Qualitative Detection of Opiates in Sweat by EIA and GC-MS

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Abstract

Sweat was collected with the PharmChek™ sweat patch, and drugs were eluted from the collection pad of the patch. A solid-phase enzyme immunoassay (EIA) using microtiter plates was modified for the analysis of opiates in sweat. After opiate administration, sweat contains primarily parent opiate (heroin, codeine) and lipophilic metabolites (6-monoacetylmorphine [6-MAM]). The immunoassay was determined to have a cross-reactivity with codeine of 588%, with hydrocodone of 143%, with diacetylmorphine of 28%, and with 6-MAM of 30% relative to 100% for the morphine calibrators. The optimum cutoff concentration for this modified assay was determined by receiver operator characteristic analysis using 215 patches from 95 subjects to be 10 ng/mL morphine equivalents. At this cutoff concentration the assay had a diagnostic sensitivity of 86.9% and a diagnostic specificity of 92.8% versus gas chromatography–mass spectrometry (GC–MS), which was the reference method. The positive predictive value at a prevalence of 50% was 86%. The intra-assay precision at 10 ng/mL was 7.8%, and the interassay coefficient of variation (CV) was 39%. Analysis of spiked patches around the cutoff gave a percent positive threshold of approximately 50% between 10 and 15 ng/mL and a 95% confidence level for a positive result by the EIA between 20 and 25 ng/mL. Eighteen possible adulterants that could be injected into or under the patch were studied. Two (tile cleaner and detergent) can cause false-positive responses in the immunoassay. Two adulterants reduced response to spiked drug (Visine eye drops and Ben Gay ointment), which could cause a false-negative response. All results were confirmed by GC–MS. The clinical sensitivity and specificity for detecting drug use by analyzing sweat collected from human subjects following known doses of codeine (0, 30, and 60 mg orally) or heroin (20 mg intravenously) were 76 and 100%, repectively.

Introduction

Opiates are excreted in sweat (1–6). As with most drugs, the parent drug is the predomi-

nate analyte found in sweat (1–6). Heroin and 6-monoacetyl-morphine (6-MAM) or codeine but little morphine are found in sweat after heroin or codeine administration as contrasted to the metabolite profile found in urine or blood (Figure 1). Heroin and 6-MAM or codeine appear in sweat within 24 h of administration of opiates in controlled studies and peak within the first three days (2). Opiate concentrations in sweat account for approximately 1–2% of the dose.

Drugs in sweat were collected by subjects wearing a Band-Aid®-like PharmChek sweat patch (Figure 2). Water (monomer and dimer forms), oxygen, carbon dioxide, and other gases pass freely through the polyurethane adhesive (Tegaderm™) covering of the patch, but transport of molecules larger than vapor-phase isopropanol is excluded by the molecular pore structure (approximately 20Å) of the plastic membrane. Opiates persisted on the PharmChek sweat patch throughout the period of wear (7 days) with a slight decrease in amount in the 5–7 days after dosing (2). Any use of opiates during patch wear was accompanied by the appearance of opiates in sweat and the accumulation of increasing amounts of drug on the collection pad of the patch (2).

In this study, a microtiter plate enzyme immunoassay (EIA) for opiates was modified to detect opiates in sweat. An immunoassay for screening for drugs in sweat must cross-react
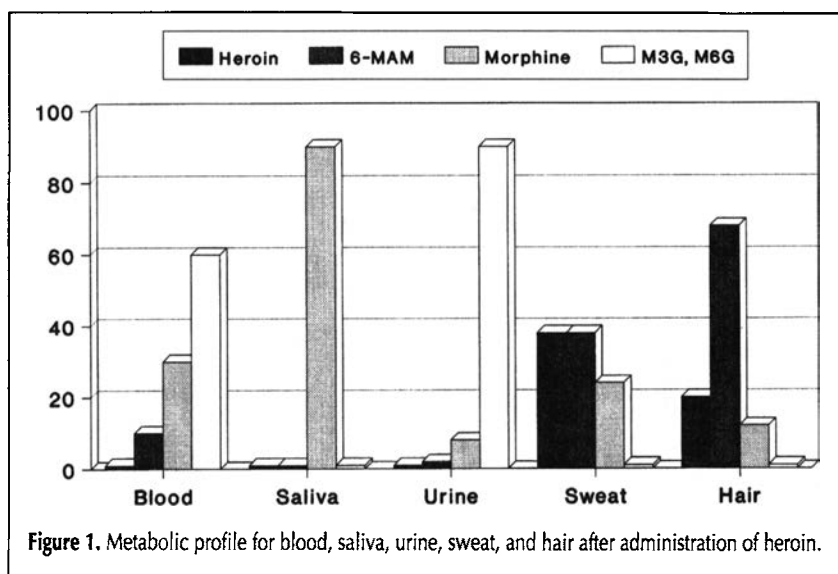

Figure 1. Metabolic profile for blood, saliva, urine, sweat, and hair after administration of heroin.

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with the parent drugs and with the lipophilic metabolites excreted in sweat. It must have a dynamic range in the concentration range encountered with the sweat patch eluate and, for qualitative screening tests, employ a cutoff concentration appropriate for the application. In this study, receiver operating characteristic (ROC) analysis (7–12) was used to choose a cutoff calibration that optimized diagnostic sensitivity, specificity, and predictive value. Gas chromatography–mass spectrometry (GC–MS) analysis was used as the measure of the presence of drug (8). In addition, the clinical sensitivity and specificity of the complete system (immunoassay screen and GC–MS confirmation) for detection of opiates in sweat were calculated from known-dose studies (9).

Materials and Methods

Specimen collection

Sweat and transdermal exudate were collected from human subjects using the PharmChek sweat patch (Sudormed, Santa Ana, CA) which was worn on the skin by the subject for 1–10 days. The PharmChek sweat patch is a semi-occlusive dressing consisting of a medical-grade cellulose blotter paper collection pad, covered by a thin layer of polyurethane (0.001 in.) and acrylate adhesives (Tegaderm Brand Transparent Dressing, 3M, St Paul, MN). A nine-digit serial number is printed under the polyurethane for use in chain of custody. The water component of sweat, vaporized by body heat, passes through the polyurethane; solids, salts, and drugs excreted in the sweat or that pass through the skin are trapped on the collection pad. The collection pad has a surface area of approximately 14 cm^2 and collects a minimum of 300 mL per day of insensible perspiration in a 22°C environment (13). Exercise, higher temperatures, or other factors that increase sweating increased the amount collected. Patches were worn by non-drug users, by known drug users in controlled administration experiments, and by suspected drug users in field studies. All human subject studies were approved in advance by the respective institutions' responsible committees.

At the end of the wear period, the collection pad was removed with disposable tweezers or gloved hands and placed in a 5-mL capped tube. The cellulose collection pad was eluted with 2.5 mL of 0.2M acetate buffer with methanol at pH 5.0 (25:75). Pads and elution buffer were mixed for 30 min on a slow-speed reciprocating shaker at 150–200 cycles per min. Aliquots of 50 mL were screened by immunoassay; aliquots of 1 mL were analyzed for morphine, codeine, heroin, and 6-MAM by GC–MS. Eluates were refrigerated for up to 10 days to batch specimens. Specimens found to be positive by GC–MS were frozen at –5 to –15°C and stored.

Immunoassay

The STC Opiates Microplate EIA (STC Technologies, Bethlehem, PA) is a solid-phase competitive immunoassay employing horseradish peroxidase labeled

with a morphine derivative. Fifty milliliters of patch eluate, control, or standard was added to each microplate well along with labeled enzyme and allowed to incubate for 30 min at room temperature. Following competition to bind to an antibody that is fixed to the surface of the well, the wells were washed six times with distilled water. Substrate (3,3',5,5'-tetramethylbenzidine) was added, and the color produced after a 30 min incubation (stopped with 100 mL of 2N sulfuric acid) was measured at an absorbance at 450 nm. Measurement of the absorbance at 630 nm was used as a reference according to the dual wavelength instructions of the manufacturer (Bio-Tek Instruments, Winooski, VT). Dual wavelength readings substantially reduce optical interference caused by scratched or fingerprinted microplates because the scratches or fingerprints similarly reduce the amount of light on both wavelengths, whereas the substrate changes affect the absorbance at only one wavelength. The absorbance is inversely proportional to the quantity of morphine or other opiates in the specimen. The calibrators and controls in the kit consisted of sweat pad elution buffer with morphine added at 0, 5, 10, and 50 ng/mL morphine. Spiked-patch controls at 5 and 15 ng/mL were provided separately or made up by the laboratory. Control patches were spiked with working solutions of morphine in methanol and dried at ambient conditions for 1 h.

The limit of detection (LOD) was defined as the concentration corresponding to the mean zero absorbance (A_0) minus three times the noise (standard deviation [SD]) ($A_{LOD} = A_0 - 3$ SD) (7). The limit of detection was determined by obtaining the absorbance values for eluate from 24 unworn, blank patches and calculating the standard deviation (SD) of the absorbance. The value for the SD of the absorbance was then multiplied by three and subtracted from the mean absorbance value to obtain the absorbance at the limit of detection. The apparent morphine concentration at the resulting absorbance is the limit of detection of the assay.

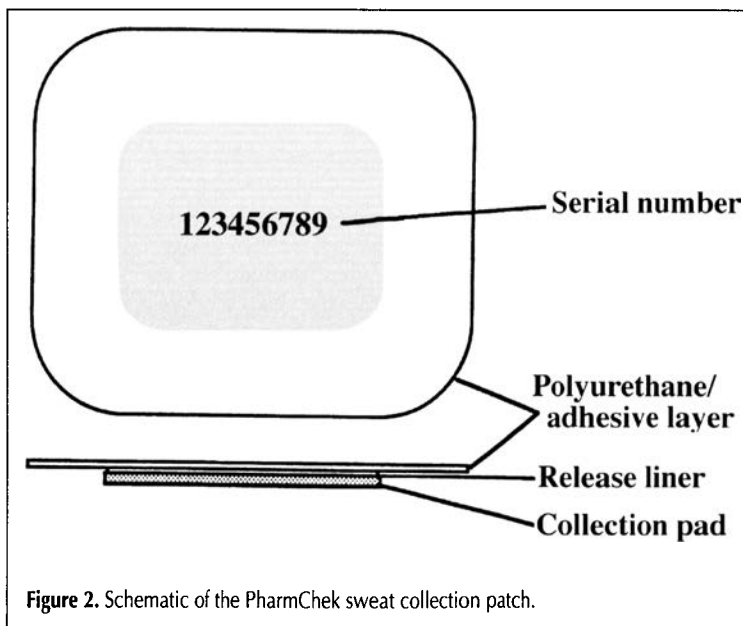The analytical sensitivity of the EIA around the cutoff concentration of 10 ng/mL was assessed by analyzing replicate

Figure 2. Schematic of the PharmChek sweat collection patch.

spiked samples at concentrations of 5, 7.5, 10, 20, and 25 ng/mL morphine. The percent of positive responses was determined for each concentration. A positive response was an absorbance lower than the cutoff calibrator mean absorbance. The cutoff calibrator mean absorbance was determined for ten replicate calibrator samples which were assayed after the calibration curve and before the spiked samples. Samples (n = 72) were run on seven different days at each concentration.

Cross-reactivity was determined by analyzing suspected cross-reactants at concentrations equal to the cutoff concentration, and at 10 times and 100 times the cutoff concentration. In addition, a series of common drugs was tested at concentrations of 10,000 ng/mL (Table I). The percent cross-reactivity was calculated as follows:

$$\% \text{ Cross-reactivity} = \frac{\text{apparent concentration from calibration curve}}{\text{spiked concentration of compound}} \times 100$$

Percentage displacement in the immunoassay measured from the negative calibrator was calculated from the following formula:

$$\% \text{ Displacement} = \frac{(\text{absorbance negative calibrator} - \text{absorbance sample})}{\text{absorbance negative calibrator}} \times 100$$

For within-run and total precision, a total of 280 collection pads were spiked with morphine. Forty pads of each concentration were prepared at concentrations of 2.5, 5, 10, 15, 17.5, 20, and 25 ng/mL. Aliquots of morphine in 100 µL methanol working solution was dropped onto the pad, and the pads were allowed to dry at ambient conditions for 1 h. Two spiked pads from each concentration were extracted and analyzed by EIA every day for a total of 20 days (14 wells × 20 days). Within-run and total precision were evaluated for the immunoassay according to calculations based on the National Committee for Clinical Laboratory Standards EP5-T2 (10).

GC–MS

Heroin, 6-MAM, codeine, and morphine were eluted from the sweat patch using 2.5 mL of methanol/acetate buffer (75:25). One-hundred microliters of an internal standard containing 0.15 µg/mL deuterated internal standards (heroin-d_3, 6-MAM-d_3, codeine-d_3, and morphine-d_3) was added, and each tube was vortex mixed. The methanol was then evaporated under nitrogen at 50°C, 1 mL tris buffer and 5 mL ethyl acetate were added, and the tube was vortex mixed. The top ethyl acetate layer was transferred, and 2 mL of 0.5M HCl was added to it. The tubes were vortex mixed and centrifuged at 2800 rpm. The top organic layer was discarded and the aqueous layer reserved and saturated with ammonium carbonate followed by another 5 mL ethyl acetate extraction, reserving the top organic layer. This organic layer was evaporated to dryness. Thirty microliters of N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) with 1% TMS was added and the tubes heated at 70°C for 20 min. The derivative extracts were evaporated under nitrogen at 50°C and then taken up in 10 µL acetonitrile and analyzed by GC–MS using selective ion monitoring (Hewlett Packard [Palo Alto, CA] HP 5890 GC with 12-m × 0.18-mm DB-1 capillary, splitless, injection port, 250°C, program: 170°C ramped 20°C/min to 300°C). The detector was a mass selective detector in selected ion monitoring (SIM) mode monitoring the following ions: 5.4–6.6 min: 343, 371, 372, 374, 375, 414, 417, 429, 430, 432; 6.6–6.8 min: 287, 290, 340, 399, 402, 414, 417, 429, 430, 432; 6.8–7.6 min: 268, 287, 290, 310, 327, 330, 340, 372, 399, 402. Retention times were as follows: codeine derivative, 6.3 min; morphine derivative, 6.6 min; 6-MAM derivative, 6.8 min; and heroin, 7.2 min. The retention times had to be within ± 2% of the retention times for the drug and the internal standard of the calibrator.

To be judged positive for codeine, ions 371, 372, and 343 had to be present in abundance ratios of within 20% of the abundance ratios for the codeine calibration standard. Drug ion

Table I. Crossreactivity of Opiates Microtiter Plate EIA*,†

Substance	Concentration added (ng/mL)	Apparent concentration (ng/mL)	% Cross-reactivity
Codeine	10	58.8	588
	100	>10,000	na
	1000	>10,000	na
Morphine	10	10	100
Hydrocodone	10	14.3	143
	100	155	155
	1000	2465	247
Heroin	10	2.8	28
	100	29.7	30
	1000	763	76
6-MAM	10	3.0	30
	100	23.3	23
	1000	535	54
Hydromorphone	10	1.6	16
	100	9.1	9
	1000	237	24
Oxymorphone	10	3.0	30
	100	1.0	1
	1000	2.2	0.2
Oxycodone	10	1.1	11
	100	1.1	1
	1000	5.7	0.6
Levorphanol	10	1.1	11
	100	3.8	4
	1000	11.0	1

* Drugs giving no signal at 10,000 ng/mL with the STC Opiates Microplate Assay: normorphine, nalorphan, morphine-3-glucuronide, alprazopam, amobarbital, amphetamine, chlordiazepoxide, clonazepam, dextromethorphan, diazepam, ephedrine, fenoprofen, flurazepam, gemfibrosil, ibuprofen, lidocaine, methylenedioxyamphetamine, methylenedioxymethamphetamine, naproxyn, pentobarbital, phenylpropanolamine, prazepam, procaine, pseudoephedrine, and temazepam.

† Abbreviations: EIA, enzyme immunoassay; na, not analyzed; 6-MAM, 6-monoacetyl-morphine.

abundances are normalized to the 371 ion. The peak areas of mass 371 and mass 374 from codeine-d_3 are used for quantitation. The qualifier ion for codeine-d_3 is 375. The limit of quantitation (LOQ) for codeine was 3 ng/mL of patch-extraction fluid.

To be judged positive for morphine, ions 429, 414, and 430 had to be present in abundance ratios within 20% of the abundance ratios for the calibration standard. Drug ion abundances were normalized to the 429 ion. The peak areas of mass 429 from morphine and mass 432 from morphine-d_3 were used for quantitation. The qualifier ion for morphine-d_3 was 417. The LOQ for morphine was 3 ng/mL of extraction fluid.

To be judged positive for 6-MAM, ions 399, 340, and 287 had to be present in abundance ratios within 20% of the abundance ratios for the calibration standard. Drug ion abundances were normalized to the 399 ion. The peak areas of mass 399 from 6-MAM and mass 402 from 6-MAM-d_3 were used for quantitation. The qualifier ion for 6-MAM-d_3 was 290. The LOQ for 6-MAM was 3 ng/mL of extraction fluid.

To be judged positive for heroin, ions 327, 268, and 310 had to be present in abundance ratios within 20% of the abundance ratios for the calibration standard. Drug ion abundances were normalized to the 327 ion. The peak areas of mass 327 from heroin and mass 330 from heroin-d_3 were used for quantitation. The qualifier ion for heroin-d_3 was 372. The LOQ for heroin was 5 ng/mL of extraction fluid.

Adulteration study

Eighteen possible adulterants were studied for their effect on the immunoassay and GC–MS (Table II). Spiked patches were prepared by applying aliquots of each adulterant to unworn patches that were then allowed to air dry. The pads were then spiked with 15 ng/mL eluate heroin, codeine, or morphine and then incubated at 37°C for 7 days. Pads were then extracted per standard procedures in the acetate buffer/methanol extraction buffer. Extracts were analyzed by the STC opiates EIA and by GC–MS.

Codeine administration

Fifteen patches were applied to lower chest, back, and biceps of the subjects starting six days before the administration of the codeine (six patches on day –6 and nine additional patches on day zero). On day 1, one of the original six patches, one of the nine day-zero patches, and a urine sample were collected for baseline. The subject then ingested either 60 or 30 mg of codeine phosphate orally (30 or 15 mL of Naldecon CXJ, respectively) accompanied by an regular meal. After a minimum 7-day washout period, a second series of patches following the same schedule was applied, and the alternative dose was administered.

Heroin administration

These studies were reported by Cone et al. (2). Heroin was administered intravenously in doses of 20 mg and orally in doses of 10 mg. Patches were removed and urine was collected at 24 and 48 h in one subject. Heroin (13.9 mg) was smoked by

Table II. Effect of Adulteration on Patch Content of Heroin, Morphine, or Codeine*

Adulterant	Heroin EIA % Recovery	Heroin GC–MS % Recovery	Morphine GC–MS % Recovery	Codeine GC–MS %Recovery
Saline	103	111	87	93
Bleach	111	100	80	92
Hand soap # 1	90	91	90	95
Hand soap #2	105	111	84	98
Liquid detergent	**171**	100	88	82
Tile cleaner	**200**	**57**	56	93
Drano	123	**57**	85	86
Vinegar	104	105	90	95
Sugar solution	95	109	83	95
Spot remover	136	**59**	83	92
Cranberry juice	89	106	91	91
Visine	73	103	88	93
Orange juice	86	113	84	90
Soda soft drink	104	108	85	96
Cough medication	**152**	105	87	91
Topical analgesic lotion	81	103	90	92
Mouthwash	115	106	87	94
Distilled water	85	114	91	105

* Spiked with 15 ng/mL. Bolded values indicate potential false-positive or false-negative results because of adulterant.
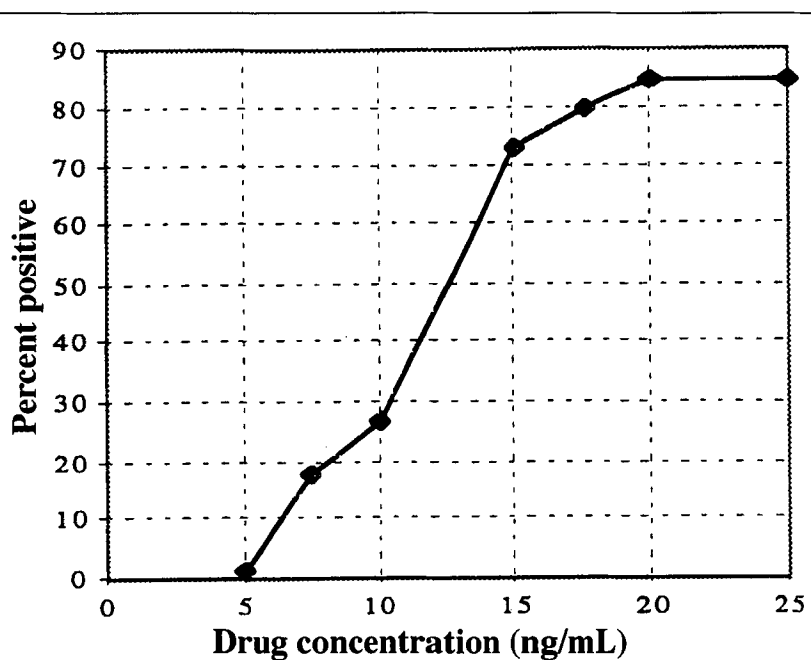

Figure 3. Percent-positive threshold analysis. The percent-positive results with the STC microtiter plate enzyme immunoassay for opiates in sweat are shown for concentrations from 5 to 25 ng/mL, n = 60 at each concentration (4 runs on 4 days of 15 replicates at each concentration). A cutoff of 10 ng/mL was used, and a sample replicate was considered positive if the absorbance was less than the mean absorbance of the 10-ng/mL calibrator.

a second subject, and patches were removed and urine was collected at 24 and 48 h (2). A 20-mg dose of heroin was administered intravenously to a third subject, and patches were removed and urine was collected at 24 and 48 h.

Poppy seed study

Subjects in this study had six patches applied to their lower chests on day zero. Following a 1-day wear period, one patch was removed for baseline measurements. Poppy-seed bagels (three different dosages of poppy seeds that were randomly assigned) or poppy-seed cake (containing 30 g of poppy seeds per dose) were consumed within 15 min. Urine specimens were collected on days 2 and 3, and patches were removed on day 3. Patches were analyzed by immunoassay.

Field trials

Patches were obtained from individuals in prison, on parole, or probation and enrollees at drug-treatment clinics and other drug-treatment programs.

ROC analysis

The number of true positives (TP), false negatives (FN), false positives (FP), and true negatives (TN) was determined for six putative cutoff concentrations (at 2.5, 5, 10, 15, 20, and 50 ng/mL morphine equivalents) by comparison of the immunoassay result to the result by GC–MS. A sample was considered a true positive if both the immunoassay and the GC–MS results were in concordance, that is, both positive for opiates (heroin, 6-MAM, morphine, or codeine present above the putative cutoff concentration) or a true negative if both were negative for opiates. Samples for which the immunoassay was positive (absorbance below the mean of the cutoff calibrator absorbance) but the GC–MS result revealed opiate concentra-

tions negative or below the LOQ were defined as false positives. Samples for which the immunoassay result was negative (absorbance above the mean of the cutoff calibrator) but the GC–MS result showed opiate concentrations above the LOQ of the assay were defined as false negatives. Sensitivity and specificity were calculated according to the following formulas (9):

$$Sensitivity = TP/(TP + FN)$$

$$Specificity = TN/(TN + FP)$$

Sensitivity was plotted versus 1 – specificity for the six possible cutoff concentrations to obtain ROC curves (9). Positive predictive value was calculated from the following formula:

$$\text{Positive predictive value} = \frac{\text{sensitivity} \times \text{prevalence}}{([1-\text{specificity}] \times [1-\text{prevalence}]) + (\text{sensitivity} \times \text{prevalence})}$$

Results

Analytical precision and accuracy

The LOD, calculated from the mean absorbance minus three times the SD of the zero calibrators ($n = 24$) was found to be 0.65 ng/mL. From this, the limit of detection for the STC opiates metabolite microplate enzyme immunoassay was lower than 1 ng/mL morphine equivalents.

Cross-reactivity of the enzyme immunoassay at 10 ng/mL was 588% for codeine, 143% for hydrocodone, 28% for heroin, and 30% for 6-MAM relative to 100% for the morphine calibrators. The cross-reactivity for heroin, 6-MAM, and codeine were nearly constant at the concentrations tested. This indicated that the binding curves are parallel and many of the same binding sites on the antibody respond to morphine, heroin, 6-MAM, and codeine. The percent crossreactivity for hydromorphone, oxymorphone, hydrocodone, oxycodone, and levorphanol differs at different concentrations. This indicates that the binding curves for these compounds with the assay antibody are not parallel to the binding curve for morphine (Table I). None of the 38 common drugs or chemicals tested at 10,000 ng/mL showed any cross-reactivity in the assay.
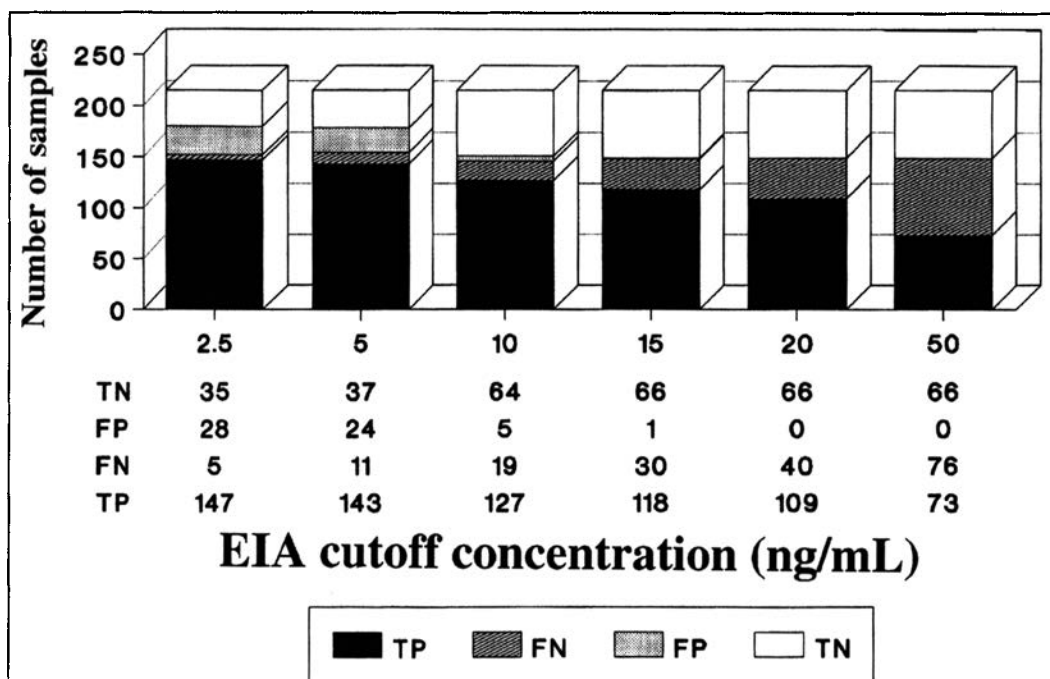

EIA cutoff concentration (ng/mL)	2.5	5	10	15	20	50
TN	35	37	64	66	66	66
FP	28	24	5	1	0	0
FN	5	11	19	30	40	76
TP	147	143	127	118	109	73

Figure 4. Accuracy of the EIA at the cutoff. The number of true-positive, false-negative, false-positive, and true-negative results for EIA cutoffs at 2.5, 5, 10, 15, 20, and 50 ng/mL with a data table for 215 specimens from 95 subjects.

The analytical sensitivity of the EIA around the cutoff concentration was assessed by analyzing replicate spiked samples at concentrations of 5, 7.5, 10, 20, and 25 ng/mL. The percent-positive response graphed for each concentration yielded the threshold response graph shown in Figure 3. The slope of the curve is the qualitative response of the EIA. From the graph, it can be seen that the 95% confidence limits for a positive response lie between 15 and 20 ng/mL. A sample containing between 10 and 15 ng/mL morphine has a 50% probability of being screened positive.

The intra-assay precision of the absorbance averaged over four days (n = 15 each day) mean CV was 10.4% for the 10 ng/mL calibrators, 7.0% at 5 ng/mL, 14% at 7.5 ng/mL, 7.8% at 10 ng/mL, 11.2% at 20 ng/mL, and 14.8% at 25 ng/mL. The interassay precision of the absorbance over 20 days was 139% at 2.5 ng/mL, 62% at 5 ng/mL, 39% at 10 ng/mL, 31% at 15 ng/mL, and 22% at 17.5 ng/mL.

Diagnostic accuracy

As shown in Figure 4, for 215 specimens from 95 subjects, using GC–MS as the reference method or "gold standard" for true presence or absence of opiates, the false-positive rate rose rapidly below a cutoff of 5 ng/mL and the false-negative rate rose steadily as the cutoff was increased from 2.5 to 50 ng/mL. The GC–MS cutoff used in this analysis was the LOQ of the GC–MS method, 5 ng/mL for heroin or 3 ng/mL for morphine, 6-MAM, and codeine. The maximum sensitivity and specificity were obtained from the ROC curve (Figure 5) at a cutoff of 10 ng/mL. Using a cutoff concentration of 10 ng/mL, the diagnostic sensitivity of the immunoassay was 127/146 = 87%, and the diagnostic specificity was 64/69 = 92.8%. For these values, the predictive value for a positive immunoassay result for opiates in sweat at 50% prevalence was 85.7%. This value was very close to the range reported for positive predictive values of commercially available laboratory immunoassay tests (radioimmunoassay [RIA] and EIA) for opiates in urine (12), which ranged from 87.6 to 98.8% at a prevalence of 50%.

Adulterants

The percent recovery of heroin, codeine, or morphine spiked onto an unworn patch after treatment with 18 substances which might be used on the skin before patch application or that could be injected into or under the patch as adulterants, is shown in Table II. Two substances (tile cleaner and household detergent) could cause a false-positive response of the immunoassay. Two adulterants reduced immunoassay recovery of spiked drug by 19% or more (Visine and Ben Gay ointment) and could cause a false negative. None of the substances caused false-positive results by GC–MS, so an immunoassay false positive that was due to these adulterants would not be reported positive after GC–MS confirmation. Three substances, tile cleaner, drain opener (Drano), and spot remover, reduced the GC–MS recovery of opiates after heroin spiking by more than 30%, probably by base hydrolysis of the drug. Tile cleaner reduced recovery by GC–MS of morphine but not codeine from spiked patches.

Poppy seed study

The poppy seeds were analyzed for opiates. Whole seeds contained 87 ng/mL morphine equivalents by RIA and 45 ng/mL morphine and 0 ng/mL codeine by GC–MS. Crushed seeds contained 123 ng/mL morphine equivalents by RIA and 64 ng/mL morphine and 1.2 ng/mL codeine by GC–MS. The two subjects who consumed three poppy-seed bagels (2.3 g each) had urine specimens positive for opiates on the first day. Their patch opiates averaged 3.8 ng/mL and were below the cutoff threshold. The five subjects who ingested poppy-seed cake produced urine specimens at or below the 300 ng/mL cutoff on day one. Their patch opiates content averaged 4.1 ng/mL, which was below the cutoff threshold.

Discussion

Validation of an immunoassay for use with alternate specimens requires that the immunoassay cross-react with the analytes found in the biological specimen, that it have a dynamic range within the concentrations of the drug found, and that it be accurate in the detection of drug present. Accuracy for a qualitative test is validated by demonstrating that the cutoff concentration has the required diagnostic sensitivity and specificity to yield a useful predictive
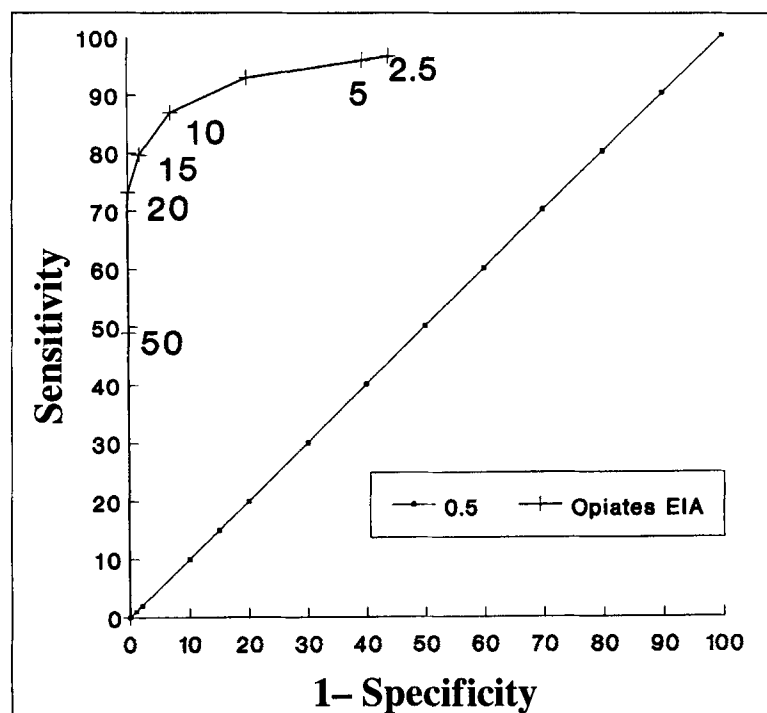

Figure 5. ROC plot for the STC microtiter plate EIA for opiates in sweat. ROC plot for the data shown in Figure 4. The sensitivity at each cutoff value is plotted versus 1 – specificity. The data points for each cutoff value, 2.5, 5, 10, 20, 30, and 50 ng/mL, are indicated on the plot.

value in the populations encountered in screening situations. Obviously, in the absence of traditional or regulatory cutoff values, diagnostic sensitivity and specificity considerations should direct the choice of a cutoff. In this study, ROC curves obtained using GC–MS for heroin, 6-MAM, morphine, or codeine as the gold standard or reference method for determining presence or absence of drug indicated either 5 or 10 ng/mL as the best cutoff. The more conservative choice was 10 ng/mL because it minimized the false positives at the expense of slightly increased false negatives.

In the application of the patch, the skin was prepared with an isopropyl alcohol wipe. This alcohol preparation removed dirt and natural skin oils and substances used on the skin, such as lotions and creams, as well as any contaminants that may have been applied to the skin to intentionally adulterate the patch. Because the polyurethane layer (Tegaderm) is impermeable to molecules larger than dimer water (molecular weight, 36) or volatile isopropanol (molecular weight, 60), contaminants and solvents applied to outside of the patch did not cross the polyurethane layer to reach the collection pad. Some solvents and treatments might destroy the polyurethane layer, but these would be noticeable by the holes that would appear in the polymer. Substances could be injected into or under the collection pad with a needle and syringe. Exposure of the skin under the patch for more than a few minutes to many of the substances studied for adulteration led to visible inflammation of the skin and discomfort for the subject. One-hundred microliters of drain opener (Drano), bleach, vinegar, tile cleaner, spot remover, and detergent (Spray and Wash) produced painful and visible trauma to the skin if contact was sustained for 20–30 min. The drug-treatment professional who removed the patches from subjects who were being monitored for drug use would be trained to recognize the inflammation caused by adulterant substances injected into patches and to note it on the chain of custody.

However, no immunoassay-positive result would be reported without confirmation. The complete system, sweat-collection patch, immunoassay, and GC–MS, was evaluated using known dosing of opiates from controlled-dosing studies (2). The concordance of positive and negative GC–MS results for opiates on sweat patch specimens with known drug doses given to patch-wearing subjects is shown in Figure 6. From the data in Figure 6, the clinical sensitivity of the overall system is 31/41 = 76%, and the clinical specificity is 20/20 = 100% or at least greater than 95%. The predictive value of a positive result reported for opiates in sweat with this system approaches 100%, and the negative predictive value, 68 at 50% prevalence. The data in Figure 6 were obtained from 61 patch specimens from 18 subjects who participated in controlled-dosing studies for doses greater than 20 mg. Codeine doses given were 30 or 60 mg codeine (Naldecon CX) administered orally. The heroin dose given was 20 mg intravenously, which is considerably lower than the doses used by heroin addicts. None of the patches from the subject administered 10 mg heroin orally were positive for opiates. The subject produced only one positive urine on the first day. The 13.9 mg heroin smoked produced only a trace of heroin on patches removed 24 and 48 h after dosing. Therefore, doses of less than 20 mg intravenously are below the detection limits of this system, and the results from the oral and smoked heroin dosing studies were not included in the calculation of the accuracy of the sweat collection and analysis system for detecting opiates in sweat.

Conclusion

Opiates can be reliably detected in sweat or transepidermal excretions collected on the PharmChek sweat patch by immunoassay screening with the solid-phase enzyme immunoassay using microfilter plates followed by GC–MS confirmation.

References

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	Patch after 24 h GC–MS	
	+	**−**
yes	31	10
no	0	20

Opiates given

Figure 6. Clinical sensitivity and specificity for PharmChek sweat patch. The concordance of the known dose with positive GC–MS results using the PharmChek sweat patch is shown for 61 patch specimens from 18 subjects who participated in controlled-dosing studies. Drug doses given were 30 and 60 mg codeine orally or 20 mg heroin intravenously.

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