Statistical analyses were performed with the Analyse-it add-in for Microsoft Excel (version 2.1; Analyse-it, www.analyse-it.com).

Fig. 1 summarizes assay imprecision. By interpolation, we estimated that the 10% CV occurred at 0.045 μg/L. Of the 1392 individuals invited to participate, 699 (50.2%) reported for screening; 336 were male. The median age was 58.0 years (interquartile range, 53–71 years). After the exclusion of ineligible individuals, we collected samples from the reference population of 309 individuals (127 men, 182 women). The median age was 53 years (range, 45–80 years; interquartile range, 49–62 years). The age distributions of the male and female participants were not statistically different for either the screening set or the reference set. There was no association between cardiac troponin I concentration and blood pressure. The troponin values of the male and female participants were not significantly different, and there was no correlation between troponin concentration and age for either the overall population or the reference set. Troponin was undetectable (no signal detectable above background) in 25 individuals and had a calculated value between 0 μg/L and 0.006 μg/L in 144 individuals; hence, 165 (53.4%) of the 309 individuals could be considered to have no measurable troponin. The 99th percentile was 0.039 μg/L.

This study used a randomly selected population of ostensibly healthy individuals, which was then screened to exclude any possibility of active cardiovascular disease. The one previous evaluation of the Siemens Ultra method showed both an age and sex dependence of the troponin values (4). That study did not use imaging and obtained a higher value for the 99th percentile. A comparable study that also measured cardiac troponin I by a high-sensitivity method demonstrated similar findings (5). In contrast, however, our study with a highly selected subset of individuals defined by nonpathologic results in cardiac-imaging analyses found no effect of sex on the 99th percentile and obtained a lower value for the 99th percentile.

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**References**


**Letters to the Editor**

Cross-Reactivity of Naloxone with Oxycodone Immunoassays: Implications for Individuals Taking Suboxone®

**To the Editor:**

In 2002, the US Food and Drug Administration approved the use of the sublingual tablet, Suboxone® (Reckitt Benckiser Pharmaceuticals), to treat opioid dependence. The formulation is available at 2 doses, 2 or 8 mg (free base) buprenorphine hydrochloride and 0.5 or 2 mg (free base) naloxone hydrochloride dihydrate (1). The combination product is designed to decrease the potential for intravenous abuse, since parenteral administration may precipitate opioid withdrawal (2).

To monitor compliance, physicians request urine testing of buprenorphine and often additional testing to ensure no illicit drug use. A client recently requested multi-
analyte testing of patients taking Suboxone. Initial testing suggested a response with the oxycodone assay. We hypothesized this response was due to the presence of naloxone. To test this hypothesis, we (a) assayed a 40-μg/L buprenorphine calibrator with the oxycodone assay and (b) assayed drug-free urine samples that had been supplemented with increasing concentrations (100–10,000 μg/L) of naloxone (Cerilliant Corporation) and naloxone glucuronide (Sigma–Aldrich).

We evaluated 2 enzyme immunoassays: the homogeneous enzyme immunoassay (HEIA) oxycodone (Immunalysis Corporation) and the DRI® oxycodone assay (Microgenics/Thermo Fisher Scientific). On the Hitachi 717 analyzer, the HEIA demonstrated 100% cross-reactivity with oxymorphone at 100 μg/L (3), and the DRI assay demonstrated 103% cross-reactivity with oxymorphone at 300 μg/L and <0.1% cross-reactivity with noroxycodone and noroxymorphone at 50,000 μg/L and 500,000 μg/L, respectively. The Microgenics package insert indicated that 200 mg/L naloxone would test negative with a 100-μg/L cutoff for the DRI assay (4). No information was provided in the Immunalysis package insert regarding cross-reactivity with naloxone. Each kit contained oxycodone calibrators at 0, 100, 300, 500, and 1000 μg/L. A cutoff of 100 μg/L was used. Reagents were tested in the semiquantitative mode on a Dade Behring/Siemens Viva-E instrument. The instrument was set up according to the program notes provided by the manufacturers.

Buprenorphine at 40 μg/L did not cross-react with either oxycodone assay. Fig. 1 illustrates the responses obtained by both assays with increasing concentrations of naloxone and naloxone glucuronide. The highest response was obtained with the HEIA for both analytes. The DRI response did not approach the cutoff, but the HEIA reached the cutoff at concentrations near 2000 μg/L for naloxone and 5600 μg/L for naloxone glucuronide. This response may be due to antibody specificity and/or to sampling parameters of the instrument. Urinary naloxone concentrations in patients taking Suboxone have not been widely reported. Hull et al. (5) suggested that typical total naloxone concentrations are <100 μg/L because of the low bioavailability of naloxone in this formulation. In 7 samples that the authors judged to be adulterated or substituted, the total naloxone concentration in urine ranged from <100 μg/L to 15,155 μg/L (5). The authors speculated that the high concentrations observed in 5 samples were due to attempts to dissolve the tablet directly in the urine sample.

In our study of 39 buprenorphine-positive urine samples, the oxycodone HEIA was positive in 27 cases (69%), and the DRI assay was positive in 2 cases. The 2 DRI cases (also positive by the HEIA) were assayed in house by solid-phase extraction and subsequent GC-MS analysis for oxycodone (limit of detection, 250 μg/L), and none was detected. Three cases (including the 2 tested in house) were sent to NMS Labs for testing. Total naloxone was quantified by liquid chromatography–tandem MS, and total oxycodone and total oxymorphone were quantified by GC-MS. Oxycodone and oxymorphone were not detected at a reporting limit of 100 μg/L. Each of the 3 urine samples was positive for total naloxone, however, with measured concentrations of 220, 820, and 580 μg/L. According to our study, these concentrations were not high enough alone to account for the positive findings. This finding suggests that another substance or substances, including unidentified metabolites, were the major contributors to the positive results. For example, naloxone metabolism also produces normaloxone (noroxymorphone) and naloxol.

It is important that users of this methodology be aware of the cross-reactivity of naloxone and naloxone glucuronide with the Immunalysis oxycodone HEIA and, to a lesser extent, with the
Letters to the Editor

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We have devised a simple, easily reproducible device (Fig. 1) that contains the following main components: (a) an electrophoresis chamber; (b) 2 UV light–emitting lamps (302 nm), which are placed below the plastic plate holding the gel; and (c) a commercial Web camera connected to a personal computer. The device can be opened to pour or remove a gel and to fill the sample wells. It is configured for connection to an external power supply and is equipped with a safety switch that shuts off the power when the device is opened. The apparatus was built with commercially available materials and may be easily reproduced at a moderate cost. Technical drawings, a list of materials used, and relative costs are available free of charge from the corresponding author.

The assembly of these components into a single unit makes our device different from the traditional apparatus; however, its key feature is the part of the electrophoresis chamber holding the gel tray, which is made of UV-transparent Plexiglas (4 mm; Rohm and Haas). This simple modification, combined with the positioning of the UV lamps under the electrophoresis chamber and the availability of a digital camera inside the apparatus, makes it possible to visualize and record separations of nucleic acids without interrupting the run and having to move the gel from the chamber.

Our apparatus has 3 substantial advantages compared with traditional devices. First, the visualization and recording of results is shortened and simplified. Digitalizing of the electrophoretic separation is accomplished by simply turning on the video camera and capturing the image. This procedure takes only a few seconds. Second, the separation of DNA molecules is easily optimized. In fact, DNA can be visualized at any moment of the electrophoresis run without interrupting it, allowing