A Quality-Control Solution for Use in the "\(\Delta A_{450}\)" Determination of Amniotic Fluid

To the Editor:

It has been well established that the bilirubin concentration in amniotic fluid is a good indicator of increased hemoglobin degradation after fetal rhesus isoimmunization (1). Frequently the bilirubin concentration is not measured directly, but instead the absorbance change at 450 nm ("\(\Delta A_{450}\)"), and this value is used as an indicator of bilirubin concentration. The technique used to measure the absorbance change is generally standardized (2), except for minor modifications. However, difficulties do arise when one attempts to implement a quality-control program.

The main problem stems from the instability of the bilirubin in amniotic fluid, which makes this material unsuitable for use as a quality control. The determination requires no reagents, so the primary reason for using a control is to provide a check on analytical technique. For this purpose, a fluid is needed that has a stable spectral response similar to that of amniotic fluid. A \(2.34 \times 10^{-3}\) mol/liter solution of 8-hydroxyquinoline (3) meets this need. Such a solution is close to the limit of solubility at room temperature (22 °C), but the solubility can be enhanced by adding a little hydrochloric acid or by using salts, such as the hemi-sulfate of 8-hydroxyquinoline. However, this is undesirable because of a spectral shift and decreased stability of the solution. We have found that the aqueous solution of 8-hydroxyquinoline is stable for at least one year at room temperature if precautions are taken to avoid excessive exposure to light (amber-colored bottle, stored away from direct sunlight). During two years use, we have established a \(\Delta A_{450}\) value of \(0.087 \pm 0.004\) (mean \(\pm 2\) SD; \(n = 300\)) for the 8-hydroxyquinoline solution. The spectral patterns of the 8-hydroxyquinoline solution and amniotic fluid so closely resemble one another that a "bilirubin" concentration can be calculated by applying a formula such as the one of Bjerre et al. (3).

Figure 1 shows the spectral tracings for a representative amniotic fluid and for the 8-hydroxyquinoline solution, Figure 2 the derivations of the \(\Delta A_{450}\) values.

References


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Interference by NaCl with the EMIT Method of Analysis for Drugs of Abuse

To the Editor:

In our Toxicology Laboratory, we encounter schemes used by drug addicts on methadone detoxification programs to avoid our detection of drugs of abuse (1). Such efforts have included incorporation of a plastic bag filled with another's urine, concealed under the addict's clothing, connected with a long piece of plastic tubing running along the trunk of the body, and, on clinic visit, substituted for his own specimen. Another stratagem is to consume large quantities of fluids 2 to 4 hours before urination, in the hope of diluting the urine to the point where the drug concentration may fall below the sensitivity of the method and thus escape detection. Methadone may be there in large quantities and may not be affected significantly by the dilution effect; thus this second scheme has limited success with both the thin-layer chromatographic or the EMIT (Syva, Palo Alto, Calif. 94304) methods for analysis for drugs of abuse.
Recently, a urine specimen to be analyzed for morphine, barbiturates, and methadone tested negative by EMIT, but positive for all three drugs by thin-layer chromatography. Further investigation revealed that the patient had added sodium chloride to the urine specimen. We undertook a preliminary investigation on the EMIT system by supplementing urine specimens known to be positive for morphine, barbiturates, and methadone with sodium chloride to concentrations up to 200 g/liter. When concentrations exceeded 50 g/liter, all specimens became negative. Thus, one should be alert for the possibility of addicts clandestinely placing salt in their urines to escape detection. Fortunately, the added salt appears to nullify all EMIT tests, so that all drugs tested will be negative, which in itself may be suspicious. Thin-layer chromatographic results are not affected (2).

pH and ionic strength play a definite role in the mechanism of enzymatic reactions (3), a role that becomes more complex in the case of EMIT (4). The effect we report here is probably attributable to an increase in ionic strength to above a critical point, at which so many ions congregate at one or more charged sites that they prevent the necessary interactions. If so, the effect is nonspecific and we would expect any salt solution that contributes a high ionic strength to work in a similar manner.

References

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Thin-Layer Chromatographic Detection of Quinine, Morphine, and Poly-Drugs

To the Editor:
We read with great interest the letter [Clinical Chemistry 22, 393 (1976)] by Wilkinson et al. in which they discussed the findings of a service laboratory that had mistakenly reported the presence of morphine and cocaine in an individual’s urine. We believe that the authors’ point with regard to the use of more than one analytical procedure for confirming positive results was a valid one. Another article, by McIntyre and Armand, which appeared in the same issue, discussed their ability to detect free morphine at a sensitivity of at least 0.5 mg/liter.

We wish to call the attention of readers of this journal to the thin-layer chromatographic technique used in this laboratory. It is capable of detecting free morphine in a concentration of 100–190 µg/liter of urine. It is used to analyze 3000 urine specimens per week for opiates (morphine, codeine, methadone, and quinine), and more than 1000 specimens for poly-drugs (15 drugs—opiates plus amphetamine, methamphetamine, phentramine, phentazamine, methylphenidate, phenothiazines, sedatives, and hypnotics). The technique has been described elsewhere (1, 2). The following modifications have been introduced: (a) urine containing ion-paper is shaken for 20–30 min, (b) ratio of chloroform and isopropanol used is 5:2, and (c) drugs are extracted by shaking for 20 min. The sensitivity of this ion-exchange paper technique was described at the Sixth International Congress of Pharmacology (3).

The use of this single-step extraction and two-stage thin-layer development system enables us to measure the entire array of drugs of abuse in urine concomitantly in the following minimum concentrations (mg/liter, expressed as base): morphine, 0.1 (volume of urine, 50 ml) and 0.15–0.19 (volume urine, 20 ml); amphetamine, 0.87; methamphetamine, 0.4; phentramine, 0.41; methylphenidate, 0.87; codeine, 0.35; methadone, 0.45–0.9; phenobarbitol, 0.5; seconobarbital, 0.36; propoxyphene, 0.90; and cocaine, 0.89. The volume of urine required for these sensitivities is 20–50 ml.

We recommend that positive results so obtained for barbiturates be confirmed by resubmitting the residue and developing in another solvent. A technician can analyze 120 urine specimens for opiates and 80–90 specimens for poly-drugs per day. The cost of analysis for performing at least 4–5 tests (opiates) per urine specimen is $0.58 and for performing 9–15 tests (poly-drugs) is $0.82 per specimen (4), including labor, chemicals, and supplies. Our current total cost of analysis, including supervisory and administrative salaries (one chief toxicologist, one laboratory manager, one chief chemist), chemicals and supplies, laboratory rental, technical and support services, is $1.38 per specimen for monitoring 3500–4000 specimens per week. Set-up costs of a toxicology laboratory facility with thin-layer chromatography and various detection procedures currently used in drug-abuse screening programs are discussed elsewhere (5, 6).

References