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Article

A New Highly Specific Buprenorphine Immunoassay for Monitoring Buprenorphine Compliance and Abuse

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Urine buprenorphine screening is utilized to assess buprenorphine compliance and to detect illicit use. Robust screening assays should be specific for buprenorphine without cross-reactivity with other opioids, which are frequently present in patients treated for opioid addiction and chronic pain. We evaluated the new Lin-Zhi urine buprenorphine enzyme immunoassay (EIA) as a potentially more specific alternative to the Microgenics cloned enzyme donor immunoassay (CEDIA) by using 149 urines originating from patients treated for chronic pain and opioid addiction. The EIA methodology offered specific detection of buprenorphine use (100%) (106/106) and provided superior overall agreement with liquid chromatography–tandem mass spectrometry, 95% (142/149) and 91% (135/149) using 5 ng/mL (EIA[5]) and 10 ng/mL (EIA[10]) cutoffs, respectively, compared to CEDIA, 79% (117/149). CEDIA generated 27 false positives, most of which were observed in patients positive for other opioids, providing an overall specificity of 75% (79/106). CEDIA also demonstrated interference from structurally unrelated drugs, chloroquine and hydroxychloroquine. CEDIA and EIA[5] yielded similar sensitivities, both detecting 96% (22/23) of positive samples from patients prescribed buprenorphine, and 88% (38/43) and 81% (35/43), respectively, of all positive samples (illicit and prescribed users). The EIA methodology provides highly specific and sensitive detection of buprenorphine use, without the potential for opioid cross-reactivity.

Introduction

Buprenorphine is a partial opioid agonist prescribed in outpatient settings to treat heroin addiction and is also increasingly used to manage chronic pain (1, 2). Following ingestion, buprenorphine is primarily metabolized to another active analgesic compound, norbuprenorphine (Figure 1) (3–5). Buprenorphine and norbuprenorphine are both highly glucuronidated to buprenorphine-glucuronide and norbuprenorphine-glucuronide, respectively, the compounds detected most frequently in urine (Figure 1). Two different buprenorphine formulations are available: Subutex, which contains buprenorphine only, and Suboxone, which contains buprenorphine and naloxone, an opioid antagonist added to the formulation to curb abuse potential (6). Despite efforts to prevent its misuse, buprenorphine diversion and abuse still occur (7). For example, up to 5% of patients treated for chronic pain who were subjected to random urine drug screening unexpectedly tested positive for buprenorphine (7).

Urine drug screens are frequently utilized in patients undergoing detoxification and pain management to monitor compliance with treatment regimens and detect the use of other prescribed, undisclosed or illicit substances (8, 9). However, few automated urine buprenorphine screening methods are available. The most extensively studied, the Microgenics cloned enzyme donor immunoassay (CEDIA) (10–13), has been shown to generate false positive responses due to nonspecific antibody cross-reactivity with other opioids, including tramadol (11), dihydrocodeine (12), morphine, codeine and methadone (13). This unwanted cross-reactivity can be particularly problematic when screening urine from heroin users and patients treated for chronic pain because opioids are frequently present in the urine in high concentrations. A more specific automated, high-throughput buprenorphine screening method is needed for monitoring buprenorphine compliance and illicit use in these populations.

In this study, the performance of the new Lin-Zhi enzyme immunoassay (EIA) was evaluated and compared with the existing CEDIA method using 149 urine specimens originating from patients treated for opioid addiction and chronic pain. Diagnostic sensitivity and specificity were determined in comparison with liquid chromatography–tandem mass spectrometry (LC–MS–MS). CEDIA and EIA cross-reactivities with chloroquine and hydroxychloroquine, two drugs identified as possible sources of CEDIA interference in our laboratories, were also evaluated.

Materials and Methods

This study was approved by the Partners Human Research Committee. Both institutions conducting this study are members of the Partners Healthcare System.

EIA and CEDIA screening

A total of 149 urine specimens sent to our laboratories for buprenorphine testing were collected and analyzed using the CEDIA (Microgenics, now Thermo Scientific, Fremont, CA) and EIA (Lin-Zhi International, Sunnyvale, CA) buprenorphine assays on Olympus AU640 or AU480 analyzers (Beckman Coulter, Brea, CA) following manufacturers' recommendations. Both the CEDIA and EIA methods rely on measurement of a change in the enzymatic activity (change in rate of absorbance increase) after combining samples with anti-drug antibody and enzyme-drug conjugate. The CEDIA assay was performed semi-quantitatively using the 0, 5, 20, 50 and 75 ng/mL buprenorphine calibrators included in the kit. CEDIA signals $\geq$5 were considered to be positive. The EIA assay was performed using a two-point calibration using the manufacturer-supplied 0 and 10-ng/mL norbuprenorphine calibrators, and the enzymatic activity of the 10 ng/mL calibrator was normalized to a value of 100. EIA was then evaluated using a 10 ng/mL cutoff (referred to as EIA[10] throughout), with signals greater than or equal to 100 (the normalized

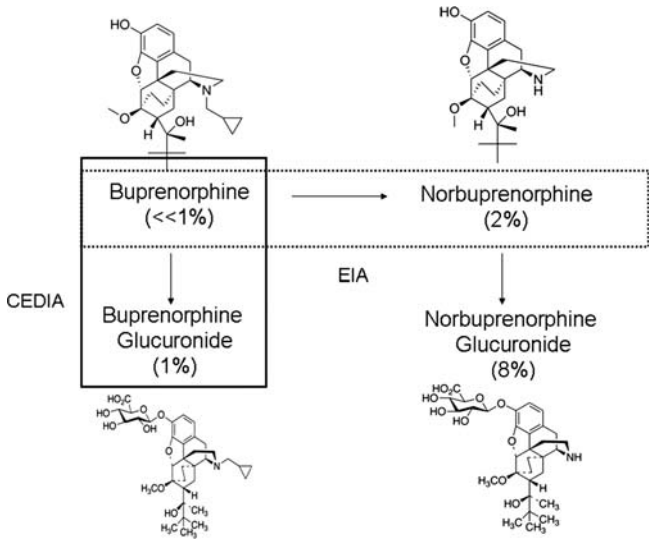

Figure 1. CEDIA and EIA assay cross-reactivity for buprenorphine and metabolites. Major buprenorphine metabolic pathways are presented, along with percentages of buprenorphine dose appearing as each compound in the urine (shown in parentheses). Compounds showing high cross-reactivity in the CEDIA and EIA assays are designated by the solid and dotted boxes, respectively. The manufacturers' stated cross-reactivities for CEDIA and EIA are: buprenorphine, 100 and 101%; norbuprenorphine, <0.5 and 100%; buprenorphine-glucuronide, 100 and 0.3%; norbuprenorphine-glucuronide, <0.5 and 1.4%, respectively.

10 ng/mL calibrator signal) considered positive, as well as with a lower qualitative threshold of 50 (referred to as EIA[5] throughout), with normalized signals ≥50% of the normalized 10 ng/mL calibrator signal considered positive.

During the study, the EIA manufacturer began providing an optional lower 5 ng/mL norbuprenorphine calibrator. To verify that the EIA[5] and EIA calibrated with the 5 ng/mL calibrator are comparable, we repeated all specimens for which we had sufficient volume (n = 95) using a two-point calibration with the 0 and 5-ng/mL norbuprenorphine calibrators and compared to those generated with the EIA[5]. We further established the comparability by verifying that the EIA calibrated with the 10 ng/mL calibrator (EIA[10]) was linear down to 5 ng/mL (or the EIA[5] cutoff) by running the 5 and 20 ng/mL buprenorphine calibrators and the 3, 5, 7, 13 and 20 ng/mL norbuprenorphine calibrators as unknowns in triplicate. The 5 and 20 ng/mL buprenorphine calibrators generated average signals of 46.4 and 181.1, respectively, and the 3, 5, 7, 13 and 20 ng/mL norbuprenorhine calibrators generated average signals of 31.1, 46.6, 71.9, 130.5 and 184.0, respectively.

CEDIA targets buprenorphine and buprenorphine-glucuronide, while EIA targets buprenorphine and norbuprenorphine, as demonstrated by the cross-reactivity and as shown in Figure 1. The manufacturers' stated cross-reactivities for CEDIA and EIA are: buprenorphine, 100% and 101%; norbuprenorphine, <0.5% and 100%; buprenorphine-glucuronide, 100% and 0.3%; norbuprenorphine-glucuronide, <0.5% and 1.4%, respectively. Both assays are FDA-approved.

Diagnostic Accuracy

A total of 149 samples were collected and analyzed by EIA and CEDIA. The majority of these samples (n = 114) originated from patients treated for chronic pain, and included 62 consecutive urine samples sent to our laboratory for our extended pain toxicology testing (which includes buprenorphine testing), and another 52 samples previously tested by LC–MS-MS and selected to include 17 samples known to contain low levels of buprenorphine and/or norbuprenorphine and 35 negatives by LC–MS-MS. Another 35 urine samples from patients treated for opioid addiction were also collected and analyzed, 33 of which were from patients who had an active buprenorphine prescription at the time of testing. For specimens that could not be tested immediately, aliquots were made within 24 hours and stored frozen (−20°C) in our laboratory until analysis.

True positive (TP), false negative (FN), true negative (TN) and false positive (FP) results were assigned based on LC–MS-MS results. Diagnostic sensitivity and specificity were calculated as $100 \times TP/(TP + FN)$ and $100 \times TN/(TN + FP)$, respectively. Diagnostic accuracy was calculated as $100 \times (TP + TN)/(TP + FN + TN + FP)$. Samples containing detectable (≥5 ng/mL) buprenorphine and/or norbuprenorphine by LC–MS-MS were considered positive.

Patients' medical records were reviewed to obtain pertinent urine drug screening results, identify other prescribed medications potentially contributing to false positive screening results and establish buprenorphine prescription status at the time of testing. Compliant patients were defined as those having a buprenorphine prescription at the time of testing who also tested positive by LC–MS-MS. Patients who had an active buprenorphine prescription and tested negative for buprenorphine and norbuprenorphine by LC–MS-MS (≤5 ng/mL) were considered non-compliant. Patients without a buprenorphine prescription who tested positive by LC–MS-MS were presumed to be illicitly using buprenorphine.

LC–MS-MS confirmation

All 149 samples were tested for total buprenorphine and total norbuprenorphine (free and conjugated forms) by LC–MS-MS at an outside laboratory (National Medical Services, Willow Grove, PA; Analysis Code U0801), using the following summarized protocol: 0.5 mL aliquots of control and patient urines were hydrolyzed using ß-glucuronidase (Patella Vulgata) for 3 h at 50°C. Internal standards (D4-buprenorphine and D3-norbuprenorphine) were added to the hydrolyzed control and patient urines, and the mixtures were acidified with acetic acid. Following solid-phase extraction (SPE) using a Strata-X-C polymer column (Phenomenex, Torrance, CA), the final eluent (2% ammonium hydroxide, 20% isopropanol, 78% ethyl acetate) was evaporated and reconstituted with a solution of mobile phase components. Samples were analyzed via a Waters ACQUITY-Quattro Micro MS UPLC–ESI–MS-MS system equipped with an electrospray ionization (ESI) probe operated in positive ionization mode. Separation was achieved using an Acquity UPLC BEH C18, 1.0 mm × 50 mm, 1.7 μm analytical column (Waters, Milford, MA) maintained at 60°C. Buprenorphine (and D4-buprenorphine) and norbuprenorphine (and D3-norbuprenorphine) eluted at approximately 3.4 and 3.7 min, respectively. Two ion transitions were monitored for each analyte and internal standard in multiple reaction monitoring mode (MRM). Transitions monitored [analyte: Q1 ion, Q3 quantitating ion (collision cell energy), Q3 qualifying ion (collision cell

energy)] were: buprenorphine: 468.2, 396.1 (40eV), 101.3 (40eV); buprenorphine-D4: 472.2, 400.1 (50eV), 101.1 (40eV); norbuprenorphine: 414.2, 100.9 (35eV), 187.1 (35eV); norbuprenorphine-D3: 417.2, 100.9 (35eV), 187.1 (40eV). The Q2 Argon gas pressure was 5.27 μbar. Each analytical run was independently calibrated using prepared standards at concentrations of 0.5, 1.0, 5.0, 20, 50 and 200 ng/mL buprenorphine and norbuprenorphine. Standards were prepared in the same manner as patient and control urine samples, except they were not subjected to hydrolysis or an additional 1:10 dilution (control and patient urine samples were diluted 10-fold during hydrolysis). The effective range of calibration for control and patient urine samples was 5 to 2,000 ng/mL. Biorad Liquichek controls containing morphine glucuronide were used to monitor the efficiency of the hydrolysis step. The LC–MS-MS method produced between run (or run-to-run) coefficients of variation of 19% and 4% for buprenorphine and 16% and 5% for norbuprenorphine at 10 and 400 ng/mL, respectively. The limit of quantitation and reporting limit for both buprenorphine and norbuprenorphine were 5 ng/mL.

Interferences

Water spiked with increasing concentrations of chloroquine and hydroxychloroquine at concentrations ranging from 75 to 10,000 ng/mL and 75 to 455,000 ng/mL, respectively, were tested by CEDIA and EIA to evaluate cross-reactivity of these drugs. Cross reactivity (%) was determined as 100 × [(immunoassay signal) / (calibrator signal)] × [(calibrator concentration) / (analyte concentration)]. Signals greater than the respective assay cutoffs were considered positive.

Results

Sensitivity, specificity and percentage agreement of EIA[5], EIA[10] and CEDIA compared to LC–MS-MS for the 149 samples are presented in Table I. EIA using both cutoffs demonstrated 100% specificity for buprenorphine and its metabolites, and provided higher overall agreements with LC–MS-MS (EIA[5] = 95%, EIA[10] = 91%) compared to CEDIA (79%) (Table I).

A total of 43 samples had detectable concentrations of buprenorphine and/or norbuprenorphine by LC–MS-MS (Table II). Of these positive samples, 81% (35/43) were detected using the lower EIA[5] cutoff, and 67% (29/43) were detected using the higher EIA[10] cutoff (Table II, Group I). Five of the EIA falsely negative samples were missed by both EIA[5] and CEDIA (Table II, Group III); of these, three had undetectable levels of buprenorphine (<5 ng/mL by LC–MS-MS), but contained low levels of the metabolite norbuprenorphine only (6–24 ng/L), and two had slightly higher buprenorphine concentrations of 8 and 9 ng/mL, respectively (Table II, Group III). The three samples missed by EIA[5], but detected by CEDIA (Table II, Group II), also had relatively low drug and metabolite concentrations (buprenorphine ≤6 ng/mL and norbuprenorphine ≤10 ng/mL).

CEDIA detected 88% (38/43) of all positive samples in this study, providing slightly higher overall sensitivity than EIA[5] (Table I). However, four samples containing buprenorphine concentrations near or below the stated CEDIA detection limit (5 ng/mL) (Samples 34–36 and 38 in Table II) also screened positive for opiates (one also screened positive for methadone), including two of the three positive samples detected by CEDIA only (Samples 36 and 38 in Table II). Therefore, CEDIA's perceived ability to detect these low level positives may have been at least partially due to nonspecific CEDIA cross-reactivity with

Table I
Sensitivity, Specificity, and Overall Agreement of EIA[5], EIA[10] and CEDIA in Comparison with LC–MS-MS

Screening method	Sensitivity	Specificity	% Agreement with LC–MS-MS
EIA[5]	81% (35/43)	100% (106/106)	95% (142/149)
EIA[10]	67% (29/43)	100% (106/106)	91% (135/149)
CEDIA	88% (38/43)	75% (79/106)	79% (117/149)

Table II
Positive Specimens (n = 43) Grouped by CEDIA and EIA[5] Screening Results*

Sample #	BUP (ng/mL)	NBUP (ng/mL)	Daily BUP Dose (mg)
Group I: CEDIA and EIA[5] positive			
1	230	660	16
2	170	380	24
3	150	270	24
4	38	480	8
5	35	150	12
6	32	110	16
7	30	250	
8	30	94	16
9	26	400	12
10	24	380	12
11	22	110	16
12	22	66	20
13	21	150	4
14	21	29	32
15	18	130	12
16	16	65	
17	14	48†	12
18	12	56	
19	10	89	8
20	10	84	12
21	10	56	12
22	10	72	
23	10	32	
24	9	44	
25	9	17†	12
26	8	85	
27	8	18†	
28	7	28	
29	6	90	
30	6	71†	8
31	6	34	6
32	<5	42†	
33	<5	31†	16
34	<5	25	
35	<5	29	
Group II: CEDIA Positive and EIA Negative			
36	6	8	
37	5	<5	
38	<5	10	
Group III: CEDIA and EIA Negative			
39	9	15	12
40	8	61	
41	<5	24	
42	<5	7	
43	<5	6	

*Measured LC–MS-MS total urine buprenorphine (BUP) and norbuprenorphine (NBUP) concentrations for the 43 samples are provided, as well as the prescribed daily buprenorphine dose (mg/day) for patients with active buprenorphine prescriptions at the time of testing. Group I: samples screening positive by CEDIA and EIA[5]. All samples in Group I also screened positive by EIA[10], unless marked with a dagger (†). Group II: positive samples screening positive by CEDIA only; Group III: samples screening negative by both CEDIA and EIA.

the other opioids in these samples, falsely increasing the overall CEDIA sensitivity.

A total of 23 LC–MS-MS positive samples (23/43) originated from patients with a buprenorphine prescription at the time of testing (Table II, Figure 2). Twenty of these samples were from patients prescribed buprenorphine substitution treatment for opioid addiction (with doses ranging from 4–32 mg/day, Table II) and three were from patients treated with buprenorphine for chronic pain (doses ranging from 16–24 mg/day; Samples #1–3 in Table II). Of the 23 positive samples from patients prescribed buprenorphine, CEDIA and EIA[5] both detected 22 (96%) (Figure 2), providing high sensitivity for detecting buprenorphine use in patients prescribed the drug. The one LC–MS-MS positive sample missed by both EIA[5] and CEDIA (Sample #39 in Table II) was very dilute (creatinine = 7 mg/dL) and had the lowest combined buprenorphine and norbuprenorphine concentrations (24 ng/mL) of all the samples originating from patients with a buprenorphine prescription. EIA using a higher cutoff, EIA[10], failed to detect four samples from patients treated with buprenorphine, providing a lower sensitivity of 83% (19/23) for detecting buprenorphine use in buprenorphine-prescribed patients (Figure 2).

Diagnostic sensitivities of EIA and CEDIA for detecting illicit buprenorphine use were estimated using the data for the 20 LC–MS-MS positive samples (20/43) in this study originating from patients who did not have a buprenorphine prescription at the time of testing (Figure 2). CEDIA, EIA[5] and EIA[10] provided sensitivities of 80% (16/20), 65% (13/20) and 55% (11/20) for detecting these samples from patients presumed to be illicitly using buprenorphine (Figure 2). The lower sensitivities of the assays for detecting illicit use were primarily attributed to the generally lower urine drug levels observed in the illicit users (Table II). Eighteen (18/20) of these positive samples from patients presumed to be illicitly using buprenorphine were preselected based on previously acquired LC–MS-MS results. However, two additional positive samples originating from patients without a buprenorphine prescription were detected in the group of 62 consecutive urines from patients treated for chronic pain. The rate of illicit buprenorphine use in our population of patients treated for chronic pain was therefore estimated to be 3% (2/62).

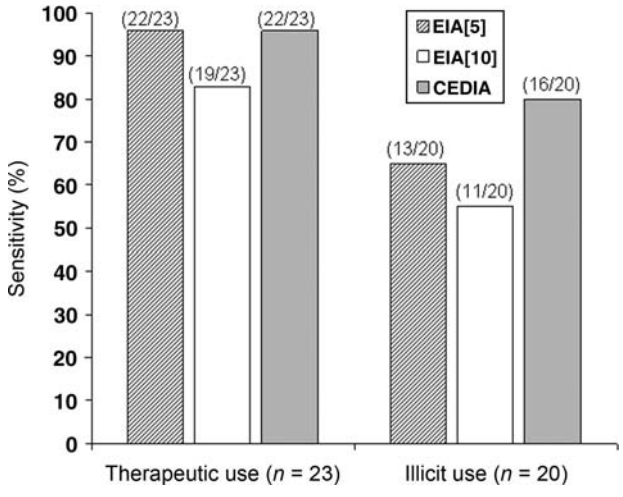

Figure 2. Sensitivities of EIA[5], EIA[10] and CEDIA for detecting therapeutic and illicit buprenorphine use. The number of samples screening positive by each method out of the total number of LC–MS-MS positive samples in each category are shown above the bars.

CEDIA produced 27 false positives in this study (i.e., CEDIA response ≥5 with undetectable buprenorphine and norbuprenorphine by LC–MS-MS), yielding an overall specificity of 75% (79/106). Twenty-two (81%) of the CEDIA false positive specimens also screened positive for compounds previously shown to interfere in the CEDIA assay, opiates (n = 13), methadone (n = 10) and/or tramadol (n = 3). Another sample had a borderline positive CEDIA signal of 5 ng/mL and borderline negative EIA[5] signal (49), and originated from a patient prescribed a relatively low dose of buprenorphine (4 mg/day), potentially representing a true positive (i.e., an LC–MS-MS and EIA false negative). Insufficient sample remained to allow retesting to determine whether this may have represented a diagnostic true positive in which buprenorphine and norbuprenorphine concentrations were present below the LC–MS-MS reporting limit (5 ng/mL). No explanation for the remaining four CEDIA false positives was found. The false positive CEDIA results all had relatively low estimated CEDIA concentrations of <20 ng/mL.

Based on our observations of several CEDIA false positive results in samples originating from patients taking hydroxychloroquine, we evaluated the potential for hydroxychloroquine interference in CEDIA and EIA. We tested for cross-reactivity by analyzing water spiked with increasing concentrations of hydroxychloroquine ranging from 75 to 455,000 ng/mL. Cross-reactivity of the related drug, chloroquine, was also tested in the same manner for concentrations ranging from 75 to 10,000 ng/mL. Hydroxychloroquine and chloroquine both demonstrated interference in the CEDIA assay with estimated cross reactivities of 0.04 and 0.5 %, respectively. EIA demonstrated no cross-reactivity with either compound over the range of concentrations tested. Based on its CEDIA cross-reactivity, hydroxychloroquine at concentrations of approximately 12,500 ng/mL would be sufficient to generate a positive CEDIA result (≥5 ng/mL). Chloroquine at a relatively low concentration of 1,000 ng/mL generated a positive CEDIA result. One of the CEDIA false positives generated in this study originated from a patient treated with 200 mg/day hydroxychloroquine; however, the sample was no longer available to allow urine hydroxychloroquine quantification.

Discussion

The aim of this study was to evaluate a new EIA automated urine buprenorphine assay as a more specific alternative to CEDIA for routine monitoring of buprenorphine use in patients treated for opioid addiction and chronic pain. The EIA was highly specific for buprenorphine and its metabolites (100%, 106/106), compared to CEDIA (75%, 79/106), and did not exhibit significant cross-reactivity with other related opioid analgesics or other prescribed and abused drugs, such as amphetamines, cocaine, benzodiazepines and cannabinoids, which were also present in many samples in this study. EIA[5] and EIA[10] both demonstrated good overall agreement with LC–MS-MS, 95% (142/149) and 91% (135/149), respectively (Table I). Overall CEDIA agreement with LC–MS-MS was lower at 78%, due to its lower specificity.

Consistent with previous reports (11–13), we observed a substantial number of CEDIA false positives, primarily in samples also testing positive for previously reported interferences, such as morphine and tramadol. We also present the first evidence of CEDIA interference from the seemingly unrelated drugs hydroxychloroquine and chloroquine. Hydroxychloroquine (Plaquenil) is a drug prescribed relatively frequently for the treatment of clinical conditions such as rheumatoid arthritis and lupus. Based on the hydroxychloroquine CEDIA cross-reactivity, urine drug concentrations of approximately 12,500 ng/mL would be sufficient to generate a positive result. Thus, patients taking the average adult dose of hydroxychloroquine of 400 mg/day, with reported urine concentrations ranging between 2,000 and 10,000 ng/mL (4), or even higher doses of 800 mg/day, may be anticipated to have urine drug and metabolite concentrations capable of triggering a positive CEDIA result. If CEDIA also cross-reacts with similarly structured metabolites of hydroxychloroquine, which also appear in the urine, it is likely that even lower drug doses would be sufficient to generate positive CEDIA results. Supporting this hypothesis, we have observed several otherwise unexplained CEDIA false positive results in patients on hydroxychloroquine doses as low as 200 mg/day. The related drug chloroquine, an anti-malarial, demonstrated even higher cross-reactivity with CEDIA, with urine levels as low as 1,000 ng/mL triggering a positive CEDIA result. However, this cross-reactivity should have minimal impact in the United States, where malaria cases are rare. Four CEDIA false positives could not be explained by opioid-related or hydroxychloroquine interferences, suggesting that additional unidentified drugs may also be contributing to false positive CEDIA results.

EIA[5] consistently provided higher overall sensitivity than EIA[10], with no loss of specificity, and is therefore emphasized throughout the remainder of the discussion. Both EIA[5] and CEDIA were highly sensitive for detecting buprenorphine use in patients treated with buprenorphine, both detecting 96% (22/23) of samples confirmed positive by LC–MS-MS (Figure 2). Only one buprenorphine-prescribed patient who tested positive by LC–MS-MS would have been missed by either CEDIA or EIA[5] (Sample 39 in Table II). This sample, from a patient prescribed 12 mg/day, was highly dilute (creatinine = 7 mg/dL) and had combined urine buprenorphine and norbuprenorphine levels lower than all other positive patients treated with buprenorphine, including those treated with much lower 4 mg/day doses.

CEDIA and EIA were less sensitive for detecting illicit buprenorphine use as opposed to therapeutic use (Figure 2), perhaps due to the lower urine concentrations of the drug and metabolite found in the illicit users (Table II). Although CEDIA appeared to provide higher sensitivity (80%; 16/20) for detecting illicit use than EIA[5] (65%; 13/20) (Figure 2), its higher observed detection rate may also be attributed to nonspecific CEDIA cross-reactivity with other opioids, which were present in many of the low-level buprenorphine positive samples in this study. It is also possible that CEDIA's higher cross-reactivity with buprenorphine-glucuronide (100% versus 0.3% for EIA) (Figure 1), a primary metabolite present in the urine could have contributed to the assay's higher sensitivity compared to EIA. EIA has high cross-reactivity for norbuprenorphine (100% versus <0.5% for CEDIA), but low cross-reactivity for glucuronide metabolites, which are the primary compounds found in urine (Figure 1). The low cross-reactivity for norbuprenorphine-glucuronide and the fact that these specimens most likely contained primarily norbuprenorphine-glucuronide (5) may explain why the specimens in Group III (Table II) generated negative results, despite total norbuprenorphine concentrations >5 ng/mL. Free buprenorphine and norbuprenorphine concentrations would have been helpful to interpret the results, but were not provided by the reference laboratory.

Unwanted CEDIA cross-reactivity with other opioids can be particularly problematic in patients treated for chronic pain and opioid addiction. In our laboratory, we have found that approximately 40% of our positive CEDIA results generated in our population of patients treated for chronic pain are falsely positive. Although in this study the highest CEDIA signal observed in the absence of buprenorphine (and norbuprenorphine) was <20 ng/mL, we have observed false positive CEDIA signals >20 ng/mL in subsequent clinical specimens sent to our laboratory for buprenorphine testing, with one clinical specimen generating a signal of 41 ng/mL. Since our implementation of CEDIA, the majority of false positive CEDIA signals have been observed in patients on high doses of morphine, with urine total morphine concentrations >50,000 ng/mL. In a six-month period, we had 19 false positive buprenorphine results, of which 14 (74%) had detectable morphine concentrations and 11 (58%) had total morphine concentrations >50,000 ng/mL. Because of the observed CEDIA specificity issues, CEDIA positives, particularly those known to contain other opioids, will often need to be confirmed by a more specific method. In these cases, clinicians should be made aware that positive CEDIA results should not be acted upon clinically until confirmation is received. Based on our findings, we confirmed all low-level positive CEDIA results (signals ≤40 ng/mL). In an effort to reduce the expense of confirming all CEDIA positives, positive CEDIA results >40 ng/mL were not confirmed unless specifically requested.

Our laboratory recently implemented EIA because of its improved specificity and overall performance compared to CEDIA. After the studies presented herein were completed, the EIA manufacturer began offering a lower 5-ng/mL norbuprenorphine calibrator option in their kit. A total of 95 of the study samples were still available and retested using the EIA 5-ng/mL cutoff, all of which yielded the same qualitative screening results as those generated with EIA[5]. At present, we recommend and are using a 5-ng/mL cutoff, although an even lower EIA cutoff may be useful for detecting lower buprenorphine levels like those frequently detected in the urine of illicit users.

In conclusion, the EIA methodology is a more specific alternative to CEDIA for high-volume buprenorphine screening and is therefore recommended for laboratories screening patients who are potentially abusing or being treated with other opioids. EIA may also be preferred in other populations, even where opioid positivity is low, because of CEDIA's observed cross-reactivity with other seemingly unrelated drugs, such as chloroquine and hydroxychloroquine.

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