*Barry Levine,*[1] *Ph.D. and John E. Smialek,*[1] *M.D.*

# Considerations in the Interpretation of Urine Analyses in Suspected Opiate Intoxications

**REFERENCE:** Levine B, Smialek JE. Considerations in the interpretation of urine analyses in suspected opiate intoxications. J Forensic Sci 1998;43(2):388–389.

**ABSTRACT:** Over the years, it has been observed that in many suspected opiate intoxications, a urine screen using the standard 300 ng/mL cutoff has produced negative results. Subsequent analysis of the blood in many of these cases, in fact, were positive for morphine. To identify the frequency of this occurrence and to determine a more appropriate urine screening cutoff, paired blood and urine specimens were tested for opiates at the above cutoffs.

Over the 6 month period of this study, 102 cases were identified where the blood morphine concentration by Roche Abuscreen was greater than 100 ng/mL of "morphine equivalents." All positive cases were confirmed as morphine by gas chromatography-mass spectrometry. Seventy nine of these cases, or 77%, had urine concentrations by Abuscreen exceeding 300 ng/mL of "morphine equivalents." The remaining 23 cases had urine morphine concentrations less than 300 ng/mL by Abuscreen. Urine specimens were then reanalyzed by Abuscreen using dilutions of the 300 ng/mL calibrator: 50, 75, and 150 ng/mL. Even with the use of a 50 ng/mL cutoff, 9 of these 23 specimens tested negative by Abuscreen.

Moreover, 23 of the 67 cases or 34% in which the cause of death was narcotic intoxication had urine opiate concentrations by Abuscreen less than the recommended 300 ng/mL cutoff. These results indicate the critical importance in cases of suspected narcotic intoxication of screening the blood in addition to urine.

**KEYWORDS:** forensic science, opiates, intoxication, postmortem, forensic toxicology

Urine is viewed as the specimen of choice in screening for abused and therapeutic drugs in forensic toxicology. It offers many advantages over other potential specimens. It is a relatively easy specimen to work with analytically. It is amenable to rapid abused drug testing by commercially available immunoassay techniques. When analyte separation is necessary, there are less interferences from endogenous components in urine than in other postmortem specimens. Solid phase extraction is much more practical with urine than with postmortem blood or tissue specimens. One other advantage of urine testing is that in general, drugs or their metabolites are present in higher concentration in urine than in blood. Drug use can also be detected for longer periods of time in urine than blood.

Heroin abuse remains a major problem in many areas. In Maryland, there were 236 fatalities from narcotic or narcotic and alcohol intoxication in 1994. In cases of suspected overdose, urine is routinely tested for abused and therapeutic drugs. Urine is tested for morphine by immunoassay at the usual cutoff of 300 ng/mL. However, over the years, it has been observed that in many of these intoxications, a urine screen using this cutoff has produced negative results. Therefore, laboratory procedures were developed such that in suspected intoxications, a full blood screen in addition to a urine screen would be performed. The blood is tested for morphine at a cutoff of 100 ng/mL.

Testing blood introduces the usual analytical problems and increases the cost of testing. To address these problems, a study was done to determine if a lower immunoassay cutoff or measurement of free morphine in urine would enable cases of intoxication to be detected without the need for testing blood.

[1]Office of the Chief Medical Examiner, State of Maryland, Baltimore, MD.

Received 6 May 1997; and in revised form 11 Aug. 1997; accepted 11 Aug. 1997.

## Experimental

### *Specimen Acquisition*

Specimens were obtained from cases investigated by the Office of the Chief Medical Examiner, State of Maryland in which autopsies were performed by the forensic pathology staff. Urine was collected from the bladder and blood was collected from the heart. After collection, specimens were sent to the Toxicology Laboratory where they were stored at 4°C or −20°C until analyzed.

### *Radioimmunoassay*

Urine specimens were initially tested by Roche Abuscreen (Roche Diagnostic Systems) using the manufacturer's instructions and at a cutoff of 300 ng/mL (1). Specimens initially screening positive were frozen and reanalyzed in batches using dilutions of the 300 ng/mL calibrator.

Blood specimens were semiquantitated using a modified Roche Abuscreen procedure (2) at a cutoff of 100 ng/mL. Specimens testing positive by Abuscreen were quantitated for free morphine by Coat-a-Count Serum Free Morphine assay (Diagnostic Products Corporation) (3).

### *Gas Chromatography-Mass Spectrometry (GC/MS)*

Morphine was confirmed in the blood or urine in each case by GC/MS using the method of Saady et al. (4).

## Results and Discussion

All cases were tested comprehensively for alcohols, therapeutic and abused drugs. Morphine was initially screened in the specimens by Roche Abuscreen, an immunoassay which responds to morphine, morphine-3-glucuronide and codeine (1). Blood specimens greater than 100 ng/mL of "morphine equivalents" were tested for free morphine using the DPC RIA which shows no cross-reactivity to morphine-3-glucuronide or codeine (3). Morphine was confirmed by GC/MS as the trifluoroacetyl derivative (4).

Over the 6 month period of this study, 102 cases were identified where the blood morphine concentration by Roche Abuscreen was greater than 100 ng/mL of "morphine equivalents." Seventy nine of these cases, or 77%, had urine concentrations by Abuscreen exceeding 300 ng/mL of "morphine equivalents," the manufacturer's cutoff. The remaining 23 cases had urine morphine concentrations less than 300 ng/mL by Abuscreen. Urine specimens were then quantitated by Abuscreen using dilutions of the 300 ng/mL calibrator; 50, 75, and 150 ng/mL calibrators were used with the 300 ng/mL calibrator to generate a standard curve for quantitative results. Table 1 gives the quantitative results for these specimens. Even with the use of a 50 ng/mL cutoff, 9 of these 23 specimens tested negative by Abuscreen. Table 1 also lists the free morphine concentration in these urine specimens. Nineteen of the 23 specimens had urine morphine concentrations above the immunoassay cutoff of 2.5 ng/mL.

These data become more significant when identifying these cases by cause of death. Forty-four of the 79 cases in which both blood and urine specimens were positive at their respective cutoffs had narcotic intoxication as the causes of death; all 23 cases in which the blood was positive and the urine was negative were narcotic intoxication cases. In other words, 23 of the 67 cases or 34% in which the cause of death was narcotic intoxication had urine opiate concentrations by Abuscreen less than the recommended 300 ng/mL cuttoff. These results indicate the critical importance in cases of suspected narcotic intoxication of either screening the blood in addition to urine or screening the urine at a cutoff much lower than 300 ng/mL. These results also emphasize the importance of providing the history of the case to the toxicology laboratory such that proper specimen selection decisions can be made by the laboratory.

Approximately 87% of a dose of morphine appears in the urine over a 72 hours period, with approximately 10% of the dose appearing as free morphine. The remaining dose appears in the urine as glucuronide or sulfate conjugates; morphine-3 glucuronide is the metabolite present in highest concentration (5). Mitchell et al. (6) found that after a single intramuscular dose of 20 mg morphine sulfate detection times in urine at the 300 ng/mL total morphine cutoff was 36–48 hours. Yeh et al. (7) used a gas chromatographic detection limit of 50 ng/mL total morphine and identified morphine in urine for 96 hours following an intravenous administration of 10 mg/70 Kg heroin hydrochloride. Given these data and the fact that most of the narcotic intoxication cases showed stigmata of chronic intravenous drug abuse, it seemed likely that those individuals who tested negative for opiates in the urine had not used opiates in the recent past, probably days prior to death. Tolerance to the effects of opiates is well known and loss of this tolerance can occur very rapidly. Therefore, a fatal intoxication may be explained by an addict returning to a dose administered while tolerant after tolerance is either partially or completely lost.

TABLE 1—*Cases with urine morphine concentrations less than 300 ng/mL by Abuscreen.*

| | Morphine Concentrations (ng/mL) | |
|---|---|---|
| No. | Abuscreen* | Free† |
| 1 | 80 | 23 |
| 2 | <50 | 4.9 |
| 3 | 95 | 8.2 |
| 4 | 60 | 3.2 |
| 5 | 69 | 8.3 |
| 6 | <50 | <2.5 |
| 7 | <50 | 5.2 |
| 8 | <50 | <2.5 |
| 9 | 130 | 5.8 |
| 10 | 115 | 8.9 |
| 11 | <50 | 3.8 |
| 12 | 90 | 6.9 |
| 13 | <50 | 4.8 |
| 14 | <50 | <2.5 |
| 15 | 210 | 41 |
| 16 | 210 | 40 |
| 17 | 70 | <2.5 |
| 18 | 60 | 11 |
| 19 | <50 | 2.9 |
| 20 | 290 | 113 |
| 21 | <50 | 6.5 |
| 22 | 140 | 116 |
| 23 | 180 | 19 |

*Abuscreen (morphine equivalents).
†Coat-a-count free morphine.

One other explanation for the acute deaths in these cases is the presence of ethanol. Previous work in this laboratory had demonstrated that the presence of small amounts of ethanol can be a significant factor in deaths due to opiate compounds (2). In only 3 of the 23 cases in which the urine morphine concentration was less than 300 ng/mL was no ethanol detected. Furthermore in 17 cases, the blood ethanol concentration exceeded 0.10 g/dL. This is contrasted by the urine morphine positive cases where 23 of the 44 narcotic intoxication cases were positive for ethanol. This strongly suggests that in these narcotic deaths in which the urine morphine concentrations by Abuscreen was less than 300 ng/mL ethanol played a role.

From these data, we conclude that screening urine exclusively at 300 ng/mL opiate equivalents is unsuitable to identify all narcotic intoxication cases. Lowering this cut-off to 50 ng/mL will identify a greater number, but not all of these cases. In cases where opiate intoxication is suspected, it is recommended that blood be screened for the presence of opiates.

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Additional information and reprint requests:
Barry Levine, Ph.D.
Office of the Chief Medical Examiner
State of Maryland
111 Penn St.
Baltimore, MD 21201