mean 32), cysteine–homocysteine mixed disulfide, 11 (expressed in internal standard aminoethylycysteine) controls: not detected); and homocysteine, 1 (controls: not detected). Together with the increase of homocystine and cysteine–homocysteine mixed disulfide, methionine was slightly decreased.

That the fetus was affected was confirmed by enzymatic study of cultured amniocytes and assay of methylmalonic acid and total homocysteine in amniotic fluid (4–7). Pregnancy was terminated 3 days later. We conclude that measurements of free amino acids in amniotic fluid can be used for the prenatal diagnosis of CblC or CblD mutants, in addition to assays of other metabolites and enzymatic study of cultured amniocytes after the 13th week of gestation.

References

OnLine® Kinetic Microparticle Immunoassay of Cannabinoids, Morphine, and Benzoylgonine in Serum, David E. Moody1 and Anthony M. Medina (Ctr. for Human Toxicol., 490 BPRB, Univ. of Utah, Salt Lake City, UT 84112; 1 author for correspondence: fax 801-581-5034)

Immunoassay has become a standard method for screening specimens for abused drugs (1). Many commercial immunoassays test urine, the specimen of choice for situations that monitor drug use. Medical emergencies, driving-under-the-influence, and forensic testing, however, often require analysis of blood, plasma, or serum (1). The use of enzyme immunoassays, fluorescence polarization immunoassays, and RIAs to analyze blood, plasma, and serum has been established (e.g., refs. 2–4). OnLine® kinetic microparticle immunoassays (KIMS; Roche Diagnostic Systems, Nutley, NJ) have recently been introduced to detect abused drugs in urine (5). We have now tested whether KIMS could also detect morphine, benzoylgonine, and 11-nor-Δ⁹-carboxy-tetrahydrocannabinol (COOH-THC) in serum.

HIV- and hepatitis-free sera from 27 individuals were purchased from Boston Biomedica (West Bridgewater, MA). Drug-free sera—as tested by RIA for opiates, cannabinoids, and cocaine metabolites (6)—were used for the specific drug class being studied. Preliminary studies were performed to test whether serum would provide a signal response comparable with that of the intended matrix, urine. Urine from two drug-free volunteers in the lab and two lots of sera were fortified with morphine or benzoylgonine (3–1000 μg/L from Alltech, Deerfield, IL), or COOH-THC (1–300 μg/L from Research Triangle Institute, Research Triangle, NC), and then analyzed on a Cobas Mira (Roche Diagnostic Systems) with OnLine reagents according to the manufacturer’s recommended procedures. Sera were a compatible matrix for analysis, and gave signal vs concentration curves similar to those for the fortified urine, although for COOH-THC these curves were at lower absorbance values.

Because drug concentrations in serum are usually lower than in urine, we attempted two adjustments to enhance the sensitivity of the assay. First, fortified serum samples were analyzed with two- and threefold increases in the sample volume (with concomitant decreases in buffer volume) programmed into the assay procedure. Alternatively, fortified 1-mL samples were extracted with 7 mL of chloroform:isopropanol (9:1, by vol), mixed for 20 min, and separated into phases by centrifugation; the organic phase was removed to a clean tube, dried under air, reconstituted to one-fifth of the final volume with ethanol, vortex mixed, and then reconstituted to a final volume of 0.25, 0.5, 0.75, or 1.0 mL with 0.1 mol/L potassium phosphate (pH 7.4). Both methods to improve sensitivity enhanced the rates of signal change at lower drug concentrations. For example, values of ΔA/min for 100 μg/L morphine divided by ΔA/min for the blank were 0.94, 0.90, and 0.84 when 6, 12, and 18 μL of sample were used, and 0.96, 0.94, 0.90, and 0.72 for extracts reconstituted in 1.0, 0.75, 0.5, and 0.25 mL of buffer, respectively. However, the dynamic range diminished with increases in sample volume. The dynamic range (i.e., maximum ΔA/min minus minimum ΔA/min) for morphine was 0.80, 0.68, and 0.50 for 6, 12, and 18 μL of unextracted sample, but was 0.83–0.86 for extracts reconstituted to 1.0–0.25 mL.

To study potential individual variations in serum matrices, we fortified several sera with the drugs being investigated. Extracted samples reconstituted in one-half the original volume were compared with unextracted samples. We found that variations were diminished by extraction. Samples fortified with 100 μg/L morphine (n = 27), 100 μg/L benzoylgonine (n = 23), or 25 μg/L COOH-THC (n = 15) had respective CVs (based on ΔA/min) of 6.8%, 7.3%, and 6.1% when unextracted, and 4.3%, 3.6%, and 4.9% when extracted.

To test the effect of individual variations in separation of positive and negative samples, we fortified sera from 10 different donors with all three drugs at zero, low, medium, and high concentrations: COOH-THC at 0, 25, 50, and 100 μg/L; morphine and benzoylgonine at 0, 30, 100, and 300 μg/L. One-milliliter aliquots were extracted and reconstituted to 0.5 mL and, along with unextracted sera, were analyzed with the three different assays (Fig. 1). Again, individual variations in signal were reduced in extracted samples. For extracted vs unextracted samples, the respective CVs ranged from 1.8–5.2% and 6.3–11.1% for the COOH-THC assay, 5.5–7.6% and 8.2–15.7% for the benzoylgonine assay, and 4.1–9.2% and 7.1–16.2% for the morphine assay. Because of this variation, only the 100 μg/L COOH-THC and 300 μg/L morphine unextracted samples could be entirely distinguished from their negatives. By contrast, all of the extracted samples containing 50 or 100 μg/L COOH-THC, 100 or 300 μg/L morphine, or 300 μg/L benzoylgonine could be distinguished from the extracted negatives (Fig. 1).

The above experiments demonstrate that COOH-THC, morphine, and benzoylgonine can be detected in fortified sera with use of the OnLine KIMS reagents. Direct measurement of unextracted sera does not detect the drug.
or metabolite concentrations expected in serum (positive sera can be reliably distinguished from negatives only at higher concentrations) but may be of use in extreme overdose cases. Extraction of serum is time consuming, but offers a method for testing serum samples at lower cutoffs that may be more reasonable for this matrix. The wide variation in signals of negative and positive samples dictates that a laboratory will need to make a pool of negative and cutoff samples from several different individuals to establish separation criteria for determination of presumptive positives when either extracted or unextracted sera are to be tested. These data on fortified sera should now provide approaches for validating the usefulness of KIMS reagents on “real” serum samples.

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References
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Enzymatic Activity in Glucose-6-phosphate Dehydrogenase-Normal and -Deficient Neonates Measured with a Commercial Kit, Michael Kaplan,1,3,4 Chava Leiter,5 Cathy Hammerman,1,2 and Bernard Rudensky2 (Shaare Zedek Med. Ctr., 1 Dept. of Neonatology, and 2 Clin. Hematol. Lab., P.O. Box 3235, Jerusalem 91031, Israel; 3 Med. School of the Hebrew Univ.-Hadassah, Jerusalem, Israel; 4 author for correspondence: fax 972-2-652-0689, e-mail kaplan@MD2.HUJI.AC.IL)

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is frequently associated with neonatal jaundice (1–3), which may cause kernicterus and death. Infants at high risk for the Mediterranean variant should be screened as soon as possible after delivery to categorize those likely to develop jaundice. Confirmation of a pathological screening test with a definitive, quantitative analysis is recommended (4).

At this Medical Center we have routinely screened high-risk newborns for many years (5, 6), using a commercial qualitative color reduction kit (visual qualitative determination of G-6-PD deficiency in red cells (no. 400; Sigma Diagnostics, St. Louis, MO). We recently attempted to improve the service offered our G-6-PD-deficient families and investigated the possibility of confirming with quantitative G-6-PD determinations those found to be enzyme deficient by the screening test. While setting up a system utilizing another commercial package (G-6-PD: quantitative, ultraviolet kinetic determination in blood at 340nm (no. 345-UV), Sigma Diagnostics), we found that the values we were reading for normal infants were in excess of the manufacturer-supplied range for adults. Although the package insert implies that values for this test in newborns may be higher than in adults, no actual values are provided. To determine standard values to make this test more meaningful, we studied the range of enzyme activity in expected G-6-PD-normal newborns of a low-risk population group and also in those found to be G-6-PD deficient by the screening test.

The principle of the quantitative test involves the oxidation of glucose 6-phosphate to 6-phosphogluconate, and the concomitant reduction of NADP+ to NADPH. These reactions occur in the presence of G-6-PD, and the rate of NADPH formation, which is proportional to G-6-PD activity, is measured spectrophotometrically. Formation of additional NADPH by 6-phosphogluconate dehydrogenase from erythrocytes (RBC) is inhibited by the use of maleimide, an inhibitor of this enzyme. One international enzyme unit (1 U) is defined as that amount of G-6-PD activity that will convert 1 μmol of substrate (glucose 6-phosphate to 6-phosphogluconate) per minute.

Term, healthy neonates in the first week postpartum were drawn from the pool of infants in the well-baby nursery. For the study of infants expected to be normal, only babies drawn from the lowest risk group for G-6-PD deficiency in our population, i.e., both of whose parents were of Ashkenazi (Eastern European) Jewish origin (7), were included. Because of the very low incidence of G-6-PD deficiency in this population, babies from this subgroup are not routinely screened. Newborns from our high-risk group (Sephardic Jewish families, who originated in Kurdistan, Iraq, Iran, Syria, Lebanon, and Tur-