SEKURE® CHEMISTRY

EN

ACETAMINOPHEN L3K® ASSAY

CATALOGUE NUMBER: 507-30 **SIZE:** R1: 3 x 10 mL, R2: 6 x 10 mL, 1 x 5 mL calibrator

Note: Changes are highlighted.

INTENDED USE

For the *in vitro* quantitative measurement of acetaminophen in serum, lithium heparin plasma and sodium heparin plasma. Measurement of acetaminophen is used in the diagnosis and treatment of acetaminophen overdose toxicity.

TEST SUMMARY

Acetaminophen (paracetamol) is used as an analgesic in many different formulations.[1] While therapeutic doses rarely cause adverse side effects, the effect of long term treatment with acetaminophen is unclear. Cases have been reported where chronic excessive use of acetaminophen has led to hepatotoxicity and nephrotoxicity.[2,3] In cases of acute overdosage, acetaminophen can cause severe hepatic damage leading to hepatic failure if untreated.[4,5,6]

The management of acetaminophen overdose requires early recognition of the drug in the bloodstream. Toxicity is generally reported at concentrations over 200 µg/mL (1324 µmol/L). N-acetylcysteine has been used as an antidote in conjunction with intensive support care. Early diagnosis of acetaminophen-induced hepatotoxicity is important since initiation of therapy within 8 hours of ingestion lessens the potential for hepatic injury and decreases the mortality rate.[7]

The majority of methods for measuring acetaminophen are based on spectrophotometric or chromatographic principles. Chromatographic methods are specific for the parent compound; however, they are not well suited to emergency laboratories. Spectrophotometric methods are simpler and more rapid, but do not always offer the desired specificity.

TEST PRINCIPLE

$$\text{Acetaminophen} \xrightarrow{\text{Acyl Amidohydrolase}} \text{p-aminophenol} + CH_3COOH$$

$$\text{p-aminophenol} + \text{2,5-dimethylphenol} \xrightarrow{Mn^{2+}} \text{4-(4-iminophenol)-2,5-dimethylcyclohexadiene-1-one}$$

The enzyme, acyl amidohydrolase, cleaves the amide bond of the acetaminophen molecule, leaving p-aminophenol and acetate. The p-aminophenol is reacted with 2,5-dimethylphenol in the presence of manganese ions to form a colored compound, 4-(4-iminophenol)-2,5-dimethylcyclohexadiene-1-one. The increased absorbance at 605 nm due to the formation of 4-(4-iminophenol)-2,5-dimethylcyclohexadiene-1-one is directly proportional to the concentration of acetaminophen in the sample.

REAGENTS

Acetaminophen Enzyme Reagent (R1): A solution containing buffer (pH 8.6 at 25°C), 0.3 mmol/L $MnCl_2 \cdot 4H_2O$, ≥ 0.9 kU/L Acyl Amidohydrolase (microbial), 50 mg/L sodium azide.

Acetaminophen Color Reagent (R2): A solution containing 0.1 mol/L sodium carbonate buffer (pH 11.5 at 25°C), 31 mmol/L 2,5-dimethylphenol, stabilizer, preservative.

Acetaminophen Calibrator: 1 x 5 mL of a solution containing buffer (pH 5.2 at 25°C), 151 µg/mL (1000 µmol/L) acetaminophen, preservatives.

Internal reference standards are created for Acetaminophen using a USP grade reference Acetaminophen material (not less than 98% and not more than 102% of paracetamol on an anhydrous basis). Acetaminophen calibrator is manufactured gravimetrically and tested against these internal reference standards.

WARNINGS AND PRECAUTIONS FOR USE

IVD

For *in vitro* diagnostic use

℞ ONLY

See Safety Data Sheet for additional information.

REAGENT PREPARATION, STORAGE AND STABILITY

Reagents are ready for use.

Supplied reagents are stable at 2-8°C until expiry date. Stability claims are based on real time studies.

Instrument specific onboard stability is listed on the application sheets.

REAGENT DETERIORATION

The reagents should be clear. Turbidity would indicate deterioration.

DISPOSAL

Reagents must be disposed of in accordance with all Federal, Provincial, State, and local regulations.

SPECIMEN

Fresh, clear, unhemolysed serum, lithium or sodium heparinized plasma. Separated samples may be stored for up to 14 days at 4 to 8°C prior to being tested. If testing will be delayed more than 14 days, separated samples may be stored frozen at ≤ -20°C for up to 45 days.[8]

LIMITATIONS/ INTERFERING SUBSTANCES (CLSI EP07-A2)[9]

Interferences from hemolysis, icterus and lipemia were evaluated for this acetaminophen method on a Roche/Hitachi® 717 using a significance criterion of > 10% variance from control.

Conjugated bilirubin concentration of up to 2 mg/dL (23.7 µmol/L) did not interfere in samples with acetaminophen concentrations of 14.8 µg/mL (98 µmol/L). Unconjugated bilirubin concentration of up to 4 mg/dL (68.4 µmol/L) did not interfere in samples with acetaminophen concentrations of 14.8 µg/mL (98 µmol/L).

Hemoglobin concentration greater than 200 mg/dL (31 µmol/L) showed a positive bias of up to 32% at acetaminophen concentration of 14.0 µg/mL (93 µmol/L). Hemoglobin produces significant interference in this method; therefore, hemolyzed samples should not be used.

NOTE: Significantly reduced acetaminophen recovery has been demonstrated in situations where testing for acetaminophen toxicity has been performed on hyperbilirubinemic samples at acetaminophen levels in the range of 5.0 - 15.1 µg/mL (33 - 100 µmol/L). It is recommended that laboratories review the Rumack-Matthews Nomogram for patient ingestion status, treatment and monitoring protocols to determine the extent of the interference.

ANALYTICAL SPECIFICITY (CLSI EP07-A2)[9]

Cross Contamination studies have not been performed on automated instruments. Certain reagent/instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Interferences from icterus, lipemia, hemolysis, ascorbic acid and N-acetylcysteine were evaluated for this acetaminophen method on the Roche/Hitachi® 717 analyzer using a significance criterion of >10% or ±1.2 µg/mL (8 µmol/L) variance from control, whichever is greater. Plasma data is expected to be similar.

Substance Tested	Highest Tested Concentration with No Significant Interference	Acetaminophen Concentration
Hemoglobin*	200 mg/dL (31 µmol/L)	4.5 µg/mL (30 µmol/L)
	200 mg/dL (31 µmol/L)	14.0 µg/mL (93 µmol/L)
	400 mg/dL (62 µmol/L)	28.4 µg/mL (188 µmol/L)
	1000 mg/dL (155 µmol/L)	130.0 µg/mL (861 µmol/L)
Conjugated Bilirubin*	2 mg/dL (23.7 µmol/L)	4.7 µg/mL (31 µmol/L)
	2 mg/dL (23.7 µmol/L)	14.8 µg/mL (98 µmol/L)
	32 mg/dL (380 µmol/L)	17.4 µg/mL (115 µmol/L)
	40 mg/dL (475 µmol/L)	30.2 µg/mL (201 µmol/L)
	40 mg/dL (475 µmol/L)	144.5 µg/mL (957 µmol/L)
Unconjugated Bilirubin*	2 mg/dL (34.2 µmol/L)	5.0 µg/mL (33 µmol/L)
	4 mg/dL (68 µmol/L)	14.8 µg/mL (98 µmol/L)
	24 mg/dL (410 µmol/L)	17.4 µg/mL (115 µmol/L)
	24 mg/dL (410 µmol/L)	30.2 µg/mL (200 µmol/L)
	40 mg/dL (684 µmol/L)	144 µg/mL (958 µmol/L)
Ascorbic Acid	3000 µg/dL (170 µmol/L)	4.5 µg/mL (30 µmol/L)
	3000 µg/dL (170 µmol/L)	16.2 µg/mL (107 µmol/L)
	3000 µg/dL (170 µmol/L)	32.5 µg/mL (215 µmol/L)
	3000 µg/dL (170 µmol/L)	142.8 µg/mL (946 µmol/L)
N-Acetylcysteine	1500 mg/L (9.2 mmol/L)	4.5 µg/mL (30 µmol/L)
	1500 mg/L (9.2 mmol/L)	16.6 µg/mL (110 µmol/L)
	1500 mg/L (9.2 mmol/L)	32.5 µg/mL (215 µmol/L)
	1500 mg/L (9.2 mmol/L)	139.2 µg/mL (922 µmol/L)
Intralipid	600 mg/dL [1800 mg/dL (20 mmol/L) Simulated Triglycerides]	4.8 µg/mL (32 µmol/L)
	1000 mg/dL [3000 mg/dL (34 mmol/L) Simulated Triglycerides]	15.1 µg/mL (100 µmol/L)
	1000 mg/dL [3000 mg/dL (34 mmol/L) Simulated Triglycerides]	30.7 µg/mL (203 µmol/L)
	1000 mg/dL [3000 mg/dL (34 mmol/L) Simulated Triglycerides]	135.6 µg/mL (898 µmol/L)

* See additional information under the heading “Limitations/ Interfering Substances”.

Samples containing elevated levels of Immunoglobulin M (IgM) or samples from patients with Waldenstrom’s Macroglobulinemia may produce unreliable results.

ANALYTICAL SPECIFICITY TO DRUGS

Interferences from the following therapeutic drugs were tested at acetaminophen concentrations of 4.2 µg/mL (28 µmol/L), 14.9 µg/mL (99 µmol/L), 32.5 µg/mL (215 µmol/L) and 143.5 µg/mL (950 µmol/L) and were evaluated for this Acetaminophen method on a Roche/Hitachi® 717 analyzer using a significance criterion of >10% or ±1.2 µg/mL (8 µmol/L) variance from control, whichever is greater.

Substance Tested	Concentration with No Significant Interference
Theophylline	222 µmol/L
Phenylbutazone	2.89 mmol/L
Ibuprofen	2425 µmol/L
Imipramine	2.5 µmol/L
Acetylsalicylic Acid	6.51 mmol/L
Levodopa	25.3 µmol/L
Ampicillin	152 µmol/L
Doxycycline	67.5 µmol/L
Amitriptyline	3.61 µmol/L
Metronidazole	701 µmol/L
Cefoxitin	1546 µmol/L
Cyclosporin	10.0 µmol/L
Methyldopa	71 µmol/L
Rifampicin	78.1 µmol/L
Salicylate	4.34 mmol/L

Samples containing NAPQI (N-Acetyl-4-benzoquinoneimine) may cause elevated levels of measured acetaminophen. Samples containing >20 mg/L metamizole may cause elevated levels of measured acetaminophen.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S.[10]

ANALYTICAL PROCEDURE

MATERIAL PROVIDED

SEKURE Acetaminophen reagents and calibrator.

MATERIALS REQUIRED (BUT NOT PROVIDED)

1. Automated analyzer capable of accurately measuring absorbance at appropriate wavelength as per instrument application.
2. Acetaminophen L3K Reagent Application files for Automated Analyzer.
3. Quality control materials.

TEST CONDITIONS

For data presented in this insert, studies using SEKISUI Diagnostics acetaminophen reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:41 and a wavelength reading of 660 nm.

For assistance with applications on automated analyzers within Canada and the U.S., please contact SEKISUI Diagnostics Technical Services at (800)565-0265. Outside Canada and the U.S., please contact your local distributor.

CALIBRATION

An acetaminophen calibrator is included and should be used as directed to calibrate the procedure. The frequency of calibration on automated systems is dependent on the system and the parameters used. See the instrument specific application sheet for the calibration stability.

QUALITY CONTROL

Appropriate concentrations of quality control materials should be analyzed as required in accordance with local, state and federal guidelines. The results should fall within the acceptable range as established by the laboratory.

CALCULATIONS

The analyzer calculates the acetaminophen concentration of each sample.

TEST LIMITATIONS

A sample with an acetaminophen concentration exceeding the linearity limit should be diluted with 0.9% saline and re-assayed incorporating the dilution factor into the calculation of the value.

REFERENCE INTERVALS[7]

Therapeutic concentration: 10-30 µg/mL (66-199 µmol/L)

Toxic concentration: > 200 µg/mL (1324 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish its own expected range.

PERFORMANCE CHARACTERISTICS

Data presented was collected on a Roche/Hitachi® 717 analyzer unless otherwise stated.

RESULTS

Acetaminophen concentration is reported as µg/mL (µmol/L).

To convert acetaminophen results to mg/L (µg/mL) or mg/dL, use the following conversion factors:

µmol/L x 0.151 = mg/L (µg/mL)
mg/dL x 10 = mg/L (µg/mL)

NOTE: 1mg/L = 1µg/mL

REPORTABLE RANGE (CLSI EP06-A)[9]

The linearity of the procedure described is 377.5 µg/mL (2500 µmol/L). The limit of quantitation of the procedure described is 1.2 µg/mL (8 µmol/L).

The reportable range is 17.4 to 377.5 µg/mL (115 to 2500 µmol/L).

PRECISION STUDIES (CLSI EP05-A3)[9]

Three levels of controls, two unaltered patient sera and two spiked patient sera were assayed in duplicate, twice a day, for twenty days (n = 80). Calibrations were performed daily. All samples were tested using 3 lots of Acetaminophen reagent.

Lot	Sample	N	Concentration (µmol/L)	Repeatability		Within Laboratory	
				SD (µmol/L)	%CV	SD (µmol/L)	%CV
A	Control 1	80	67.2	1.8	2.7	2.5	3.8
	Control 2	80	237.3	4.4	1.9	6.1	2.6
	Control 3	80	764.3	13.4	1.8	14.3	1.9
	Unaltered P1	80	170.3	2.1	1.2	2.4	1.4
	Unaltered P2	80	195.5	2.4	1.2	2.6	1.4
	Spiked 1	80	1295.9	11.5	0.9	19.7	1.5
	Spiked 2	80	2278.6	17.3	0.8	51.0	2.2
B	Control 1	80	66.6	1.1	1.7	2.4	3.5
	Control 2	80	237.6	3.9	1.7	5.8	2.4
	Control 3	80	765.2	14.2	1.9	14.2	1.9
	Unaltered P1	80	169.5	1.5	0.9	2.1	1.2
	Unaltered P2	80	195.4	2.6	1.3	2.6	1.3
	Spiked 1	80	1294.3	12.8	1.0	21.4	1.7
	Spiked 2	80	2281.5	15.9	0.7	52.3	2.3
C	Control 1	80	68.2	1.4	2.0	2.4	3.5
	Control 2	80	245.1	4.0	1.6	5.8	2.4
	Control 3	80	789.0	13.6	1.7	14.2	1.8
	Unaltered P1	80	175.0	1.6	0.9	2.0	1.2
	Unaltered P2	80	202.0	2.7	1.3	3.0	1.5
	Spiked 1	80	1339.3	13.8	1.0	22.5	1.7
	Spiked 2	80	2357.9	17.5	0.7	50.0	2.1

ACCURACY (CLSI EP09-A3)[9]

The performance of this method (y) was compared with the performance of a similar acetaminophen method (x) on a Roche/Hitachi® 717. A combination of 105 natural and spiked patient serum samples ranging from 17.5 – 369.5 µg/mL (116 – 2447 µmol/L) were tested. The Deming and Passing-Bablok data is summarized below.

Deming: This method = 0.974 (reference method) + 0.7 µg/mL (4.7 µmol/L) R = 0.9999

Passing-Bablok: This method = 0.975 (reference method) + 0.3 µg/mL (2.3 µmol/L) R = 0.9999

The performance of this method with plasma (y) was compared to the performance of this method with serum (x) on a Roche/Hitachi® 717. A combination of 40 natural and spiked serum and plasma samples ranging from 17.4 – 350.6 µg/mL (115-2322 µmol/L) were tested. The Deming and Passing-Bablok data is summarized below.

Deming: This method (plasma) = 1.015 [This method (serum)] - 0.3 µg/mL (1.7 µmol/L) R = 0.9997

Passing-Bablok: This method (plasma) = 1.012 [This method (serum)] - 0.6 µg/mL (3.7 µmol/L) R = 0.9997

TRADEMARK

L3K® is a registered US trademark of SEKISUI Diagnostics.

Symbols

LOT
Batch code


Manufacturer


Consult instructions for use

IVD
In vitro diagnostic medical device


Use-by Date
YYYY-MM-DD or YYYY-MM

REF
Catalogue number


Temperature limit

℞ONLY
Caution: Federal law restricts this device to sale by or on the order of a physician

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