LZI 6-Acetylmorphine (6AM) Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0290 (100/37.5 mL R_1/R_2 Kit)
0291 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) 6-Acetylmorphine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of 6-acetylmorphine in human urine at a cutoff value of 10 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

6-Acetylmorphine (6AM), otherwise known as 6-monoacetylmorphine (6MAM), is a unique metabolite of heroin (3, 6-diacetylmorphine). Due to the fact that it is a unique metabolite of heroin and because 6AM cannot be synthesized in the human body from either codeine or morphine, its detection can be used as a specific assay for the use of heroin (3, 4, 5). The specificity of this assay is significant, as traditional immunoassays for heroin test for the presence of morphine, which is also a metabolite of a number of different opiates including codeine, morphine, and heroin (6, 7). Within the body, heroin is rapidly metabolized via deacetylation into 6AM. 6AM is then further hydrolyzed into morphine then morphine glucuronides and finally excreted in urine (8, 9). Clearance rates for 6AM are dependent on factors such as frequency of drug use, amount of drug used, and metabolism rates. Previous studies have found detectable levels of 6AM within urine for up to 8 hours (4). Previous studies have also shown that following IV infusion of heroin, the urinary metabolites accumulated over a 40-hour period include morphine (4.2 %), conjugated morphine (38.3 %), 6AM (1.3 %), and unchanged heroin (0.1 %) (10).

Assay Principle

The LZI 6-acetylmorphine assay is a homogeneous enzyme immunoassay using a ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (11). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, 6-acetylmorphine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody binds to the free drug and the unbound 6-acetylmorphine-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains a mouse monoclonal anti-6-acetylmorphine antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.
Enzyme-drug Conjugate Reagent (R_2): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with 6-acetylmorphine in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and controls are sold separately and contain negative human urine with sodium azide as a preservative.*

6-ACETYLMORPHINE Calibrators	REF
6AM Negative Calibrator	0299
Low Calibrator: Contains 5 ng/mL 6-acetylmorphine	0292
Cutoff Calibrator: Contains 10 ng/mL 6-acetylmorphine	0293
Intermediate Calibrator: Contains 20 ng/mL 6-acetylmorphine	0294
High Calibrator: Contains 40 ng/mL 6-acetylmorphine	0295

6-ACETYLMORPHINE Controls	REF
Level 1 Control: Contains 7.5 ng/mL 6-acetylmorphine	0297
Level 2 Control: Contains 12.5 ng/mL 6-acetylmorphine	0298

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (12).*
- *Do not use the reagents beyond their expiration dates.*
- Rx ONLY *For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use.

Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (13). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (14, 15). For longer storage, keep samples frozen at -20ºC and then thaw before use. Studies have shown 6-acetylmorphine analytes in urine are stable at -20ºC for up to six months (16). Samples should be equilibrated to room temperature (18-25ºC) for testing. Samples with high turbidity should be centrifuged before analysis.
Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing. *Handle all urine specimens as if they are potentially infectious.*

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay. Performance characteristics presented in this package insert have been validated on the Hitachi 717. If other instruments are used, performance will need to be validated by the laboratory (17, 18).

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. Typical assay parameters used for the Hitachi 717 analyzer include a 20 µL sample, 200 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) in 37ºC incubation temperature, 30-35 reading frames, and a 340 nm primary wavelength. For qualitative analysis, use the 10 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 7.5 ng/mL and 12.5 ng/mL.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A positive test result does not necessarily mean that a person took illegal drugs and a negative test result does not necessarily mean that a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 10 ng/mL of 6-acetylmorphine, is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance (ΔmA/min) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmA/min) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS, LC/MS or (2) permitting laboratories to establish quality control procedures.
When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of 6-acetylmorphine in the sample may then be estimated from the calibration curve.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of 6-acetylmorphine. The test is not intended for quantifying this single analyte in patient samples.*
2. *A preliminary positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take illegal drugs.*
4. *There is a possibility that other substances and/or factors not listed above may interfere with the test and cause incorrect results (e.g., technical or procedural error, fluid intake, endogenous or exogenous interferents).*
5. *Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
6. *The test is designed for use with human urine only.*
7. *The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The assay range from 0 ng/mL to 20 ng/mL was tested in qualitative (mA/min) and semi-quantitative (ng/mL) mode using a modified NCCLS protocol. Results shown below were obtained by testing all samples in replicate of two, two runs per day for 22 days on the Hitachi 717 automated clinical chemistry analyzer.

Precision:

Qualitative analysis: Typical results (mA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	358.6	2.2	0.6 %	358.6	2.4	0.7 %
2.5 ng/mL	380.0	2.5	0.7 %	380.0	2.8	0.7 %
5.0 ng/mL	399.4	2.2	0.6 %	399.4	2.4	0.6 %
7.5 ng/mL	419.5	2.4	0.6 %	419.5	2.7	0.6 %
10 ng/mL	437.4	2.2	0.5 %	437.4	2.6	0.6 %
12.5 ng/mL	454.8	2.0	0.4 %	454.8	3.0	0.7 %
15 ng/mL	470.4	3.8	0.8 %	470.4	4.6	1.0 %
17.5 ng/mL	489.2	2.3	0.5 %	489.2	3.0	0.6 %
20 ng/mL	500.1	2.2	0.4 %	500.1	2.8	0.6 %

Additional Qualitative analysis: The following table summarizes the interpretation of the absorbance (mA/min) results as being either positive or negative results:

10 ng/mL Cutoff		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
2.5 ng/mL	25 %	22	22 Neg	88	88 Neg
5.0 ng/mL	50 %	22	22 Neg	88	88 Neg
7.5 ng/mL	75 %	22	22 Neg	88	88 Neg
10 ng/mL	100 %	22	12 Neg/ 10 Pos	88	47 Neg/ 41 Pos
12.5 ng/mL	125 %	22	22 Pos	88	88 Pos
15 ng/mL	150 %	22	22 Pos	88	88 Pos
17.5 ng/mL	175 %	22	22 Pos	88	88 Pos
20 ng/mL	200 %	22	22 Pos	88	88 Pos

Semi-quantitative analysis: Typical results (ng/mL) are as follows:

Concentration	Within Run (N=22)		Total Precision (N=88)	
	Mean	Qualitative Response	Mean	Qualitative Response
0 ng/mL	0.2	-	0.2	-
2.5 ng/mL	2.8	-	2.8	-
5.0 ng/mL	5.1	-	5.1	-
7.5 ng/mL	7.4	-	7.4	-
10 ng/mL	9.7	-	9.7	-
12.5 ng/mL	12.2	+	12.2	+
15 ng/mL	14.6	+	14.6	+
17.5 ng/mL	17.7	+	17.7	+
20 ng/mL	19.9	+	19.9	+

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 2 ng/mL and is supported by the recovery study.

Accuracy: Eighty (80) unaltered clinical urine specimens were tested with LZI 6-Acetylmorphine Enzyme Immunoassay and confirmed with GC/MS. Specimens having a 6-acetylmorphine concentration greater than 10 ng/mL by LC/MS are defined as positive, and specimens with concentrations lower than 10 ng/mL by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ± 50 % of the cutoff value):

Qualitative Accuracy Study:

10 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	0	7	31	95 %
Negative	10	14	16	2*	0	100 %

The following table summarizes the result for the two discordant samples:

10 ng/mL Cutoff	Assay Result:		6AM Sample Testing Method	
Concentration	LC/MS	LZI EIA	LC/MS (ng/mL)	LZI EIA (mA/min)
Sample #41*	+	-	10	437.1
Sample #43*	+	-	11	440.3

Discrepant samples are based on a 10 ng/mL cutoff concentration with a 444.9 mA/min absorbance value.

Semi-Quantitative Accuracy Study:

10 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	0	6	31	93 %
Negative	10	14	16	3*	0	100 %

The following table summarizes the result for the three discordant samples:

10 ng/mL Cutoff	Assay Result:		6AM Sample Testing Method	
Concentration	LC/MS	LZI EIA	LC/MS (ng/mL)	LZI EIA (mA/min)
Sample #41*	+	-	10	7.5
Sample #43*	+	-	11	7.9
Sample #48*	+	-	13	9.4

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free urine pool spiked with pure 6-acetylmorphine was serially diluted. Each sample was run in 10 replicates and the average was used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follows:

$y = 0.9632x + 0.2491$, $r^2 = 0.9961$

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
0 ng/mL	0.4	N/A
2 ng/mL	2.5	123.5 %
5 ng/mL	5.5	109.6 %
10 ng/mL	9.8	97.7 %
15 ng/mL	14.2	94.8 %
20 ng/mL	19.2	95.9 %
30 ng/mL	29.1	97.1 %
35 ng/mL	32.3	92.4 %
40 ng/mL	40.5	101.3 %

Specificity: Cross-reactivity of various potential interfering drugs were tested by spiking various concentrations of each substance into drug-free urine, and then evaluated with the assay's calibrated dose-response curve. The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 10 ng/mL cutoff or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator.

Structurally Related Opiate Compounds*:

Compound	Equivalent [ ] to 10 ng/mL (ng/mL)	Dose [ ] (ng/mL)	% Cross-Reactivity
Codeine	500,000	1.20	0.00 %
Dextromethorphan	100,000	0.35	0.00 %
Dihydrocodeine	500,000	7.30	0.00 %
Heroin	200	7.7	3.83 %
Hydrocodone	300,000	1.00	0.00 %
Hydromorphone	100,000	2.50	0.00 %
Imipramine	200,000	1.10	0.00 %
Levorphanol	100,000	1.40	0.00 %
Meperidine	800,000	0.95	0.00 %
Morphine	100,000	3.45	0.00 %
Morphine-3-Glucuronide	600,000	1.30	0.00 %
Morphine-6-Glucuronide	600,000	1.65	0.00 %
Nalorphine	100,000	3.45	0.00 %
Naloxone	500,000	2.25	0.00 %
Naltrexone	300,000	1.55	0.00 %
Norcodeine	600,000	1.30	0.00 %
Normorphine	100,000	2.75	0.00 %
Oxycodone	500,000	1.30	0.00 %
Oxymorphone	100,000	1.60	0.00 %

***There is a possibility that metabolites of the compounds listed above may interfere with 6-acetylmorphine immunoassays and cause false results.*

Structurally Unrelated Pharmacological Compounds:

Compound	Equivalent [ ] to 10 ng/mL (ng/mL)	Dose [ ] (ng/mL)	% Cross-Reactivity
11-Nor-Delta-9-THC-COOH	100,000	0.3	0.000 %
Acetaminophen	500,000	0.3	0.000 %
Acetylsalicylic Acid	500,000	0.3	0.000 %
Amitriptyline	500,000	0.4	0.000 %
Benzoylecgonine	500,000	0.5	0.000 %
Brompheniramine	100,000	0.2	0.000 %
Caffeine	500,000	0.9	0.000 %
Chlorpromazine	250,000	0.9	0.000 %
Desipramine	500,000	1.0	0.000 %
Diazepam	100,000	0.4	0.000 %
Digoxin	100,000	0.5	0.000 %
Diphenhydramine	100,000	0.1	0.000 %
Doxepin	100,000	0.1	0.000 %
Fluoxetine	500,000	0.1	0.000 %
Hydroxyzine	500,000	0.4	0.000 %
Ibuprofen	500,000	0.5	0.000 %
Methadone	500,000	0.8	0.000 %
Methamphetamine	500,000	0.5	0.000 %
Oxazepam	500,000	0.7	0.000 %
Phencyclidine	100,000	0.4	0.000 %
Phenobarbital	500,000	0.9	0.000 %
Propoxyphene	100,000	0.3	0.000 %
Ranitidine	500,000	1.4	0.000 %
Secobarbital	500,000	0.7	0.000 %
Triprolidine	100,000	1.0	0.001 %

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

Interference: Endogenous Substances

The following endogenous compounds were spiked into negative urine and the two levels of controls (7.5 ng/mL and 12.5 ng/mL) for the assay. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 10 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	0 ng/mL (ng/mL)	7.5 ng/mL Control (ng/mL)	12.5 ng/mL Control (ng/mL)
Acetone	1000	1.9	8.6	15.2
Ascorbic Acid	400	0.0	7.0	12.7
Creatinine	500	0.6	8.5	14.4
Ethanol	1000	0.8	8.3	13.0
Galactose	10	0.2	8.3	14.3
γ-Globulin	500	0.2	8.0	13.2
Glucose	1500	0.3	8.6	13.7
Hemoglobin	300	1.2	8.2	14.3
Human Serum Albumin	500	0.6	8.0	13.9
Oxalic Acid	100	0.4	7.2	11.8
Sodium Chloride	3000	0.0	5.5	11.8
Urea	2000	0.4	8.1	10.9

Specific Gravity: Samples ranging in specific gravity from 1.0025 to 1.0300 were tested with the assay in the presence of 0 ng/mL, 7.5 ng/mL, and 12.5 ng/mL of 6-acetylmorphine, and no interference was observed.

Note: All endogenous substances listed above, including specific gravity, were also tested in qualitative mode. No interference is observed. The results are identical to the semi-quantitative mode as all samples gave correct positive/negative results corresponding to the cutoff value of 10 ng/mL.

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Additions, deletions, or changes are indicated by a change bar in the margin.

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