Urinary 5-Hydroxyindoleacetic Acid by HPLC with Electrochemical Detection

To the Editor:

We note recent claims by Davidson and Williamson (1) for the improved assay of urinary 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection. We are familiar with a very similar method, which also involves protein precipitation and has a comparable mobile phase (2). In our experience, precipitation of proteins from urine with ethanol was not always successful but was time consuming, and back-extraction of 5-HIAA into water was found to be necessary to avoid peak broadening. These additional steps introduced further pipetting stages, with the possible loss of sensitivity and recovery, and they increased analysis time. To compensate for any loss in sensitivity a higher detector electrode potential may be used, but this increases the risk of interfering peaks, e.g., possible paracetamol (acetaminophen) metabolites (1). The necessity for protein precipitation is questionable. Three studies (3–5) have reported minimal loss of column efficiency, even after many injections of undiluted (3, 4) and diluted (5) urine, a finding that we have subsequently confirmed—even without the use of a guard column.

We cannot comment on Davidson and Williamson’s claim for an improved assay, because no reference was made to previously published methods. However, we acknowledge that their method is superior to the colorimetric methods, which are time consuming, insensitive, and prone to drug interference.

We suggest that elaborate sample preparation (1, 2) is unnecessary and offers no improvement over previously reported methods (3–5). In addition, the mobile phase used by Davidson and Williamson does not improve resolution and has the disadvantage of increased retention times.

We report here a method that involves little sample preparation and faster chromatography, and in our experience is unaffected by endogenous substances, drugs, or metabolites. In one case of massive paracetamol overdose (560 mg/L at 4 h) we observed a peak co-eluting with the internal standard. A Varian 5010 liquid chromatograph was used with a Severn Analytical Model 5100A electrochemical detector. The detector potential of 0.4 V was considerably lower than that used in previous methods (1–3, 5) and may explain why we have not observed any interference. The analytical column was packed with Spherisorb S5 ODS 2 (Phase Sep, Queensferry, Clwyd, U.K.) and eluted with phosphate buffer (pH 2.3, 50 mmol/L)ethanol/isopropanol (100/102 by vol) at a flow rate of 1.0 mL/min. The stock internal standard contained 50 mg of 5-hydroxyindole-2-carboxylic acid in 100 mL of ethanol. A working internal standard was prepared by diluting 1 mL of this stock to 100 mL with a 0.1 mol/L solution of HCl. The working standard contained 100 µmol of 5-HIAA per liter of 0.1 mol/L HCl. We added 200 µL of standard, control, or patient’s sample to 1 mL of working internal standard, mixed, and injected 10 µL into the chromatograph. Figure 1 shows a chromatogram of a normal patient’s urine (5-HIAA = 9 µmol/L) and of that from a patient with carcinoid syndrome (5-HIAA = 108 µmol/L).

The standard curve is linear up to at least 1200 µmol/L. Our mean analytical recovery was 98.6%. Within-batch CVs were 3.2% and 5.1% for concentrations of 30 and 185 µmol/L, respectively. Between-batch CVs were 3.3% and 10.8% for concentrations of 23 and 132 µmol/L, respectively.

Six patients’ samples with appropriate standards and controls can be analyzed in 1 h. The method is simple to operate, sensitive, specific, and economical. It has been used routinely in our laboratory for the past year.

References

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Prostate-Specific Antigen and the Female Prostate

To the Editor:

Having had the opportunity to immunologically identify human prostate tissue-specific antigens (1, 2), one as prostate tissue-specific acid phosphatase (PSAP) and the second as prostate tissue-specific antigen, currently referred to as prostate antigen or prostate-specific antigen (PSA) (3), I read the recent report (4) and follow-up communications (5) on PSA in the journal with considerable interest.

In these reports (4, 5), concern has been raised over detection of PSA in normal women, in some cases relatively high values, e.g., up to 0.8 g/L (4). These investigators have suggested reasons for the identification of PSA in women, ranging from a discrepancy of nonspecific binding in the radioimmunoaassay (4, 5) to "... artifacts produced by PSA antibodies in their normal female subjects" (5) as perhaps, a consequence of the formation of "... antibodies in some women in the same manner that antibodies to sperm are produced" (5). Although such reasoning may not be completely untenable, I hasten to direct these investigators’ attention to the biological fact that women have a prostate gland (6) (also known as paraurethral and (or) Skene’s glands, the latter after Alexander Skene, who in 1850 (7) extended and redescribed the early observations of de Graaf, made in 1672 (6)). So women may have PSA in their circula-
tion, as is the case in normal men.

In addition to demonstrable PSA (8), the female prostate also contains PSAP (8, 9). In this regard, consider as a caveat the forensic implications of prior alleged cases of rape, wherein, in the absence of knowledge of the female prostate, and the presence of PSAP (or for that matter, now also PSA) in the female ejaculatory fluid, the identification in vaginal secretions of a marker heretofore considered male specific may have been "... a fait accompli" (10).

For further particulars on the female prostate, the reader is referred to the detailed reports of Huffman on anatomy (11) and clinical significance (12).

References

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Strategy for Diagnosis of Monoclonal Gammopathies in Serum

To the Editor:
Keren et al. (1) describe an approach for the detection and characterization of serum paraproteins by high-resolution electrophoresis (HRE) on agarose gel together with quantification of immunoglobulin heavy and light chains as an alternative to immunofixation electrophoresis (IEP) or immunofixation electrophoresis (IFE) in some cases.

Although it clearly is effective when a serum contains a large amount of monoclonal immunoglobulin, their approach has the following serious limitations:
1. It will not detect the simultaneous presence of both intact immunoglobulin and free monoclonal light chain in the serum.
2. Of the sera they examined, 32 (9.5% of the total screened) contained a visible band on electrophoresis but had a normal kappa/lambda ratio, whereas nine samples had an abnormal kappa/lambda ratio but no detectable monoclonal band, for a false-positive rate of 22% and a false-negative rate of 78%. Evidently the kappa/lambda ratio performs very poorly as a screening procedure for small paraproteins (see below), and its usefulness as an adjunct to HRE or as an alternative to IFE is thus questionable.
3. About two-thirds of patients with paraproteinemia have very small monoclonal immunoglobulin bands (<5 g/L) (2, unpublished results), which would therefore probably not be detected by the kappa/lambda ratio. The presence of such small paraproteins is often not suspected on clinical grounds, so use of the kappa/lambda ratio instead of IFE as a screening procedure would result in many cases being missed.
4. Many laboratories use radial immunodiffusion rather than nephelometry or turbidimetry for immunoglobulin quantification. To my knowledge, there is no RID method for light-chain measurement, and in any case the imprecision of RID would almost certainly preclude its use for this purpose.

A different approach to the judicious use of IFE, illustrated in Figure 1, has been described previously (2, 3). Clearly, IFE need not be performed on previously characterized paraproteins unless the electrophoretic pattern undergoes a significant change over time. Only the laboratory staff may order IFE, taking into account the account the clinical diagnosis as well as the electrophoretic findings. For samples with a suspicious abnormality on electrophoresis (e.g., an isolated increase in transferrin, hypogammaglobulinemia), or to rule out paraproteinemia in certain clinical situations (e.g., lymphoma, polynuropathy), I perform a screening IFE, using antisera against kappa and lambda only, to minimize costs. Using these criteria, IFE would definitely have been performed on at least one of the three cases reported as false negative by HRE, and probably also on the other two, if the transferrin or C3 had appeared increased because of the IgA paraproteins co-migrating with them.

Immunoochemical measurement of immunoglobulin heavy- and light-chain isotypes before IFE is unnecessary. As reported previously (2), adopting a single set of serum dilutions (1:10 for IgG, 1:5 for IgA, IgM, and kappa, and 1:2 for lambda) suffices to characterize practically all paraproteins that are visible on HRE. For those that are not visible on HRE, the concentration of the anomalous immunoglobulin is likely to be within a range (~0.1–5.0 g/L) that does not necessitate other dilutions of the serum, again making prior quantification redundant.

The poor sensitivity of the light-chain ratio for detecting small paraproteins more than offsets the slight decrease in turnaround time (for a non-urgent procedure) and in cost for a majority of samples. The available data support the position that appropriate, cost-effective use of IFE requires only critical inspection of HRE patterns in relation to the clinical situation of the patient.

References
1. Keren DF, Warren JS, Lowe JB. Strategy to diagnose monoclonal gammopathies