The Emit® II Plus Opiate Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine. The assay is based on competition between drug in the specimen and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

### 4 REAGENTS

Reagents contain the following substances:

- Sheep polyclonal antibodies to morphine (4.2 µg/mL), glucose-6-phosphate (10 mM), nicotinamide adenine dinucleotide (6 mM), bovine serum albumin, morphine labeled with G6PDH (0.47 U/mL), Tris buffer, preservatives, and stabilizers.

#### Precautions

- **For in vitro diagnostic use.**
- **Reagent 1 contains nonsterile sheep antibodies.**
- **Reagent 2 contains nonsterile mouse antibodies.**
- **Reagents 1 and 2 contain nonsterile bovine serum albumin.**
- **Do not use after expiration date.**
- **Turbid or yellow reagents may indicate contamination or degradation and must be discarded.**

#### Preparation of Reagents

<table>
<thead>
<tr>
<th>Desired Cutoff Level (ng/mL)</th>
<th>Qualitative Analysis</th>
<th>Concentration of Morphine (ng/mL)</th>
<th>Semiquantitative Analysis</th>
<th>Concentration of Morphine (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>Level 0</td>
<td>0</td>
<td>Level 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Level 1</td>
<td>300</td>
<td>Level 1</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Level 5</td>
<td>4000</td>
<td>Level 2</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>Level 5</td>
<td>4000</td>
<td>Level 3</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>Level 3</td>
<td>2000</td>
<td>Level 3</td>
<td>2000</td>
</tr>
</tbody>
</table>

**Note:** The Emit® Calibrators/Controls contain the stated concentrations of morphine listed in Table 1. Emit® Calibrator/Control Levels 1, 2, 3, and 5 contain additional drugs of abuse that do not affect the assay. See the Emit® Calibrators/Controls instructions for use. For any individual cutoff level, a calibrator/control is used either as a calibrator or as a control when the assay is used for qualitative analysis. When a calibrator/control is used as a calibrator for an individual cutoff level, the other level calibrators/controls (above or below it, as listed above) are used as controls.

### 1 INTENDED USE

The Emit® II Plus Opiate Assay is a homogenous enzyme immunoassay with a 300 ng/mL cutoff. The assay is intended for use in the qualitative and semiquantitative analyses of opiates in human urine. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

The Emit® II Plus Opiate 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. Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

### 2 SUMMARY

Opiates are a class of compounds that includes morphine, codeine, and heroin. Morphine and codeine are naturally occurring alkaloids that are found in opium, a substance exuded from the unripe seed pod of the opium poppy Papaver somniferum. Heroin is a semisynthetic derivative of morphine.

Morphine is a potent analgesic. Codeine is used in analgesic preparations and as a cough suppressant. Heroin is an even more potent analgesic than morphine. Both morphine and codeine are legitimate drugs. Heroin is a drug of abuse that may be snorted, smoked, or dissolved and injected subcutaneously or intravenously.

Opiates are absorbed rapidly. Heroin is converted almost immediately to morphine, which is excreted in urine both unchanged and as a glucuronidated metabolite. Excretion takes place over a period of a couple of days. Codeine is excreted in urine as a glucuronidated conjugate, as free and conjugated norcodeine, and as morphine. The presence of opiates in urine indicates the use of heroin, morphine, and/or codeine.

The Emit® II Plus Opiate Assay tests for morphine, morphine-3-glucuronide, and codeine in human urine and gives a positive result if any of these opiates are present. It also detects synthetic opiates related to morphine, such as hydromorphone, and high concentrations of the analgesic meperidine and the narcotic antagonist nalorphine. Positive results for specimens containing other compounds structurally unrelated to opiates have not been observed.

Methods historically used for detecting opiates in biological fluids include thin-layer chromatography, gas chromatography, high-performance liquid chromatography, fluorometry, microcrystallography, enzyme immunoassay, and radioimmunoassay. While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result.

### 3 METHODOLOGY

The Emit® II Plus Opiate Assay is a homogenous enzyme immunoassay technique used for the analysis of specific compounds in human urine. The assay is based on competition between drug in the specimen and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

### 5 SPECIMEN COLLECTION AND PREPARATION

- **Urine specimens may be collected in plastic (ie, polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics, other than those listed, can adsorb certain drugs.**
- **If not analyzed immediately, specimens may be stored unrefrigerated for up to 7 days following collection. After 7 days, specimens should be stored frozen at -20°C.**
- **Frozen specimens must be thawed and mixed thoroughly prior to analysis.**
- **Specimens with high turbidity should be centrifuged before analysis.**
- **The recommended pH range for urine specimens is 3.0–11.0.**
Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain another specimen.

Human urine specimens should be handled and treated as if they were potentially infectious.

6 Procedure

Materials Provided

Emit® II Plus Opiate Assay
Reagent 1
Reagent 2

Materials Required But Not Provided

Emit® Calibrators/Controls
Commercially available controls (see Quality Control, Semiquantitative Analysis)

Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.

Calibration

Qualitative Analysis

Run the Emit® Calibrator/Control—Level 1 (300 ng/mL) or Level 3 (2000 ng/mL) in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the analyzer User’s Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis: 300 ng/mL Cutoff

Prepare a calibration curve by running Emit® Calibrators/Controls Level 0 (0 ng/mL), Level 1 (300 ng/mL), Level 2 (1000 ng/mL), and Level 3 (2000 ng/mL). Validate the calibration by running controls (see Quality Control). Refer to the analyzer User’s Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis: 2000 ng/mL Cutoff

Prepare a calibration curve by running Emit® Calibrators/Controls Level 0 (0 ng/mL), Level 2 (1000 ng/mL), Level 3 (2000 ng/mL), and Level 5 (4000 ng/mL). Validate the calibration by running controls (see Quality Control). Refer to the analyzer User’s Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Quality Control

Qualitative Analysis

Validate the calibration by assaying controls. Ensure that the result from Emit® Calibrator/Control Level 0 (0 ng/mL) or Level 5 (4000 ng/mL) relates appropriately to the cutoff calibrator result from the selected cutoff calibrator (Level 1 [300 ng/mL] or Level 3 [2000 ng/mL]). Once calibration is verified, run urine specimens.

Semiquantitative Analysis

For a selected cutoff level (300 ng/mL or 2000 ng/mL), validate the calibration curve by assaying commercial controls. Ensure that the control results fall within acceptable limits as defined by your laboratory. Once the calibration curve is validated, run urine specimens.

Evaluation and Interpretation of Results

When the Emit® II Plus Opiate Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given specimen cannot be estimated. The assay results distinguish between positive and negative specimens—positive indicating specimens contain opiates; negative indicating specimens do not contain opiates, or opiates are present in concentrations below the cutoff level for this assay.

• A specimen that gives a change in rate value equal to or higher than the rate of the selected cutoff calibrator level is interpreted as positive.

• A specimen that gives a change in rate value lower than the rate of the selected cutoff calibrator level is interpreted as negative.

When used semiquantitatively, the Emit® II Plus Opiate Assay yields the approximate concentration of the drug detected by the assay (See Section 8, Specific Performance Characteristics, Analytical Recovery or Specificity). The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for confirmation by GC/MS. Semiquantitation also permits the laboratory to establish quality control procedures and assess control performance.

7 Limitations of the Procedure

• The assay is designed for use only with human urine.

• A positive result from the assay indicates the presence of opiates but does not indicate or measure intoxication.

• Poppy seeds can contain opiates, and ingestion of products containing poppy seeds can cause a positive test result at the 300 ng/mL cutoff.6

• Boric acid is not recommended as a preservative for urine.

• Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

• Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.

• Immunoassays that produce a single result in the presence of a drug and its metabolites cannot fully quantitate the concentration of individual components.

8 Specific Performance Characteristics

The data appearing in this section were collected on the AU4000/AU6000® Clinical Chemistry System using the Emit® II Plus Opiate assay. Positive specimens were confirmed by GC/MS. Refer to the Application Sheets for other AU Clinical Chemistry Systems and for additional information. Results may vary due to analyzer-to-analyzer differences. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

Precision

Within-run precision was determined by assaying two replicates of controls (at concentrations +/-25% of either cutoff [300 ng/mL or 2000 ng/mL]) twice a day for 20 testing days. Table 2 summarizes the findings at the 300 ng/mL cutoff and Table 3 summarizes the findings at the 2000 ng/mL cutoff.

Table 2 — Within-Run Precision at 300 ng/mL

<table>
<thead>
<tr>
<th>Opiate</th>
<th>300 ng/mL Cutoff</th>
<th>Control 75%</th>
<th>Control 125%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mAU/min)</td>
<td>741</td>
<td>781</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1.7</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 — Within-Run Precision at 2000 ng/mL

<table>
<thead>
<tr>
<th>Opiate</th>
<th>2000 ng/mL Cutoff</th>
<th>Control 75%</th>
<th>Control 125%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mAU/min)</td>
<td>815</td>
<td>864</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>2.4</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

Total precision was determined by assaying two replicates of controls (at concentrations +/-25% of either cutoff [300 ng/mL or 2000 ng/mL]) twice a day for 20 testing days, which spanned 30 calendar days. Table 4 summarizes the findings at the 300 ng/mL cutoff, and Table 5 summarizes the findings at the 2000 ng/mL cutoff.

Table 4 — Total Precision at 300 ng/mL

<table>
<thead>
<tr>
<th>Opiate</th>
<th>300 ng/mL Cutoff</th>
<th>Control 75%</th>
<th>Control 125%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mAU/min)</td>
<td>741</td>
<td>781</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>4.6</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 — Total Precision at 2000 ng/mL

<table>
<thead>
<tr>
<th>Opiate</th>
<th>2000 ng/mL Cutoff</th>
<th>Control 75%</th>
<th>Control 125%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mAU/min)</td>
<td>815</td>
<td>864</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>6.6</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td>0.8</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Comparative Analysis

Clinical urine specimens were analyzed on the AU4000/AU6000 Clinical Chemistry System and on the SYVA®-30R Biochemical System. Table 6 summarizes the number of positive/negative results identified and the percent agreement between both analyzers.

Table 6 — Summary of Comparative Analysis

<table>
<thead>
<tr>
<th>Assay</th>
<th>Positive</th>
<th>Negative</th>
<th>% Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opiate 300 ng/mL</td>
<td>69</td>
<td>51</td>
<td>99</td>
</tr>
<tr>
<td>Opiate 2000 ng/mL</td>
<td>21</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Analytical Recovery

Negative human urine specimens were spiked with known concentrations of morphine and tested in duplicate in four runs over one or two days. Specimens that were analyzed qualitatively were correctly identified as positive or negative 100% of the time. Table 7 summarizes the results obtained using a semiquantitative analysis at the 300 ng/mL cutoff; Table 8 summarizes the results obtained at the 2000 ng/mL cutoff.
Concentration Target (ng/mL) | Mean (ng/mL)
--- | ---
0 | 0
75 | 100
125 | 73
200 | 153
270 | 225
330 | 408
500 | 668
600 | 701
700 | 821
900 | 988
1400 | 1608
1800 | 1608

**Specificty**
The Emit® II Plus Opiate Assay detects morphine and morphine-3-glucuronide (the major metabolites of heroin) and codeine in human urine.

Table 9 gives the compounds this assay detects and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator/Control Level 1 [300 ng/mL] or Level 3 [2000 ng/mL]). Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 9 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Therapeutic doses of ofloxacin (Floxin) or levofloxacin (Levaquin), non-opiates, may produce positive results with this assay. A positive result from an individual taking ofloxacin or levofloxacin should be interpreted with caution and confirmed by another method.

**Table 7 — Semiquantitative Analysis of Morphine-Spiked Samples at the 300 ng/mL Cutoff**

**Table 8 — Semiquantitative Analysis of Morphine-Spiked Samples at the 2000 ng/mL Cutoff**

**Table 10 lists the concentrations of compounds that show a negative response to the Emit® II Plus Opiate Assay at both cutoff levels.**

**Table 10 — Concentrations of Compounds Showing a Negative Response to the Cutoff**

**Sensitivity**
The sensitivity level of the Emit® II Plus Opiate Assay using the 300 ng/mL cutoff is 16 ng/mL, and the sensitivity using the 2000 ng/mL is 140 ng/mL. These levels represent the lowest concentration of opiate that can be distinguished from 0 ng/mL with a confidence level of 95%.

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* Therapeutic or toxic urinary levels of levallorphan and naltorphine are not reported in the literature.

† Meperidine urinary concentrations of 150000 ng/mL have been measured in cases of fatal meperidine overdosage.
9 REFERENCES


8. SAMHSA Guidelines for Federal Workplace Testing - Section 2.4 Laboratory Analysis p. 73. Effective Date October 1, 2004.