CEDIA® Buprenorphine Assay

INTENDED USE
The CEDIA® Buprenorphine Assay is a homogenous enzyme immunoassay for qualitative or semi-quantitative determination of the presence of buprenorphine in human urine at cutoff concentration of 5 ng/mL. The assay provides a simple and rapid analytical screening procedure to detect buprenorphine in human urine.

THE ASSAY PROVIDES ONLY A PRELIMINARY ANALYTICAL TEST RESULT. A MORE SPECIFIC ALTERNATIVE CHEMICAL METHOD MUST BE USED TO OBTAIN A CONFIRMED ANALYTICAL RESULT. GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) IS THE PREFERRED CONFIRMATORY METHOD. CLINICAL AND PROFESSIONAL JUDGMENT SHOULD BE APPLIED TO ANY DRUG OF ABUSE TEST RESULT, PARTICULARLY WHEN PRELIMINARY RESULTS ARE USED.

SUMMARY AND EXPLANATION OF THE TEST
Buprenorphine is a semi-synthetic opioid analgesic derived from thebaine, a component of opium. Buprenorphine resembles morphine structurally but has both antagonist and agonist properties. Buprenorphine has a longer duration of action than morphine and can be administered sublingually as an analgesic. Subutex®, a higher dose buprenorphine formulation, is widely used in Europe and elsewhere as a substitution treatment for opiate addiction. Recently, the FDA has approved the use of Subutex and Suboxone® containing buprenorphine as active drug, for the treatment of opiate dependence in the US. The antagonist potency was reported as equivalent to naltrexone. Subutex and Suboxone are the first narcotic drugs shown that buprenorphine has abuse potential and may itself cause dependency. In addition, a number of deaths have been recorded as a result of overdose with intravenously injected buprenorphine in conjunction with other psychotropic drugs such as benzodiazepines. Buprenorphine is metabolized primarily by N-dealkylation to form norbuprenorphine and by conjugation to form glucurono-buprenorphine and glucurono-norbuprenorphine.

The CEDIA® Buprenorphine Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system. The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. The assay is performed by allowing the analyte in the sample in the presence of enzyme donor and enzyme acceptor to form fully active enzymes that, in the assay format, cleave a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment (enzyme donor) of β-galactosidase for a limited number of antibody binding sites. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragment free to form active enzyme. If the analyte is not present in the sample, antibody binds to analyte conjugated on the inactive fragment, inhibiting the re-association of inactive β-galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.

REAGENTS
1  EA Reconstitution Buffer: Buffer salts, 0.35 mg/L mouse monoclonal anti-buprenorphine antibody, stabilizer, and preservative.
1a ED Reagent: 0.171 g/L Enzyme Acceptor (micr opioidal), buffer salts, and preservative.
2  ED Reconstitution Buffer: Buffer salts, stabilizers, and preservative.
2a EA Reagent: 25 mg/L Enzyme Donor (micr opioidal) conjugated to buprenorphine, 1.67 g/L chlorophenol red-g-D-galactopyranoside, stabilizers, and preservative.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED:

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit Description</th>
</tr>
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<tbody>
<tr>
<td>100241</td>
<td>CEDIA Buprenorphine S1 Calibrator (0 ng/mL)</td>
</tr>
<tr>
<td>100242</td>
<td>CEDIA Buprenorphine S2 Calibrator (5 ng/mL)</td>
</tr>
<tr>
<td>100243</td>
<td>CEDIA Buprenorphine S3 Calibrator (20 ng/mL)</td>
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<tr>
<td>100244</td>
<td>CEDIA Buprenorphine S4 Calibrator (50 ng/mL)</td>
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<tr>
<td>100245</td>
<td>CEDIA Buprenorphine S5 Calibrator (75 ng/mL)</td>
</tr>
<tr>
<td>100246</td>
<td>CEDIA Buprenorphine Low and High Controls</td>
</tr>
</tbody>
</table>

PRECAUTIONS AND WARNINGS
The reagents contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Seek immediate medical attention if reagents are splashed in eye or ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

PROTECTIVE CONSIDERATIONS
Store CEDIA Buprenorphine reagents at 2-8°C. DO NOT FREEZE.

STORAGE CONDITIONS
For stability of the unopened components, refer to the box or bottle labels for the expiration date.

To ensure reconstituted EA reagent stability, protect it from prolonged continuous exposure to bright light.

R1 SOLUTION: 60 days refrigerated on analyzer or at 2-8°C.
R2 SOLUTION: 60 days refrigerated on analyzer or at 2-8°C.

SPECIMEN COLLECTION AND PREPARATION
Urine samples are suitable for use in the CEDIA Buprenorphine Assay. Treat human urine as potentially infectious material. Collect urine using standard sampling cups and procedures. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed.

Cap samples immediately after collection, store at 2-8°C and assay within 7 days after collection. If the assay can’t be performed within 7 days, or if the sample is to be shipped, cap the sample, and keep it frozen. Store sample at -20°C and assay within 2 weeks.

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. Centrifuge specimen with high turbidity or visible particulate matter before testing.

OBTAIN ANOTHER SAMPLE FOR TESTING IF ADULTERATION OF THE SAMPLE IS SUSPECTED. ADULTERATION OF URINE SPECIMENS CAN AFFECT THE TEST RESULT.

ASSAY PROCEDURE
The CEDIA Buprenorphine Assay is intended for use on automated clinical analyzers capable of maintaining a constant temperature, pipetting, mixing reagents, measuring enzymatic rates at an absorbance of 680 nm, and timing the reaction can be used to perform this assay. Specific application performance data are on file at Microgenics Corporation, a part of Thermo Fisher Scientific. For application parameter settings on your analyzer, refer to the applicable application diskette, barcode transfer sheet or instrument specific application sheet available at Microgenics Corporation. The performance of applications not obtained from Microgenics Corporation is not warranted and must be defined by the user.
Calibrators and Controls
The approximate concentration of buprenorphine for each of the five calibrators and two controls used in the CEDIA Buprenorphine Assay are as follows:

- S1: CEDIA Buprenorphine Calibrator (0 ng/mL)
- S2: CEDIA Buprenorphine Calibrator (5 ng/mL)
- S3: CEDIA Buprenorphine Calibrator (20 ng/mL)
- S4: CEDIA Buprenorphine Calibrator (50 ng/mL)
- S5: CEDIA Buprenorphine Calibrator (125 ng/mL)
- C1: CEDIA Buprenorphine Low Control (1 ng/mL)
- C2: CEDIA Buprenorphine High Control (2 ng/mL)

Calibration Frequency
Recalibration is recommended
- After reagent bottle change
- After calibrator or reagent lot change
- After instrument maintenance is performed
- As required following quality control procedures

See below for calibration frequency recommendations for Hitachi analyzers. For other analyzers, refer to the instrument-specific application sheet.

Reportable Range
The CEDIA Buprenorphine Assay is designed for semi-quantitative use in the range between 5 ng/mL, the lowest calibrator of the assay containing buprenorphine, and the value of the S5 calibrator.

The minimum detectable concentration on the Buprenorphine Assay is 1.25 ng/mL.

Out of Range Samples
Specimens giving concentration greater than the S5 calibrator can be reported as greater than the value of the high calibrator or diluted one part sample with one part of negative calibrator and re-assayed for dilutions up to 1:100.

The value obtained on re-assay should be derived as follows:
Actual Value = (dilution factors x diluted value) - concentration of negative calibrator

Specimens giving values below the cutoff concentration should be reported as negative.

Quality Control
Each laboratory should establish its own control frequency.

Good laboratory practice suggests that at least two levels of quality controls (one below and one above the cutoff of the assay) be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters. Contact Customer Technical Support for further assistance and recommendations on suitable control material. All QC requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements.

NOTE: Reassess control targets and ranges following a change of reagent lot.

Calculation
Refer to the appropriate operator’s manual or analyzer-specific application protocol for detailed calculation information.

Limitations
1. It is possible that other substances or factors other than those investigated in the specificity study may interfere with the test and cause false results.
2. A positive result using the CEDIA Buprenorphine Assay indicates only the presence of buprenorphine or cross-reactant and does not necessarily correlate with the extent of physiological and psychological effects. An assay result may not be able to distinguish between therapeutic use and abuse of buprenorphine.
3. Performance characteristics for the CEDIA Buprenorphine Assay performance have not been established with body fluids other than human urine.
4. Care should be taken when reporting results since there are many factors, e.g., fluid intake and other biologic factors, that may influence a urine test result. The assay may be run in semi-quantitative mode for estimating dilutions for GC/MS confirmation or for quality control purposes.
5. This CEDIA Buprenorphine Assay was validated on analyzers utilizing an integral cell wash. If your analyzer does not have an integral cell wash, contact your local Thermo Fisher Scientific representative.

Results and Expected Values
The published data may be used as a reference for therapeutic and toxic values until a laboratory has established its own ranges. The results obtained in your laboratory may differ from these data.

Qualitative results
The CEDIA Buprenorphine Assay cutoff calibrator, containing 5 ng buprenorphine/mL, is used as a reference for distinguishing between positive and negative samples. A sample having an observed absorbance value (A) equal to or greater than that obtained with cutoff calibrator is considered positive. Conversely, a sample having an observed absorbance less than the cutoff calibrator is considered negative. Refer to the analyzer specific application sheet for additional information.

Semi-quantitative results
Use of all the CEDIA Buprenorphine Assay Calibrators enables estimation of a relative concentration of buprenorphine in urine. The approximate concentration of buprenorphine in a specimen can be obtained by comparing the absorbance observed for the specimen, comparing it to the standard calibration curve, and interpolating its estimated concentration.

When the estimated sample concentration is greater than the highest calibrator, the sample can be diluted with negative calibrator and retested as previously described. Care should be taken when reporting results since there are many factors, e.g., fluid intake and other biologic factors, that may influence a urine test result. The assay may be run in semi-quantitative mode for estimating dilutions for GC/MS confirmation or for quality control purposes.

Specific Performance Characteristics
Typical performance data obtained on the Hitachi 717 analyzer are shown below:

<table>
<thead>
<tr>
<th>N</th>
<th>Low</th>
<th>Med</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

| X (ng/mL) | 4.4 | 6.8 | 36.5 |
| SD (ng/mL) | 0.3 | 0.3 | 1.0 |
| % CV | 5.7 | 3.9 | 2.6 |

Linearity
A urine pool containing a known high concentration of buprenorphine was serially diluted in 10% increments (successive 1:10 dilutions) with a human urine pool free of buprenorphine. Buprenorphine concentration for each of the resulting 10 dilutions was determined and the percent recovery was calculated as the quotient of the observed to expected value. Results shown below demonstrate that the observed buprenorphine concentrations for serially diluted specimens are within ±10% of expected values. When comparing observed (y) and expected (x) values using least squares fitting techniques, the observed regression equation (y = 1.025x - 0.021) and correlation (r = 0.9986) support the linearity of the assay with successively diluted specimens originating from a single high pool.

Results
The CEDIA Buprenorphine Assay cutoff calibrator, containing 5 ng buprenorphine/mL, is used as a reference for distinguishing between positive and negative samples. A sample having an observed absorbance value (A) equal to or greater than that obtained with cutoff calibrator is considered positive. Conversely, a sample having an observed absorbance less than the cutoff calibrator is considered negative. Refer to the analyzer specific application sheet for additional information.
Cutoff Characterization
A human urine pool free of buprenorphine was spiked with a stock solution having a high buprenorphine concentration to produce two sets of 21 specimens each, one set with a buprenorphine concentration 25% greater (6.25 ng/mL) and the other 25% less (3.75 ng/mL) than the assay cutoff of 5 ng buprenorphine/mL. Each set of 21 aliquots was assayed using the CEDIA Buprenorphine Assay. Cutoff characterization was deemed acceptable if the observed buprenorphine concentration for 95% of the 21 specimens from each of the two sets assayed was greater or less than the concentration observed for the 5 ng/mL cutoff calibrator. As is shown in the table below, the buprenorphine concentration observed for all specimens were appropriately greater or less than the concentration of 5.4 ng buprenorphine/mL observed for the 5 ng/mL cutoff calibrator.

### Specificity

#### Interference with Endogenous Substances
The potential interference of endogenous physiologic substances on recovery of buprenorphine using the CEDIA Buprenorphine Assay was assessed by spiking known amounts of potentially interfering substances into urine specimens having a known buprenorphine concentration. Buprenorphine concentration for each specimen (substance and final concentration noted in the table below) was determined and the percent recovery calculated as the quotient of the observed buprenorphine concentration to into urine specimens having a known buprenorphine concentration.

### Buprenorphine Degradation Products
Potential cross-reactants evaluated included buprenorphine-3-β-D glucuronide, norbuprenorphine, and norbuprenorphine-3-β-D glucuronide. Potential cross-reactivity was determined by adding known amounts of each cross-reactant to buprenorphine-free urine specimens. A metabolite was determined to cross react with native buprenorphine if recovery observed for the metabolite spiked specimen was greater than 1% of the estimated target concentration. As indicated by the results provided, when prepared specimens are assayed using the CEDIA Buprenorphine Assay, buprenorphine 3-β-D glucuronide exhibits nearly 100% cross-reactivity with buprenorphine while norbuprenorphine and its conjugated glucuronide show no evidence of significant cross-reactivity.

### Cross Reactivity with Pharmacologic Substances
The potential cross-reactivity posed by drugs commonly co-administered with buprenorphine was evaluated by adding a final concentration of 100000 ng/mL of each substance to buprenorphine-free urine. The observed difference in quantitation between a control and the sample with the added drug was then used to calculate cross-reactivity. All of the pharmacologic compounds evaluated are included in the following table and were < 0.015% cross-reactive in the CEDIA Buprenorphine Assay.

### Accuracy

#### Method Comparison - Semi-quantitative
The relationship between buprenorphine concentrations assayed using both the CEDIA Buprenorphine and gas chromatography/mass spectrometry methods was evaluated using linear regression techniques for 96 urine specimens representing the dynamic range of the assay (from 1.25 to 75.0 ng buprenorphine/mL). The correlation coefficient (r) of 0.988 as well as Deming and least squares regression parameters, shown in the following table and associated figure demonstrate overall excellent, unbiased agreement between the CEDIA Buprenorphine (y) and GC/MS (x) assay results.

### Table

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Aliquot</th>
<th>High-Aliquot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Dose</td>
<td>2.7</td>
<td>6.6</td>
</tr>
<tr>
<td>SD</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>% C.V.</td>
<td>6.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Cut off dose</td>
<td>5.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

### Equations

**1. Cross-reactivity (%)**

\[
\text{Cross-reactivity} = \left( \frac{\text{Observed concentration of cross-reactant}}{\text{Estimated target concentration of cross-reactant}} \right) \times 100
\]

**2. Equations for Deming’s and Least Squares Regression**

Deming’s

\[
y = \frac{y_1 + y_2}{2} = \frac{9933x + 0.10}{2}
\]

Least Squares

\[
y = 0.981x + 0.27
\]

**S.E.E.**

3.08

**r**

0.988

BG = Buprenorphine-3-β-D glucuronide; NG = Norbuprenorphine-3-β-D Glucuronide
Method Comparison - Qualitative

The same 96 urine specimens described in the previous section were also qualitatively evaluated using a threshold of 5 ng buprenorphine/mL as the cutoff discriminating a negative or positive test result. In this analysis all specimens having a buprenorphine concentration greater than or equal to 5 ng/mL (≥5 ng/mL) were defined as positive for both methods while samples with concentrations of 4.99 ng/mL or lower (<5 ng/mL) were defined as negative. The results shown in Table VII-9 demonstrate excellent overall concordance of 99.0% (95/96 = 98.95%, Yates-corrected χ²=89.17, p < 0.0001) between GC/MS and the CEDIA Buprenorphine Assay.

<table>
<thead>
<tr>
<th>CEDIA Positive</th>
<th>GC/MS Positive</th>
<th>GC/MS Negative</th>
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<tbody>
<tr>
<td></td>
<td>45</td>
<td>1</td>
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<tr>
<td>46</td>
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<td>CEDIA Negative</td>
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<td>45</td>
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References