.
- Calibration procedure must be performed for reagents used for the first time or requiring recalibration.
- Set detection parameters according to the following data.

Wavelength	600nm/None (Primary/Secondary)	Sample	4μL	Reaction Method	Two-point End-point Assay
Cuvette Light Path	1cm	R1	150μL	Calibration Type	Spline/Logit-log(4p)
Reaction Temperature	37℃	R2	50μL	Reaction Direction	Downward

An example of the assay procedure is indicated below.

	Calibrator	Sample
Calibrator	4μL	-
Sample	-	4μL
R1	150μL	150μL
Mix and incubate at 37°C for 3-5 minutes		
R2	50μL	50μL
Mix, incubate at 37°C for about 20 seconds, read absorbance A1, incubate for 5 minutes, read absorbance A2, calculate the concentration of Polymyxin-B based on the ΔA=A2-A1		

Sample Dilution Procedure

For samples with Polymyxin-B concentration > 8.00μg/mL, dilute the sample as follows:

- Mix 150μL sample with 150μL Calibrator A in a centrifuge tube.
- Test Result = Measured Value × 2.

Calibration

Perform a full calibration (6-point) procedure using the PETIA Polymyxin-B Calibrators A, B, C, D, E, and F; test calibrators in duplicate. The calibration curve is established by plotting the calibrator concentration against the absorbance change (ΔA) using non-linear fitting to evaluate the Polymyxin-B concentration in the sample.

- Calibration Range: 0.00-8.00μg/mL.
- After the system accepts and saves the calibration curve, subsequent samples can be tested without further calibration unless:
  1) A new batch number of the kit is used.
  2) The quality control value is out of the target range.

Quality Control

Certified quality control products can be used for quality control. The concentration of quality control products measured by each laboratory may be inconsistent, but it should be within the target range; if the measured value is out of the target range, please check:

- Whether the parameter settings are correct;
- Whether the sample needle and reagent needle are contaminated or blocked;
- Whether the light source is aging and the cuvette is clean;
- Whether the flow path of the instrument pipeline system is unobstructed;
- Whether the reaction temperature is correct;
- Whether the kit is expired.

10 RESULTS

The result units are reported as μg/mL. A therapeutic range for Polymyxin-B has not been well established. A steady-state concentration range across 24h ($C_{ss,\,avg}$) of 2 to 4μg/mL has been proposed, corresponding to an $AUC_{ss,\,24\,h}$ of 50–100 mg·h/L [1].

The assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures. A comprehensive clinical evaluation of the patient is required before determining or changing the treatment regimen.

11 LIMITATIONS OF PROCEDURE

This assay is designed for use with EDTA-anticoagulated plasma. It is generally good practice to use the same method consistently for individual patient care due to the potential for method-to-method variabilities. Like other immunoassays, the PETIA Polymyxin-B Assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy and dosage adjustments.

12 PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained on the Hitachi 7180 Biochemical analyzer. Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer.

- **Sensitivity**

1) The difference in absorbance between standard solution (0.5 μg/mL) and another standard solution (0μg/mL) is ⩾ 0.03.
2) The ratio of absorbance change of the 8μg/mL calibrator (ΔA_8) to that of the zero-value calibrator (ΔA_0) is < 15%.

- **Measurement Range**

The measurement range of the PETIA Polymyxin-B Assay is 0.5 – 8.0μg/mL. Specimens containing Polymyxin-B in higher concentrations (>8.0μg/mL) may be assayed by dilution of the specimen into the measurement range for a quantitative result or otherwise reported as detected above the measurement range.

- **Recovery**

Analytical recovery was assessed by adding concentrated Polymyxin-B drug into human plasma negative for Polymyxin-B, to obtain three samples with Polymyxin-B at (1.5 ± 0.5)μg/mL, (3.0 ± 0.5)μg/mL and (7.5 ± 0.5)μg/mL. Six replicates of each sample were assayed. The results were averaged and compared to the target concentration; the obtained Polymyxin-B concentration should be in the range of 85–115% of the expected values.

- **Precision**

Tri-level samples [*(1.5±0.5)μg/mL, (3.0±0.5)μg/mL and (7.5±0.5) μg/mL)*] were assayed in quadruplicate twice a day for 12 days. Each of the runs per day was separated by at least three hours. The within run, between day percent CVs were ≤15.0% for sample at (1.5±0.5)μg/mL, and ≤10.0% for samples at (3.0±0.5)μg/mL and (7.5±0.5)μg/mL.

- **Specificity**

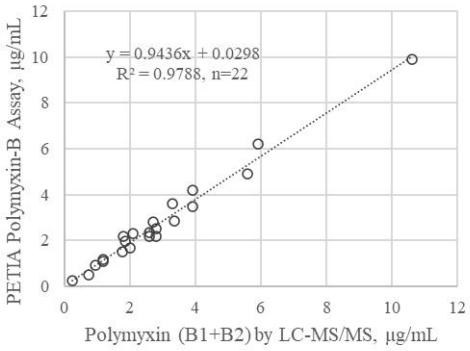
Polymyxin-B is a multi-component antibiotic with polymyxin B1 and B2 as its major components [2]. The PETIA Polymyxin-B Assay shows similar reactivity for polymyxin B1 and B2, and the cross-reactivity with polymyxin-E is less than 0.3%.

- **Interfering Substances**

Clinically high concentrations of the potentially interfering substances (bilirubin at 100mg/dL, Cholesterol at 600mg/dL, Hemoglobin at 1000mg/dL, and Triglycerides at 1000mg/dL) in serum with known levels of Polymyxin-B (3.0 and 6.0μg/mL) were evaluated. Each sample was assayed using the PETIA Polymyxin-B Assay. Measurement of Polymyxin-B resulted in ≤15% error in the presence of interfering substances.

- **Method Comparison**

Results from the PETIA Polymyxin-B Assay were compared with results from LC-MS/MS by measuring 22 specimens with Polymyxin-B concentrations ranged 0.2μg/mL to 10.6μg/mL. Passing-Bablok regression [3] statistics are shown below.



13 REFERENCES

1) Xiaofen LIU, Chenrong HUANG, Phillip J. BERGEN, et al. Chinese consensus guidelines for therapeutic drug monitoring of polymyxin B, endorsed by the Infection and Chemotherapy Committee of the Shanghai Medical Association and the Therapeutic Drug Monitoring Committee of the Chinese Pharmacological Society. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2023, 24(2):130-142.

2) Xiaofen Liu, Zhenwei Yu, Yu Wang, et al. Therapeutic drug monitoring of polymyxin B by LC–MS/MS in plasma and urine. Bioanalysis, 2020; 12: 845-55.

3) Bablok W, Passing H, Bender R. et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry. Part III. J Clin Chem Clin Biochem., 1988, 26:783-790.

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