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PETIA *Busulfan Assay*

(Particle-enhanced immunoturbidimetric inhibition assay for plasma/serum Busulfan)

This package insert for the PETIA Busulfan 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 Busulfan 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 Busulfan Assay is intended for the quantitative determination of Busulfan in human plasma on automated clinical chemistry analyzers.

Busulfan is an alkylating antineoplastic agent, mainly used for myeloablative conditioning prior to hematopoietic stem cell transplantation. It exerts antitumor effects by damaging the DNA structure of tumor cells and inhibiting cell proliferation. Busulfan has an extremely narrow therapeutic window: subtherapeutic concentrations may lead to transplantation failure and disease recurrence, while supratherapeutic concentrations frequently cause severe irreversible adverse reactions, including hepatic veno-occlusive disease (HVOD), myelosuppression, neurotoxicity, and pulmonary toxicity. These toxicities are often indistinguishable from other symptoms during the conditioning phase. Therefore, busulfan TDM is a core clinical practice to ensure transplantation safety and optimize dosing regimens.

The PETIA Busulfan Assay is designed for rapid and convenient monitoring of busulfan levels in patient's sample.

4 PRINCIPLES OF THE PROCEDURE

The PETIA Busulfan Assay is based on the principle of latex-enhanced competitive immunoturbidimetry. During the assay, Busulfan in the sample competes with Busulfan conjugated to BSA for binding to anti-Busulfan monoclonal antibodies coated on the surface of latex particles. When the antibody-coated latex is added in the reaction vessels, Busulfan-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 Busulfan in the sample, the Busulfan concentration can be determined by measuring the change of absorbance at 600nm.

5 REAGENTS

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

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

Calibrators: Solutions with different concentrations of Busulfan; Preservative: Sodium azide.

6 STORAGE AND SHELF LIFE

The PETIA Busulfan 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 Busulfan 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

- The blood should be centrifuged within 3h of collection. Serum and plasma can be used. 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 24 hours on ice. If testing will be delayed more than 6 days, specimens can 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
R2	50μL	50μL
Mix, incubate at 37℃ for about 20 seconds, read absorbance A1, incubate for 5 minutes, read absorbance A2, calculate the concentration of busulfan based on the ΔA=A2–A1		

Sample Dilution Procedure

For samples with Busulfan concentration > 5000ng/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 Busulfan 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 Busulfan concentration in the sample.

- Calibration Range: 0-5000ng/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 ng/mL. A therapeutic range for Busulfan has not been well established. A steady-state concentration range across 24h ($C_{ss,\ avg}$) of 600 to 900ng/mL has been proposed, corresponding to an $AUC_{ss,\ 24\ h}$ of 1200–1800 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 Busulfan 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 (300ng/mL) and another standard solution (0 ng/mL) is ≥ 0.03.
2) The ratio of absorbance change of the 5000ng/mL calibrator (ΔA_8) to that of the zero-value calibrator (ΔA_0) is < 15%.

- **Measurement Range**

The measurement range of the PETIA Busulfan Assay is 300 – 5000ng/mL. Specimens containing Busulfan in higher concentrations (>5000ng/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 Busulfan drug into human plasma negative for Busulfan, to obtain three samples with Busulfan at (500±50)ng/mL, (800±50)ng/mL and (4000±50)ng/mL. Six replicates of each sample were assayed. The results were averaged and compared to the target concentration, the obtained Busulfan concentration should be in the range of 85–115% of the expected values.

- **Precision**

Tri-level samples [*(500±50)ng/mL, (800±50)ng/mL and (4000±50) ng/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 (500±50ng/mL, and ≤10.0% for samples at (800±50)ng/mL and (4000±50) ng/mL.

- **Specificity**

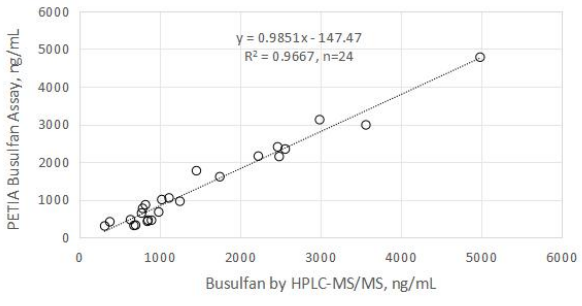
The assay shows good specificity to busulfan. No cross-reactivities were observed with 20 μ g/mL of Bu metabolites including tetramethyl sulfone, tetrahydrothiophene, and tetrahydrothiophene-3-ol-1,1-dioxide.

- **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 Busulfan (800 and 4000ng/mL) were evaluated. Each sample was assayed using the PETIA Busulfan Assay. Measurement of Busulfan resulted in ≤15% error in the presence of interfering substances.

- **Method Comparison**

Results from the PETIA Busulfan Assay were compared with results from LC-MS/MS by measuring 24 specimens with Busulfan concentrations ranged 314ng/mL to 4985ng/mL. Passing-Bablok regression [3] statistics are shown below.



13 REFERENCES

1. European Society for Blood and Marrow Transplantation (EBMT) Pharmacist Committee. A practical guide to therapeutic drug monitoring in busulfan. Bone Marrow Transplant, 2024.
2. Gibbs JP, et al. Gas chromatographic–mass spectrometric method for the detection of busulfan and its metabolites in plasma and urine. J Chromatogr B Biomed Sci Appl. 1993; 617(2):245–252.
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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