the total urinary estrogens in pregnancy, such interference is acceptable.

We evaluated within-assay precision from duplicate assays, and reproducibility by assaying the same samples on different days (Table 1). We performed 524 assays of specimens from 122 normal pregnant women in the 20th to 42nd week of gestation. Table 2 summarizes our results.

We compared estriol values obtained by the present method and by a reference gas-chromatographic method (5). The correlation was highly significant \( n = 115; r = 0.989; y = 0.11 + 1.09 x \) and the fluorometric results \((y-axis)\) were slightly higher, probably because of the larger number of extraction steps in the chromatographic method.

Acid hydrolysis and fluorescence reaction at 120 °C permit a drastic shortening of analysis time as compared with methods done at lower temperatures (Figures 1 and 2). Moreover, all steps up to fluorometry are performed in the same test tube, eliminating losses caused by transfers and minimizing contamination from glassware. Fluorescence is measured after a simple dilution, avoiding time-consuming extraction steps with organic solvents.

The Kober reaction product is stable for at least an hour at 0 °C before the fluorogenic reagent has been added, but, starting with this step, fluorescence remains constant for only about 20 min, then begins to decline, even at 0 °C but more rapidly at room temperature.

Our method appears to provide reliable responses in less than 1 h, and a well-trained technician can easily process 30 samples in duplicate in half a working day. Only easily available equipment and low-cost, non-hazardous reagents are involved.

References

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Urinary Total Estrogen Methodology for Pregnancy Urine

To the Editor:

We have several comments concerning the Letter appearing in Clinical Chemistry 24, 2217 (1978) by Yang et al., who compare two techniques for extraction of the Kober chromatogram during determination of total pregnancy estrogens (TPE) in urine. In their preferred technique tetrachloroethane containing trichloroacetic acid (TCA) is used, whereas the Stanbio Laboratory (1) adaptation of the method of Rouke et al. (2) involves addition of aqueous TCA followed by extraction of the color into chloroform.

Although the Royal Columbian Hospital group's suggested solvent combination does, in our hands, increase absorbance values by 25–30%, the corrected absorbance gives the same final answer. Never in the many years of our experience with repeat assays has there been a difference approaching 70%, as suggested for the aqueous TCA–chloroform form. In our combined experience of almost 50 000 assays we have recorded a between-assay coefficient of variation of 5.75% as determined by our on-going computer program. This agrees with our previously published data (3). Their grossly high difference must have involved such low urinary TPE concentrations that the slightest deviation in technique would result in a vast percent discrepancy. If in fact higher estrogen concentrations were found also to differ by as much as 70% between-runs, this would further suggest erratic technique or general inexperience with the method.

Because the clinical value of urinary TPE assays depends on the trend of a series of results, it is of utmost importance that consistency of technique be maintained. We believe that this may be best accomplished by using the results of a series of urinary estrogen (E) and creatinine (C) determinations, each performed on single voided specimens, and reporting the E/C ratios. This precludes the inconvenience of 24-h urine collection and gives information more promptly to the requesting physician (4).

Yang et al acknowledge tetrachloroethane to be more toxic than chloroform, but they imply that because of vapor-pressure differences the exposure to hazardous concentrations of the former is less. In all fairness to laboratories that may be influenced to use tetrachloroethane, we believe that reference to three toxicity statements must be made:

a. The Merck Index (5)—tetrachloroethane “is considered to be the most toxic of the common chlorinated hydrocarbons.”

b. Manufacturing Chemists Assn. (MCA) warning label (6)—tetrachloroethane is “highly toxic by ingestion, inhalation and skin absorption; tolerance, 5 ppm in air.”

c. NFPA Instant Warning System for Dangerous Materials (7,8)—tetrachloroethane: 4.0-0.0 (Health-Flammability-Reactivity; 4: can cause death or major injury despite medical treatment; 0: will not burn; 0: normally stable, not reactive with water).

In light of the above considerations, we think that with proper attention to technique and laboratory safety, urinary total pregnancy estrogens are most effectively determined by the method of Rouke et al. (2) or the Stanbio Laboratory adaptation (1).

References
1. Stanbio TPE Total Pregnancy Estrogen


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The authors of the Letter in question respond:

To the Editor:

Safety in the laboratory is a prime concern for all workers, and we appreciate this re-emphasis of our comment regarding the toxicity of tetrachloroethane. Concern regarding the toxicity of a compound must be kept in the context of its use, however, and a bald statement of the toxicity of this organic solvent can be misleading. Risks in the use of tetrachloroethane in estril determination are related chiefly to inhalation exposure. We use dispensers to apportion solvents in our laboratory, and certainly mouth pipetting is not condoned in any laboratory that gives attention to proper technique and laboratory safety. Both chloroform and tetrachloroethane are given a "3" (high) rating for inhalation exposure in the toxic hazard rating code used in the 1975 edition of Dangerous Properties of Industrial Materials (N. Irving Sax, Ed.), this rating meaning "may cause death or permanent injury after very short exposure to small quantities."

Thus the hazards of chloroform should not be ignored and we refer the reader's attention also to the 1976 edition of The Merck Index, which notes the carcinogenicity of chloroform in animals, its toxicity for humans, and the limitations placed on its use by the FDA. Regarding tetrachloroethane, it notes that, "its narcotic action is stronger than that of chloroform, but because of its low volatility, narcosis is less severe and much less common in industrial poisoning than in the case of other chlorinated HC." Thus the lower vapor pressure of tetrachloroethane than of chloroform is not insignificant, and we reiterate that there is less inhalation exposure to hazardous material as used in this test than with chloroform. Nonetheless, we emphasize that, with either material, work should be done in a fume hood.

Like Huang et al., we record a CV of about 5% by our ongoing computer system for quality-control specimens in which chloroform is used; with tetrachloroethane the CV is smaller.

The point that we wish to emphasize and perhaps did not make sufficiently clear in our Letter, and which prompted our investigation in the first place, was not our daily quality-control results, however, but repeat measurements of estrogen concentrations, which differed in occasional patients' specimens by as much as ±70%. These variations were seen for all estril concentrations, by both our most experienced and our less-experienced technologists. They were detected only if all patients' specimens were analyzed in two separate runs, a procedure we routinely used with chloroform extraction because of the potential seriousness of reporting or failing to report a significant decline in estril excretion in a series of results for a patient. (Within-run duplicate assays never showed this discrepancy.) Since we began using trichloroacetic acid in tetrachloroethane as we recommend, this problem has been eliminated. Kober chromatogen extraction is more complete and the color is much more stable. We have described this modification, not to deprecate the original method, but to point out and provide a solution for the serious problem of occasional spurious, potentially dangerous results, which can be generated and go undetected in individual patient's specimens, even with careful use of quality-control programs.

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Enzymatic vs. Jaffé (Continuous-Flow) Assay of Creatinine in Serum

To the Editor:

Many methods for assay of creatinine are based on the classic alkaline picrate technique of Jaffé; such methods may be subject to interference (1) and thus specific enzymatic approaches have been attempted.

Several such methods are theoretically possible. End-point (2, 3) and kinetic (4) assays involving creatinase (EC 3.5.2.10) have been described that are based on measurement of the resulting creatine by a series of coupled reactions catalyzed by creatine kinase (EC 2.7.3.2), pyruvate kinase (EC 2.7.1.40), and lactate dehydrogenase (EC 1.1.1.27). Because 1 mol of NAD+ is generated per mole of creatinine, the decrease in absorbance at 340 nm is proportional to the concentration of creatinine. It has been suggested that such assays have acceptable precision and, because results are lower than those by alkaline picrate methods, an analytical recovery of creatinine is good, that they have good accuracy. However, the many reagents required may make such assays impracticable for routine use.

Recently, reagents for this assay have become available in a kit set (Test-Combination Creatinine Enzymatic, Boehringer Mannheim GmbH), and we have investigated the use of the kit in the routine clinical chemistry laboratory. The evaluation protocol was based upon previously published recommendations (5–7) and on our own experience. The kits were from Boehringer Mannheim Australia Pty. Ltd., Mount Waverly, Vic. 3149, Australia.

Manual analyses were done exactly as described by the supplier of the reagent set. Absorbances were measured in 1-cm cuvettes, with a Model 300T-1 spectrophotometer (Gilford Instrument Labs. Inc., Oberlin, OH 44074).

The comparison method in this study was continuous-flow analysis with an SMA 6/60 (Technicon Instruments Corp., Tarrytown, NY 10591) by the alkaline picrate technique (Technicon Method SF4-0011FH4).

Creatinine (SRM 914) for use as a standard was obtained from National Bureau of Standards, Washington, DC 20234.

Precision. Day-to-day precision was determined by assaying three different concentrations of pooled, reconstituted (lyophilized) quality-control materials once a day for 20 days; within-run precision was determined by assaying the same three sera 10 times as a single batch on each of three days. The sera were stored in aliquots at −20 °C and thawed as required. The results are shown in Table 1. The poor precision