



The importance of norbuprenorphine cross-reactivity in a buprenorphine urine immunoassay

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Introduction

Buprenorphine, an opiate antagonist is metabolized by N-dealkylation to norbuprenorphine, followed by glucuronide conjugation of both buprenorphine (BUP) and norbuprenorphine (nor-BUP) for excretion in urine.

Immunoassays which do not detect norbuprenorphine are subject to higher false negative rates since not all specimens from buprenorphine users contain the parent drug.

Objectives

- To evaluate the analytical performance of two commercially available urine buprenorphine immunoassays: Immunoanalysis (IMM) & Microgenics (MCG)

- To assess the importance of the different characteristics of the assays

IMM has almost identical cross-reactivity (100%) with both free BUP and nor-BUP; MCG has cross reactivity with free BUP and conjugated BUP but no nor-BUP cross-reactivity.

Methods

In the MCG assay: since extensive cross-reactivity with opiates and opiate glucuronides has been observed, the use of an elevated cut-off concentration of 30ng/mL rather than 5ng/mL has been suggested (1).

- MCG screening cut-off set to 20ng/mL as proposed by a reference laboratory rather than 30ng/mL as suggested in the literature, in an attempt to minimize both false negatives due to non-detection of nor-BUP; and false positives due to cross-reactivity with opiates and other glucuronides

- IMM screening cut-off set to the IMM recommendation of 5ng/mL (since no opioid cross reactivity was observed at this level)

- Specimens from patients treated with BUP and from patients taking other opiates were analyzed using both homogeneous enzyme immunoassays (HEIA)

- All specimens were additionally analyzed by LC-MS/MS for BUP, nor-BUP and corresponding glucuronides at a cut-off concentration of 5ng/mL

Results and Discussion

		MCG 20ng/mL	
		+	-
IMM 5ng/mL	+	49	13
	-	1	37
		LC-MS/MS 5 ng/mL	
		+	-
IMM 5ng/mL	+	62	1
	-	0	37

14 specimens were discrepant using the MCG assay. 13 were confirmed as false negatives of which five contained free norbuprenorphine at concentrations >20ng/mL; the rest had free buprenorphine <20ng/mL. One was a false positive compared to LC-MS/MS. The IMM assay produced one false negative result. In further specificity testing, the MCG assay gave false positive results with 100 µg/mL concentrations of dihydrocodeine, codeine and norpropoxyphene, while the IMM assay showed no response at that concentration.

Summary

At the concentrations described, the performance of the assays was as follows:

	IMM	MCG
Sensitivity	100%	79%
Specificity	97%	97%
Accuracy	99%	86%

When compliance testing is the main goal of an application, a high false negative rate due to the inability to detect the metabolite norbuprenorphine is a drawback. In 515 specimens taken from daily users of BUP the positive rates for BUP, Nor-BUP, BUP-gluc and Nor-BUP gluc were 1.2%, 63.9%, 89.9% and 96.5% respectively (2). While MCG assay detects BUP-gluc, other opiate glucuronides potentially present in urine may lead to false positives.

Reference

1. Pavlic M et al. *Int J Legal Med* 119(6); 2005:378-81
2. Kacinko S. et al. *Clin Chem* 55(6); 2009: 1177-87