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PETIA *Acetaminophen Assay*

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

This package insert for the PETIA Acetaminophen (N-acetyl-p-aminophenol, APAP) 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 Acetaminophen 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 Acetaminophen Assay is intended for the quantitative determination of acetaminophen in human plasma/serum on automated clinical chemistry analyzers.

Acetaminophen is a widely used antipyretic and analgesic agent for mild to moderate pain and fever control, commonly applied to relieve headache, arthralgia, postoperative pain and pyrexia of various origins. Overdose can cause severe, potentially fatal hepatic necrosis. Timely measurement of plasma/serum acetaminophen concentration is critical for assessing risk and guiding N-acetylcysteine treatment [1, 2, 3, 4].

The measurement of an APAP levels after overdose should be as soon as possible in order to timely confirm the patient's risk of toxicity and thus the need to initiate NAC (N-acetylcysteine). The PETIA Acetaminophen Assay is designed for rapid, automated determination of APAP levels in human serum or plasma. It aids in the diagnosis and management of acetaminophen overdose and guides antidotal therapy.

The PETIA Acetaminophen Assay should only be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

4 PRINCIPLES OF THE PROCEDURE

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

5 REAGENTS

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

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

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

6 STORAGE AND SHELF LIFE

The PETIA Acetaminophen 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 Acetaminophen 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 or serum 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 following data.

Wavelength	600nm/None (Primary/Secondary)	Sample	3μL	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.

Assay procedure	Calibrator	Sample
Calibrator	3μL	-
Sample	-	3μL
R1	150μL	150μL
Mix and incubate at 37℃ for 3-5 minutes		
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 acetaminophen based on the ΔA=A2-A1		

Sample Dilution Procedure

For samples with acetaminophen concentration > 200μg/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 Acetaminophen 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 Acetaminophen concentration in the sample.

- Calibration Range: 0-200μg/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

- Results are calculated automatically by the analyzer. No additional manipulation of data is required unless samples have been manually diluted.
- The concentration of acetaminophen in serum or plasma depends on the time of drug ingestion, concomitant drug therapy, sample condition, time of sample collection, and individual variations in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results.
- In acute acetaminophen overdose, a single serum or plasma level determination, plotted on the Rumack-Matthew nomogram, provides a good indication of whether overdose therapy is required [5, 6].
- 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 or serum. 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 Acetaminophen Assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should monitor patients carefully 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 (6 μg/mL) and another standard solution (0 μg/mL) is ⩾ 0.03.
2) The ratio of absorbance change of the 100μg/mL calibrator (ΔA_8) to that of the zero-value calibrator (ΔA_0) is < 15%.

- **Measurement Range**

The measurement range of the PETIA Acetaminophen Assay is 2-200μg/mL. Specimens with APAP higher than 200 μg/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 acetaminophen drug into human plasma negative for acetaminophen, to obtain three samples with acetaminophen at (5.0 ± 0.5) μg/mL, (20 ± 2) μg/mL and (80 ± 8) μg/mL. Six replicates of each sample were assayed. The results were averaged and compared to the target concentration; the obtained acetaminophen concentration should be in the range of 85–115 % of the expected values.

- **Precision**

Tri-level samples [*(5.0±0.5)μg/mL, (20±2)μg/mL and (80±8) 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 (5.0±0.5)μg/mL, and ≤10.0% for samples at (20±2)μg/mL and (80±8)μg/mL.

- **Specificity**

The cross reactivities (at the 50 % displacement level) with the major metabolites of APAP were listed below.

Substance	Cross Reactivity (%)
APAP	100
APAP sulfate	< 0.09%
APAP glucuronide	< 0.01%
Acetylcysteine	< 0.01%
N-acetyl-p-benzoquinoneimine	< 0.05%

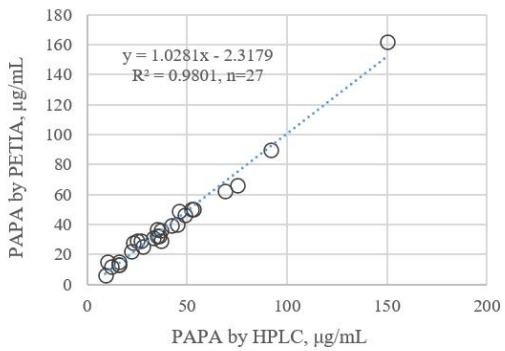
- **Interfering Substances**

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 acetaminophen (3.0 and 20μg/mL) were evaluated. Each sample was assayed using the PETIA Acetaminophen Assay. Measurement of acetaminophen resulted in ≤15% error in the presence of interfering substances.

- **Method Comparison**

Results from the PETIA Acetaminophen Assay were compared with results from HPLC by measuring 27 specimens with Acetaminophen concentrations ranged 6.2μg/mL to151 μg/mL.

Passing-Bablok regression statistics are shown below.



13 REFERENCES

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Manufacturer: Shanghai Baiwei Biotechnology Co., Ltd.

Address: Unit C401-3, Building 7, Pujiang Wisdom Valley, No.1188 Lianhang Road, Pujiang Town, Minhang District, Shanghai, China.

URL: bowei-biotech.com

Contact: +8615221067868