



# Stanbio Acetaminophen LiquiColor®<sup>®</sup>, Proc. No. 2400

For the *In Vitro* Quantitative Determination of Acetaminophen in Serum

## Summary and Principle

Acetaminophen (Tylenol, paracetamol, P-hydroxyacetanilide) is used in many formulations<sup>1</sup> as an analgesic generally with no adverse effects. Although the consequences of long-term treatment with acetaminophen are unclear, cases of hepatotoxicity and nephrotoxicity following chronic excessive use have been reported.<sup>2,3</sup> However, in cases of overdose, it can cause severe hepatic damage leading to hepatic failure if untreated.<sup>4,5,6</sup> Incidents of self poisoning as well as poisoning in small children have been increasing, now exceeding the incidence of aspirin poisoning.

The management of acetaminophen overdose requires an early recognition of the drug in the blood stream at certain concentrations. The toxic range is generally reported at over 200 mg/L. N-acetylcysteine has been used as an antidote in conjunction with intensive supportive care. Early diagnosis of acetaminophen induced hepatotoxicity is important because initiation of therapy within 16 hours of ingestion lessens the potential for hepatic injury and decreases the mortality rate.<sup>7</sup> A specific, simple and quick method to determine cases of overdose is most helpful so that proper treatment can quickly be made available. An enzymatic method offers these advantages over other methods.

Enzymes have been known to hydrolyze n-arylacylamides.<sup>8,9</sup> In the Stanbio method, acetaminophen is hydrolyzed by an arylacylamidase enzyme to yield p-aminophenol and acetate. Subsequently, the p-aminophenol is measured colorimetrically by its conversion to an indophenol in the presence of o-cresol where periodate is used as a catalyst.<sup>7</sup>

## Reagents

### Reagent A - Enzyme, Ref. No. 2401

Arylacylamidase < 7.5 KU/L  
o-Cresol < 9 mmol/L

### Reagent B - Catalyst, Ref. No. 2402

Sodium m-Periodate ≤ 3.75 mmol/L

### Standard - 300 mg/L, Ref. No. 2403

Acetaminophen 300 mg/L

## Precautions

For *in vitro* diagnostic use only. Avoid skin contact with the reagents. If skin contact occurs, wash immediately with large amounts of water. Handle serum and blood specimens as potentially infectious samples and follow the guidelines established by the Centers for Disease Control (CDC), Atlanta, Georgia, for blood collection and handling.

## Reagent Preparation:

Prepare the working reagent by adding 1 part reagent B to 2 parts reagent A, in that order. Mix before use. The working reagent is stable at 2-8 °C for 2 days or at 20-25°C for 4 hours when kept in stoppered amber vial.

## Reagent Storage and Stability:

Reagents A and B are stable at 2-8°C for 24 months after manufacture when kept separated. The aqueous Acetaminophen Standard is stable at 2-8 °C for 24 months after manufacture. Do Not Freeze.

## Specimen Collection and Preparation

Serum is the recommended specimen.

## Sample Stability:

**Interfering Substances:** No interference was observed when the following drugs at a 1mM concentration were added to serum containing 100 mg/L (0.661 mM) acetaminophen.

acetylsalicylic acid	p-ethoxyacetanilide
amylobarbitol	pentazocine
caffeine	phenobarbital
chlordiazepoxide	promethazine
diazepam	salicylamide
diphenhydramine	salicylic acid
indomethacin	salicylicuric acid
lorazepam	secobarbital
meprobamate	sodium barbital
methadone	theophylline
nitrazepam	tolbutamide
p-aminosalicylic acid	

Ethanol was tested at a concentration of 0.4% and found not to interfere. Acetylsalicylic acid and salicylic acid at concentrations of 5 mM were found not to interfere. N-acetyl-L-cysteine was tested at a concentration of 1000 mg/L and found not to significantly interfere. Concentrations of bilirubin at 10 mg/dL, creatinine at 25 mg/dL, or hemolytic serum with OD<sub>540</sub> of 2.5 do not interfere significantly with the measurement of acetaminophen. However, at extremely high concentrations of bilirubin (25 mg/dL, the acetaminophen concentrations of 90, 180, and 270 mg/L, the observed values were 131 mg/L, 208 mg/L and 284 mg/L.

EDTA at a concentration of 0.15% results in 10% lower reported values at concentrations of 100 and 200 mg/L acetaminophen.

In serum contain 90 mg/L acetaminophen, a 1 mM concentration of the drugs amitriptyline, amphetamine, imipramine and p-thoxyaniline gave biased values of -22%, -24%, -14% and +34%, respectively.

## Procedure

The Acetaminophen LiquiColor®<sup>®</sup> can be used in a 10 minute procedure as well as in a kinetic procedure, as described in Procedure 1 and Procedure 2, respectively. Both procedures give the same results provided that the spectrophotometer used reads absorbance to the third decimal place.

### Procedure #1- 10 Minute Test

Conditions: Wavelength.....615 nm  
 Temperature.....37°C  
 Mode.....10 minute rate  
 Sample Volume.....100 µL  
 Reagent Volume.....2.0 mL  
 Sample to reagent ratio.....1/20

Procedure: 1) Prepare the required amount of working reagent by adding 1 part Reagent B to 2 parts Reagent A, in that order.  
 2) Incubate the working reagent at 37°C for 3 minutes  
 3) To two 3 mL cuvettes, add 2 mL of working reagent and measure OD<sub>615</sub>(T<sub>0</sub>).  
 4) To cuvette #1, add 100 µL of test serum and measure OD<sub>615</sub>(T<sub>1</sub>).  
 5) To cuvette #2, add 100 µL of acetaminophen standard and measure OD<sub>615</sub>(T<sub>2</sub>).  
 6) Exactly 10 minutes after adding serum or standard, measure OD<sub>615</sub>(T<sub>0</sub>).  
 7) Subtract T<sub>0</sub> from T<sub>1</sub> to get ΔOD<sub>10</sub>

Calculations:

$$\frac{\Delta OD_{10} \text{ of test serum}}{\Delta OD_{10} \text{ of standard}} \times 300 \text{ mg/L} \times \text{dil of serum} = \text{Acetaminophen mg/L}$$

An acetaminophen concentration of 100 mg/L should give ΔOD<sub>10</sub> of about 0.24 - 0.26 in the 10 minute test mode.

## Procedure #2 - Kinetic Test

Conditions: Wavelength.....615nm  
 Temperature.....37°C  
 Mode.....Kinetic  
 Sample Volume.....100 µL  
 Reagent volume.....2.0 mL  
 Sample to reagent ratio.....1/20

Procedure: 1) Prepare the required amount of working reagent by adding 1 part Reagent B to 2 parts Reagent A, in that order.  
 2) Incubate the working reagent at 37°C for 3 minutes  
 3) To two 3 mL cuvettes, add 2 mL of working reagent.  
 4) To cuvette #1, add 100 µL of test serum.  
 5) To cuvette #2, add 100 µL of acetaminophen standard.  
 6) Determine the ΔOD/min between 3 and 5 mins at 37°C.

## Calculation:

$$\frac{\Delta OD/\text{min of test serum}}{\Delta OD/\text{min of standard}} \times 300 \text{ mg/L} \times \text{dil of serum} = \text{Acetaminophen mg/L}$$

NOTES: a) Precise measurements of temperature and time are required to obtain accurate results.  
 b) The working reagent when first prepared has a light yellow color which gradually deepens over the lifetime of the working reagent. Test results are not affected by this coloration.

## Quality Control

In order to assure consistent performance, it is recommended that a normal and an abnormal serum control be assayed with each run.

## Expected Values

Although toxic manifestations have been observed at serum concentrations of > 100 mg/L. The therapeutic range varies, and has been reported at 10-30 mg/L.<sup>7</sup>

## Performance Characteristics

**Linearity:** Both procedures described above are linear to 300 mg/L acetaminophen. For higher concentrations, dilute the sample with distilled water. Repeat the assay and multiply the results by the dilution factor.

**Sensitivity:** Concentrations of acetaminophen of 90, 100 and 110 mg/L can be clearly distinguished at the 99.9% confidence limit with either method. Due to the variation between laboratory instruments, and the reduced absorbance change with acetaminophen concentrations below 10 mg/L, test results below 10 mg/L should be reported as "<10 mg/L."

**Precision:** Precision studies were conducted using two serum pools containing 50 and 200 mg/L acetaminophen. The following results are an average of eighteen determinations.

10 Minute Test:	Mean(mg/L)	Standard Deviation	CV(%)
Within Day	50.0	0.71	1.42
	202.9	3.05	1.50
Between Day	50.0	1.79	3.58
	202.7	6.36	3.14

## References

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