



# BUP

## Buprenorphine Enzyme Immunoassay

### Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
09067752190	Buprenorphine Enzyme Immunoassay (240 tests)	System-ID 2067 001 <b>cobas c 503</b>
Materials required (but not provided):		
08239886190	Buprenorphine Semi-Quantitative Calibrators Set BUP Negative Calibrator 0 ng/mL (1 x 5 mL) Code 20641 BUP Low/Cutoff Calibrator #1 5 ng/mL (1 x 5 mL) Code 20642 BUP Cutoff Calibrator #2 10 ng/mL (1 x 5 mL) Code 20643 BUP Intermediate Calibrator #1 20 ng/mL (1 x 5 mL) Code 20644 BUP Intermediate Calibrator #2 40 ng/mL (1 x 5 mL) Code 20645 BUP High Calibrator 75 ng/mL (1 x 5 mL) Code 20646	
08356661190	Buprenorphine 5 Qualitative Calibrator Set BUP Cutoff Calibrator 5 ng/mL (1 x 5 mL) Code 20636	
08239894190	Buprenorphine 10 Qualitative Calibrator Set BUP Cutoff Calibrator 10 ng/mL (1 x 5 mL) Code 20637	
08356670190	Buprenorphine 5 Control Set Negative Control 3 ng/mL (2 x 15 mL) Code 20110 Positive Control 7 ng/mL (2 x 15 mL) Code 20239	
08239908190	Buprenorphine 10 Control Set Negative Control 7 ng/mL (2 x 15 mL) Code 20244 Positive Control 13 ng/mL (2 x 15 mL) Code 20245	
09330119190	DAT Opiates Multi Control I Set (for 5 ng/mL assay) Negative Control 3 ng/mL (2 x 15 mL) Code 20159 Positive Control 7 ng/mL (2 x 15 mL) Code 20160	
04908856160 <sup>a</sup>	Open/Close tool (5 pieces)	

a) Catalog number is for USA only. Open/Close tool is available upon request in other countries.

### English

**Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.**

### System information

**BUP5Q:** ACN 20671: for qualitative assay, 5 ng/mL

**BUP1Q:** ACN 20670: for qualitative assay, 10 ng/mL

**BUPS:** ACN 20672: for semi-quantitative assay, 5/10 ng/mL

### Intended use

The Buprenorphine Enzyme Immunoassay is an in vitro diagnostic test intended for the qualitative and semi-quantitative determination of norbuprenorphine (a buprenorphine metabolite) in human urine. The cutoff for both the qualitative and semi-quantitative modes of the assay is 5 ng/mL or 10 ng/mL of norbuprenorphine. The assay is designed for prescription use with the Roche **cobas c** automated clinical chemistry analyzers.

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 gas or liquid chromatography/mass spectrometry (GC-MS or LC-MS) or (2) permitting laboratories to establish quality control procedures.

**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.<sup>1,2</sup> Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.**

### Summary

Buprenorphine is a semi-synthetic opioid derived from thebaine, an alkaloid of the poppy plant, *Papaver somniferum*. It is an analgesic often used as a substitution treatment for heroin addiction or opiate dependence. Buprenorphine structurally resembles morphine but has both antagonist and agonist properties.<sup>3</sup> As an opioid partial agonist, buprenorphine can produce typical opioid effects and side effects such as euphoria and respiratory depression. However, its maximal effects are less than those of full agonists like heroin and methadone. At low doses, buprenorphine

produces sufficient agonist effects to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. The agonist effects of buprenorphine increase linearly with increasing doses of the drug until they reach a plateau and no longer continue to increase with further increases in dosage. Buprenorphine also acts as an antagonist, blocking other opioids, while allowing for some opioid effect of its own to suppress withdrawal symptoms and cravings.<sup>4</sup> Buprenorphine is metabolized in the human liver by N-dealkylation to the pharmacologically active norbuprenorphine, which, along with the parent compound, is conjugated with glucuronic acid<sup>5</sup>, and excreted in urine. Clearance rates are dependent on many factors, such as frequency of drug use, the amount of drug taken, metabolism rates, and even body fat content. For typical opioid-dependent patients who received a stable daily sublingual dose of 16 mg of buprenorphine and 4 mg of naloxone for at least 2 weeks, 24-hour urinary elimination is approximately 11 % of daily dose.<sup>6</sup> Therapeutically, buprenorphine is as effective as methadone but exhibits a much lower level of physical dependence. However, studies have shown that buprenorphine has abuse potential and may itself cause dependency.<sup>7</sup>

### Test principle

The Buprenorphine Enzyme Immunoassay assay is a homogeneous enzyme immunoassay ready-for-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.<sup>8</sup> 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, buprenorphine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when free drug is present in the sample, antibody binds to the free drug; the unbound buprenorphine-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.

**Buprenorphine Enzyme Immunoassay****Reagents - working solutions**

- R1** Contains a mouse monoclonal anti-buprenorphine antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.
- R2** Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with buprenorphine in buffer with sodium azide (0.09 %) as a preservative.

For timing of reagent pipetting refer to the test definition section.

**Precautions and warnings**

- This test is for *in vitro* diagnostic use only. Harmful if swallowed.
- Reagents used in the assay contain sodium azide as a preservative, which may react with lead or copper plumbing to form potentially explosive metal azide. When disposing of 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.<sup>9</sup>
- Do not use the reagents beyond their expiration dates.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

**Reagent handling**

Ready for use



Filling the **cobas c** pack:

- Turn the **cobas c** pack toward you.
- Position B of the **cobas c** pack is on the left side and position C on the right side.
- Unscrew the screw cap of the bottle in position B on the left side of the **cobas c** pack using the Open/Close tool.
- Use one of the enclosed funnels to pour the content of the R1 bottle (24.0 mL) into the opened bottle of the **cobas c** pack (position B). Discard the funnel.
- Close the bottle tightly using the Open/Close tool.
- Unscrew the screw cap of the bottle in position C on the right side of the **cobas c** pack using the Open/Close tool.
- Use one of the enclosed funnels to pour the content of the R2 bottle (10.1 mL) into the opened bottle of the **cobas c** pack (position C). Discard the funnel.
- Close the bottle tightly using the Open/Close tool.

The BUP **cobas c** pack is now ready for use.

**NOTE:** Solutions must be at the reagent compartment storage temperature of the analyzer before performing assays.

**Note**

Always use a new **cobas c** pack when preparing fresh reagents. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results. If the **cobas c** pack bottles are not filled correctly, this may result in faulty reagent pipetting and could cause erroneous results.

**Storage and stability**

- Shelf life at 2-8 °C: See expiration date on **cobas c** pack label
- On-board in use and refrigerated on the analyzer: 92 days

**Do not freeze.****Specimen collection and preparation**

Urine samples may be collected in plastic or glass containers. Some plastics may adsorb drugs. Use of plastics such as polyethylene is recommended.<sup>10</sup> Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8 °C for up to seven days.<sup>11,12</sup> For longer storage, keep sample frozen at -20 °C and then thaw before use. Studies have shown buprenorphine analytes in urine are stable at -20 °C up to 85 days.<sup>8</sup> Samples should be equilibrated to room temperature (18-25 °C) 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 both samples should be forwarded to a laboratory for testing.

See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

*Handle all urine specimens as if they were potentially infectious.*

**Materials provided**

See "Reagents – working solutions" section for reagents.

**cobas c** pack, funnels

**Materials required (but not provided)**

See "Order information" section

General laboratory equipment

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

**Applications for urine****Test definition - 5 ng/mL and 10 ng/mL cutoff assays**

	Semi-Quantitative	Qualitative
Reporting time	10 min	10 min
Wavelength (sub/main)	415/340 nm	415/340 nm

**Reagent pipetting**

R1	65 µL	65 µL
R3	25 µL	25 µL

**5 ng/mL cutoff assay**

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (H <sub>2</sub> O)
Normal	7.3 µL	–	–
Decreased	7.3 µL	–	–
Increased	7.3 µL	–	–

**10 ng/mL cutoff assay**

Sample volumes	Sample	Sample dilution
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**Buprenorphine Enzyme Immunoassay**

		Sample	Diluent (H <sub>2</sub> O)
Normal	7.3 µL	–	–
Decreased	7.3 µL	–	–
Increased	7.3 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

**Calibration**

Calibrators	<p><i>Semi-Quantitative applications</i></p> <p>5 ng/mL and 10 ng/mL cutoff assays</p> <p>S1: Buprenorphine Calibrator 0</p> <p>S2: Buprenorphine Calibrator 5</p> <p>S3: Buprenorphine Calibrator 10</p> <p>S4: Buprenorphine Calibrator 20</p> <p>S5: Buprenorphine Calibrator 40</p> <p>S6: Buprenorphine Calibrator 75</p> <p>0, 5, 10, 20, 40, 75 ng/mL</p> <p><i>Qualitative applications</i></p> <p>5 ng/mL cutoff assay</p> <p>S1: Buprenorphine Calibrator 5</p> <p>10 ng/mL cutoff assay</p> <p>S1: Buprenorphine Calibrator 10</p> <p>The drug concentrations of the calibrators have been verified by GC-MS or LC-MS.</p>
Calibration K Factor	For the qualitative application a K factor of 10000 is predefined in the application settings.
Calibration mode	<p><i>Semi-Quantitative applications</i></p> <p>Result Calculation Mode (RCM)</p> <p><i>Qualitative applications</i></p> <p>Linear</p>
Calibration frequency	<p>Full calibration</p> <ul style="list-style-type: none"> <li>every 14 days</li> <li>after reagent lot change</li> <li>as required following quality control procedures</li> </ul>

For the cutoff calibrator a value of "0" is encoded in the e-barcode in order to ensure flagging of positive samples with >Test and negative absorbance values for negative samples.

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against a primary reference method (GC-MS or LC-MS).

**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 Lin-Zhi International, Inc. (LZI) technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 14 days.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

**Results**

**NOTE:** A preliminary 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 5 ng/mL or 10 ng/mL of norbuprenorphine, is used as a reference for distinguishing preliminary positive from negative samples. Samples producing a positive or "0" absorbance value are considered preliminary positive. Preliminary positive samples are flagged with the >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign. Results of this assay distinguish preliminary positive ( $\geq 5$  ng/mL or  $\geq 10$  ng/mL depending on the cutoff) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

**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 6 calibrators. The concentration of norbuprenorphine in the sample may then be estimated from the calibration curve.

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

As with any sensitive test for drugs of abuse on automated clinical chemistry analyzers, the possibility exists for analyte carry-over from a sample with an extremely high concentration to a normal (negative) sample which immediately follows it.

For the semi-quantitative applications **cobas c** systems automatically calculate the drug or metabolite concentration of each sample in the unit ng/mL. Results equal to or greater than the respective cutoff value are considered preliminary positive. Concentration values below the respective cutoff indicate a negative result.

**Limitations**

1. A preliminary positive result from the assay indicates only the presence of norbuprenorphine.
2. The test is not intended for quantifying this single analyte in patient samples.
3. A preliminary positive result does not necessarily indicate drug abuse.
4. A negative result does not necessarily mean a person did not take illegal drugs.
5. 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).
6. Preliminary positive results should be confirmed by other affirmative, analytical chemical methods (e.g., chromatography), preferably GC-MS or LC-MS.
7. The test is designed for use with human urine only.
8. The test is not for therapeutic drug monitoring.
9. There is a possibility that metabolites of other opiate drugs may interfere with the test.

**ACTION REQUIRED**

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

**Specific performance data**

Representative performance data on a Roche/Hitachi 717 analyzer are given below. Data was validated on a Roche **cobas c** 501, **cobas c** 502, and **cobas c** 503 analyzer and did not show any significant differences

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between the analyzers. Results obtained in individual laboratories may differ.

### Precision on Roche/Hitachi 717

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 replicates of two, two runs per day for 22 days on a Roche/Hitachi 717 automated clinical chemistry analyzer.

### Repeatability / Intermediate precision

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

Concentration [ng/mL]	Within run (N = 22)			Total precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
0	400.9	3.2	0.8	400.9	5.1	1.3
2.5	419.6	2.7	0.6	419.6	4.0	0.9
5.0	439.2	3.2	0.7	439.2	5.0	1.1
7.5	461.2	3.3	0.7	461.2	4.7	1.0
10	479.2	3.2	0.7	479.2	4.5	0.9
12.5	495.4	3.3	0.7	495.4	4.8	1.0
15	511.7	3.3	0.6	511.7	4.6	0.9
17.5	526.8	3.2	0.6	526.8	4.5	0.9
20	540.3	3.5	0.6	540.3	4.5	0.8

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

5 ng/mL Cutoff		Within run		Total precision	
Concentration [ng/mL]	% of Cutoff	# Samples	EIA result	# Samples	EIA result
0	0	22	22 Neg	88	88 Neg
2.5	50	22	22 Neg	88	88 Neg
5.0	100	22	13 Neg/ 9 Pos	88	45 Neg/ 43 Pos
7.5	150	22	22 Pos	88	88 Pos
10	200	22	22 Pos	88	88 Pos

10 ng/mL Cutoff		Within run		Total precision	
Concentration [ng/mL]	% of Cutoff	# Samples	EIA result	# Samples	EIA result
0	0	22	22 Neg	88	88 Neg
2.5	25	22	22 Neg	88	88 Neg
5.0	50	22	22 Neg	88	88 Neg
7.5	75	22	22 Neg	88	88 Neg
10	100	22	4 Neg/ 18 Pos	88	29 Neg/ 59 Pos
12.5	125	22	22 Pos	88	88 Pos
15	150	22	22 Pos	88	88 Pos
17.5	175	22	22 Pos	88	88 Pos
20	200	22	22 Pos	88	88 Pos

*Semi-Quantitative analysis:* Typical results were measured in ng/mL. Preliminary positive/negative results are as follows:

Concentration [ng/mL]	Within run (N = 22)			Total precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
0	0.5	0.6	N/A	0.5	0.6	N/A

Concentration [ng/mL]	Within run (N = 22)			Total precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
2.5	3.0	0.3	9.5	3.0	0.4	14.4
5.0	5.2	0.3	6.5	5.2	0.5	9.4
7.5	7.7	0.3	4.3	7.7	0.5	6.0
10	10.0	0.4	4.0	10.0	0.5	5.3
12.5	12.1	0.4	3.4	12.1	0.5	4.4
15	14.5	0.6	4.4	14.5	0.8	5.5
17.5	17.0	0.5	3.0	17.0	0.6	3.7
20	19.9	0.7	3.4	19.9	0.8	4.0

### Precision on Roche cobas c 503 analyzer

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 replicates of two, two runs per day for 22 days on a **cobas c 503** analyzer.

*5 ng/mL Cutoff:*

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

Concentration [ng/mL]	Within run (N = 22)			Total precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
0	397.1	0.9	0.2	397.1	1.6	0.4
2.5	416.0	1.1	0.3	416.0	1.8	0.4
5.0	435.3	1.5	0.3	435.3	2.6	0.6
7.5	454.8	1.3	0.3	454.8	2.1	0.5
10	474.4	1.1	0.2	474.4	1.9	0.4
12.5	486.9	1.4	0.3	486.9	2.2	0.5
15	503.0	1.6	0.3	503.0	2.7	0.5
17.5	517.3	1.5	0.3	517.3	3.0	0.6
20	526.1	1.6	0.3	526.1	2.8	0.5

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

5 ng/mL Cutoff		Within run		Total precision	
Concentration [ng/mL]	# Samples	EIA result	# Samples	EIA result	
0	22	22 Neg	88	88 Neg	
2.5	22	22 Neg	88	88 Neg	
5.0	22	19 Neg/ 3 Pos	88	74 Neg/ 14 Pos	
7.5	22	22 Pos	88	88 Pos	
10	22	22 Pos	88	88 Pos	
12.5	22	22 Pos	88	88 Pos	
15	22	22 Pos	88	88 Pos	
17.5	22	22 Pos	88	88 Pos	
20	22	22 Pos	88	88 Pos	

*10 ng/mL Cutoff:*

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

Concentration [ng/mL]	Within run (N = 22)			Total precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
0	362.7	1.2	0.3	362.7	2.4	0.7
2.5	381.7	1.3	0.4	381.7	2.3	0.6
5.0	400.6	1.5	0.4	400.6	2.8	0.7

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Concentration [ng/mL]	Within run (N = 22)			Total precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
7.5	420.2	1.6	0.4	420.2	2.7	0.6
10	440.1	1.2	0.3	440.1	2.0	0.5
12.5	452.4	1.4	0.3	452.4	2.0	0.4
15	468.3	1.5	0.3	468.3	2.4	0.5
17.5	483.0	1.6	0.3	483.0	2.4	0.5
20	491.6	1.2	0.2	491.6	2.3	0.5

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

10 ng/mL Cutoff	Within run		Total precision	
Concentration [ng/mL]	# Samples	EIA result	# Samples	EIA result
0	22	22 Neg	88	88 Neg
2.5	22	22 Neg	88	88 Neg
5.0	22	22 Neg	88	88 Neg
7.5	22	22 Neg	88	88 Neg
10	22	20 Neg/ 2 Pos	88	85 Neg/ 3 Pos
12.5	22	22 Pos	88	88 Pos
15	22	22 Pos	88	88 Pos
17.5	22	22 Pos	88	88 Pos
20	22	22 Pos	88	88 Pos

**Semi-quantitative analysis:** The following concentrations were determined with reference curves from six calibrators. Typical results were measured in ng/mL. Preliminary positive/negative results are as follows:

Concentration [ng/mL]	Within run (N = 22)		Total precision (N = 88)	
	Mean	Qualitative response	Mean	Qualitative response
0	0.1	-	0.1	-
2.5	2.5	-	2.5	-
5.0	4.7	-	4.7	-
7.5	6.9	-	6.9	-
10	9.5	-	9.5	-
12.5	11.3	+	11.3	+
15	14.1	+	14.1	+
17.5	17.4	+	17.4	+
20	19.5	+	19.5	+

### Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 2 ng/mL for both the 5 ng/mL and 10 ng/mL cutoffs.

### Analytical recovery on Roche/Hitachi 717

To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free urine pool spiked with pure norbuprenorphine 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:

$$y = 1.0026x + 0.9053, r^2 = 0.991$$

Expected value [ng/mL]	Observed value [ng/mL]	% recovery
2	2.3	115.0
5	5.0	99.6
10	9.9	99.4
20	20.7	103.7
30	33.2	110.8
40	44.4	111.1
50	52.7	105.3
60	56.3	93.8
70	71.4	102.1

### Accuracy on Roche/Hitachi 717

A total of ninety (90) unaltered clinical specimens were tested with the Buprenorphine Enzyme Immunoassay and confirmed with GC-MS. Specimens having a norbuprenorphine concentration greater than or equal to 5 ng/mL or 10 ng/mL by GC-MS are defined as positive, and specimens with lower concentrations by GC-MS are defined as negative in the table below. Near cutoff samples are defined as  $\pm 50\%$  of the cutoff value. The correlation results are summarized as follows:

5 ng/mL Cutoff	Neg	< 50 % below the Cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the Cutoff	% agreement
<b>Positive</b>	0	0	2*	7	47	96.4
<b>Negative</b>	16	6	12	0	0	100

\*Discrepant between 50 % below the cutoff and the cutoff concentration (2.5-4.9 ng/mL)

10 ng/mL Cutoff	Neg	< 50 % below the Cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the Cutoff	% agreement
<b>Positive</b>	0	0	2**	8	31	95.1
<b>Negative</b>	16	20	12	1***	0	98.0

\*\*Discrepant between 50 % below the cutoff and the cutoff concentration (5-9.9 ng/mL)

\*\*\*Discrepant between cutoff and 50 % above the cutoff concentration (10-14.9 ng/mL)

The following table summarizes the results for the five discordant samples:

Cutoff value ng/mL	Assay value	Sample composition	
		NBUP (GC-MS) ng/mL	BUP (GC-MS) ng/mL
5	+	4.1	0.0
5	+	4.4	0.0
10	+	8.5	3.2
10	+	9.5	0.0
10	-	10.2	0.0

### Accuracy on Roche cobas c 503 analyzer

#### 5 ng/mL Cutoff

A total of ninety-six (96) urine samples were tested with the LZ1 Buprenorphine Enzyme Immunoassay and confirmed with LC-MS. Specimens having a total norbuprenorphine and buprenorphine concentration greater than or equal to 5 ng/mL by LC-MS are defined as preliminary positive and specimens with concentrations lower than 5 ng/mL by LC-MS are defined as negative in the table below. The correlation results from a Roche **cobas c 503** analyzer are summarized as follows (near cutoff samples are defined as  $\pm 50\%$  of the cutoff value).

**Buprenorphine Enzyme Immunoassay****Clinical Samples Qualitative Correlation Results:**

5 ng/mL Cutoff	Neg	< 50 % below the Cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% agreement
<b>Positive</b>	0	0	2*	7	42	98.0
<b>Negative</b>	20	16	8	1**	0	95.7

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

Samples	LC-MS	EIA	LC-MS (ng/mL)
Sample #45*	-	+	4.7
Sample #46*	-	+	4.9
Sample #54**	+	-	6.7

\*Discrepant between 50 % below the cutoff and the cutoff concentration (2.5-4.9 ng/mL)

\*\*Discrepant between cutoff and 50 % above the cutoff concentration (5-7.4 ng/mL)

**Clinical Samples Semi-Quantitative Correlation Results:**

5 ng/mL Cutoff	Neg	< 50 % below the Cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% agreement
<b>Positive</b>	0	0	3*	7	42	98.0
<b>Negative</b>	20	16	7	1**	0	93.5

The following table summarizes the results for the four discordant samples:

Samples	LC-MS	EIA	LC-MS (ng/mL)
Sample #40*	-	+	3.1
Sample #45*	-	+	4.7
Sample #46*	-	+	4.9
Sample #54**	+	-	6.7

\*Discrepant between 50 % below the cutoff and the cutoff concentration (2.5-4.9 ng/mL)

\*\*Discrepant between cutoff and 50 % above the cutoff concentration (5-7.4 ng/mL)

**Qualitative comparison of Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer**

A total of forty-two (42) urine samples were tested with the LZ1 Buprenorphine Enzyme Immunoassay on both the Roche **cobas c 503** analyzer and the Roche **cobas c 501** analyzer in qualitative mode.

Twenty (20) out of twenty-three (23) urine samples which were LC-MS confirmed as negative, were screened as negative at the 5 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the twenty (20) samples screened as negative on the Roche **cobas c 503** analyzer, all twenty (20) were also negative on the Roche **cobas c 501** analyzer. There is a 100.0 % concordance between the **cobas c 503** analyzer and **cobas c 501** analyzer for negative clinical samples in qualitative mode.

Eighteen (18) out of nineteen (19) urine samples which were LC-MS confirmed as positive, were screened as preliminary positive at the 5 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the eighteen (18) samples screened as preliminary positive on the Roche **cobas c 503** analyzer, all eighteen (18) were also screened as preliminary positive on the Roche **cobas c 501** analyzer. There is a 100.0 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for preliminary positive clinical samples in qualitative mode.

**Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer Qualitative Correlation Results:**

	cobas c 501 analyzer	
	+	-

cobas c 503 analyzer	+	18	0
	-	0	20

**Semi-Quantitative comparison of Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer**

A total of forty-two (42) urine samples were tested with the LZ1 Buprenorphine Enzyme Immunoassay on both the Roche **cobas c 503** analyzer and the Roche **cobas c 501** analyzer in semi-quantitative mode. Twenty (20) out of twenty-three (23) urine samples which were LC-MS confirmed as negative, were screened as negative at the 5 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the twenty (20) samples screened as negative on the Roche **cobas c 503** analyzer, nineteen (19) were also negative on the Roche **cobas c 501** analyzer. There is a 95.0 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for negative clinical samples in semi-quantitative mode. Eighteen (18) out of nineteen (19) urine samples which were LC-MS confirmed as positive, were screened as preliminary positive at the 5 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the eighteen (18) samples screened as preliminary positive on the Roche **cobas c 503** analyzer, all eighteen (18) were also screened as preliminary positive on the Roche **cobas c 501** analyzer. There is a 100.0 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for preliminary positive clinical samples in semi-quantitative mode.

**Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer Semi-Quantitative Correlation Results:**

		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	18	0
	-	1	19

**10 ng/mL Cutoff**

A total of ninety-five (95) urine samples were tested with the LZ1 Buprenorphine Enzyme Immunoassay and confirmed with LC-MS. Specimens having a total norbuprenorphine and buprenorphine concentration greater than or equal to 10 ng/mL by LC-MS are defined as preliminary positive and specimens with concentrations lower than 10 ng/mL by LC-MS are defined as negative in the table below. The correlation results from a Roche **cobas c 503** analyzer are summarized as follows (near cutoff samples are defined as  $\pm 50\%$  of the cutoff value).

10 ng/mL Cutoff	Neg	< 50 % below the Cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% agreement
<b>Positive</b>	0	0	6*	9	29	100.0
<b>Negative</b>	20	26	5	0	0	89.5

The following table summarizes the results for the six discordant samples:

Sample	LC-MS	EIA	LC-MS (ng/mL)
Sample #48*	-	+	5.7
Sample #49*	-	+	6.0
Sample #52*	-	+	6.4
Sample #55*	-	+	7.9
Sample #56*	-	+	9.7
Sample #57*	-	+	9.9

\*Discrepant between 50 % below the cutoff and the cutoff concentration (5-9.9 ng/mL)

**Clinical Samples Semi-Quantitative Correlation Results:**

10 ng/mL Cutoff	Neg	< 50 % below the Cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% agreement
<b>Positive</b>	0	0	4*	9	29	100.0

**Buprenorphine Enzyme Immunoassay**

<b>Negative</b>	20	26	7	0	0	93.0
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The following table summarizes the results for the four discordant samples:

Samples	LC-MS	EIA	LC-MS (ng/mL)
Sample #48*	-	+	5.7
Sample #49*	-	+	6.0
Sample #56*	-	+	9.7
Sample #57*	-	+	9.9

\*Discrepant between 50 % below the cutoff and the cutoff concentration (5-9.9 ng/mL)

#### Qualitative comparison of Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer

A total of ninety-five (95) urine samples were tested with the LZ1 Buprenorphine Enzyme Immunoassay on both the Roche **cobas c 503** analyzer and the Roche **cobas c 501** analyzer in qualitative mode.

Fifty-one (51) out of fifty-seven (57) urine samples which were LC-MS confirmed as negative, were screened as negative at the 10 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the fifty-one (51) samples screened as negative on the Roche **cobas c 503** analyzer, fifty-one (51) were also negative on the Roche **cobas c 501** analyzer. There is a 100.0 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for negative clinical samples in qualitative mode.

Thirty-eight (38) out of thirty-eight (38) urine samples which were LC-MS confirmed as positive, were screened as preliminary positive at the 10 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the thirty-eight (38) samples screened as preliminary positive on the Roche **cobas c 503** analyzer, all thirty-eight (38) were also screened as preliminary positive on the Roche **cobas c 501** analyzer. There is a 100.0 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for preliminary positive clinical samples in qualitative mode.

#### Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer Qualitative Correlation Results:

		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	38	0
	-	0	51

#### Semi-Quantitative comparison of Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer

A total of ninety-five (95) urine samples were tested with the LZ1 Buprenorphine Enzyme Immunoassay on both the Roche **cobas c 503** analyzer and the Roche **cobas c 501** analyzer in the semi-quantitative mode.

Fifty-three (53) out of fifty-seven (57) urine samples which were LC-MS confirmed as negative, were screened as negative at the 10 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the fifty-three (53) samples screened as negative on the Roche **cobas c 503** analyzer, fifty-two (52) were also negative on the Roche **cobas c 501** analyzer. There is a 98.1 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for negative clinical samples in semi-quantitative mode.

Thirty-eight (38) out of thirty-eight (38) urine samples which were LC-MS confirmed as positive, were screened as preliminary positive at the 10 ng/mL cutoff on a Roche **cobas c 503** analyzer. Out of the thirty-eight (38) samples screened as preliminary positive on the Roche **cobas c 503** analyzer, all thirty-eight (38) were also screened as preliminary positive on the Roche **cobas c 501** analyzer. There is a 100.0 % concordance between the **cobas c 503** analyzer and the **cobas c 501** analyzer for preliminary positive clinical samples in semi-quantitative mode.

#### Roche cobas c 503 analyzer vs. Roche cobas c 501 analyzer Semi-Quantitative Correlation Results:

		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	38	0
	-	1	52

#### Analytical specificity on Roche/Hitachi 717

Cross-reactivity of various potentially interfering drugs was tested by spiking each substance into drug-free urine and then evaluated with the assay's calibrated dose-response curve. Cross-reactivity of the parent drug buprenorphine and its glucuronidated acid metabolite is listed below as well. The assay detects the parent drug buprenorphine equally to norbuprenorphine, but has only minimal cross-reactivity to either of the glucuronides.

The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 5 ng/mL and 10 ng/mL norbuprenorphine cutoffs. For compounds tested at 100000 ng/mL and which gave an immunoassay result below 2 ng/mL, which is the assay's limit of detection, equivalent concentration and percent cross-reactivity are listed as "not detectable" ("ND").

#### Structurally related buprenorphine compounds:

Compound	Equivalent to 5 [ng/mL]	Equivalent to 10 [ng/mL]	% cross- reactivity
Buprenorphine	4.9	9.9	101
Buprenorphine-glucuronide	3846	7692	0.1
Norbuprenorphine-glucuronide	556	1111	0.9

#### Structurally related opiate compounds\*:

Compound	Equivalent to 5 [ng/mL]	Equivalent to 10 [ng/mL]	% cross- reactivity
6-Acetylcodeine	ND	ND	ND
Codeine	ND	ND	ND
Dextromethorphan	ND	ND	ND
Dihydrocodeine	ND	ND	ND
Heroin	192308	384615	0.003
Hydrocodone	ND	ND	ND
Hydromorphone	ND	ND	ND
Levorphanol	70423	140845	0.007
6-Monoacetylmorphine	ND	ND	ND
Morphine	ND	ND	ND
Morphine-3-glucuronide	ND	ND	ND
Morphine-6-glucuronide	ND	ND	ND
Nalorphine	ND	ND	ND
Naloxone	ND	ND	ND
Naltrexone	ND	ND	ND
Norcodeine	ND	ND	ND
Noroxycodone HCl	ND	ND	ND
Noroxymorphone HCl	ND	ND	ND
Oxycodone	ND	ND	ND
Oxymorphone	ND	ND	ND

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

#### Structurally unrelated pharmacological compounds\*\*:

Compound	Equivalent to 5 [ng/mL]	Equivalent to 10 [ng/mL]	% cross- reactivity
α-Methadol	ND	ND	ND
Citalopram	ND	ND	ND



# BUP

## Buprenorphine Enzyme Immunoassay

Compound	Equivalent to 5 [ng/mL]	Equivalent to 10 [ng/mL]	% cross-reactivity
EDDP	ND	ND	ND
EMDP	185185	370370	0.003
Fluoxetine	ND	ND	ND
Gabapentin	ND	ND	ND
Imipramine	ND	ND	ND
LAAM	ND	ND	ND
Meperidine	ND	ND	ND
Methadone	ND	ND	ND
Norpropoxyphene	ND	ND	ND
Paroxetine	ND	ND	ND
Sertraline	ND	ND	ND
Tramadol	ND	ND	ND

**\*\*It is possible that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedure errors.**

### Interference: Endogenous substances

The following endogenous compounds were spiked into negative urine and the 3 levels of controls (3 ng/mL, 7 ng/mL, and 13 ng/mL) for the assay. The spiked solutions were evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiologically relevant concentrations as all spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 5 ng/mL or 10 ng/mL. The table listed below shows the maximal concentration of the compound tested without interference on a Roche/Hitachi 717 analyzer.

Interfering substances	Spiked	0 ng/mL Urine	3 ng/mL Control	7 ng/mL Control	13 ng/mL Control
	[mg/dL]	[ng/mL]	[ng/mL]	[ng/mL]	[ng/mL]
Acetone	1000	0.0	3.2	6.5	12.2
Ascorbic acid	400	0.4	3.3	6.5	13.2
Creatinine	500	0.9	3.8	7.1	13.0
Galactose	10	0.4	3.5	6.4	11.7
γ-Globulin	500	0.0	3.0	8.1	11.2
Glucose	1500	0.0	3.1	7.1	11.5
Hemoglobin	300	0.4	4.0	8.2	13.0
NaCl	6000	0.9	3.9	7.8	13.0
Oxalic acid	100	0.5	3.3	6.7	11.8
Human serum albumin	500	0.0	3.2	7.1	11.8
Riboflavin	7.5	0.0	3.0	7.9	12.5
Urea	2000	0.0	3.3	7.2	11.6
Ethanol	1000	0.0	3.0	8.1	11.9
pH 3	N/A	0.0	3.3	5.9	13.6
pH 11	N/A	0.0	3.7	8.0	12.7

**Specific gravity:** Samples ranging in specific gravity from 1.001 to 1.027 were tested with the assay in the presence of 0 ng/mL, 3 ng/mL, 7 ng/mL, and 13 ng/mL of norbuprenorphine, and no interference was observed.

**Note:** All endogenous substances listed above, including specific gravity, were also tested in qualitative mode. No interference was observed. The results were identical to the semi-quantitative mode, as all samples gave correct preliminary positive/negative results corresponding to the cutoff value of 5 ng/mL or 10 ng/mL.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see [dialog.roche.com](http://dialog.roche.com) for definition of symbols used):

**CONTENT**

Contents of kit

**GTIN**

Global Trade Item Number

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# BUP

Buprenorphine Enzyme Immunoassay



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