ed three days before surgery, the day of surgery, and a day after surgery were examined, no hCG was detected in any with Assays B, F, or G. Taken together, these observations indicate that the positive Bio-RIA assay results were aberrant.

As shown above, when discordant results occur, they usually have the following characteristics: (a) the positive test (e.g., RIA) usually gives a low value (<200 int. units/L and usually <30 int. units/L); (b) the positive result seems inconsistent with clinical information (e.g., no evidence of pregnancy, trophoblastic disease, or tumor); (c) the sequential hCG titer does not change with time in a given procedure; and (d) the result is negative in at least one alternative quantitative test for hCG.

When a low-positive result is obtained for a serum and the low-positive result does not seem consistent with the clinical information on the patient, the possibility of discordant results should be further tested, either by analysis in an alternative procedure, by sequential hCG analysis, or both. Although many alternative hCG detection procedures with different antibodies to hCG are available from which to choose, we recommend using a method that also involves a different technology than the initial procedure. This strategy minimizes the possibility of non-hCG interference (e.g., hyperproteinemia, hyperlipidemia, autoantibodies, DNA, etc.) in both assays. For example, if one procedure is an RIA with polyethylene glycol and (or) second antibody as precipitation agent, we recommend a solid-phase RIA or an IEMA (e.g., Assay G) as the other procedure.

In our experience, when a low-positive hCG value is obtained for a serum by RIA, but no hCG is detectable by at least one alternative procedure (e.g., Assay G), both pregnancy (ectopic or intrauterine) and gestational trophoblastic disease can be ruled out, and the clinical treatment program should be designed accordingly. This usually means conservative clinical follow-up and monitoring the serum hCG at two-to-three-week intervals. Similarly, if sequential analysis (of low hCG titers) fails to show an increase of at least 66% in hCG titer in two days, a normal intrauterine pregnancy can be ruled out (8, 9). If the patient is a male with a testicular tumor that secretes free β-subunit of hCG but not complete hCG (I, 2), the use of alternative procedures of differing and complementary specificity (e.g., Assay G along with RIA) would still permit detection of the free β-subunit.

References

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Heparinized “Microtainer” Tube Evaluated for Collection of Capillary Blood

To the Editor:

Our Children’s Hospital has long used the Caraway tube containing heparin anticoagulant in collection of capillary blood for clinical chemical analyses. Although with Caraway tubes collection of small samples (350 μL) is convenient and inexpensive, their use is time-consuming, and the need to break the tubes to remove the plasma increases the risk of transmission of blood-borne infections. The advent of Microtainer Tubes (Becton-Dickinson, Rutherford, NJ) containing heparin prompted us to compare this new product with the Caraway tubes, to determine the accuracy for eight common chemistry tests, the yield of plasma, the incidence of hemolysis, and the ease of collection and handling of specimens.

Blood samples were collected from patients, ages one day to 19 years, admitted to St. Louis Children’s Hospital or Barnes Hospital newborn nursery. Blood was collected from either the heel or finger tips by a standard skin puncture technique (1, 2). Approximately 1.5 mL of blood was obtained from each participant from a single skin puncture, divided between three Caraway tubes and one Microtainer Tube with a randomized order of filling. The yield of plasma was determined by weighing all tubes when empty, after filling, and after removal of plasma. Caraway tubes were centrifuged at 1000 × g for 5 min, and the plasma was removed by scooping and breaking the tubes at the plasma–cell interface. Microtainer Tubes were centrifuged in an Eppendorf Microfuge for 5 min. Plasma samples from all tubes were decanted into 12 × 75 mm plastic conical tubes before analysis. We measured eight common chemistry analytes as follows: sodium and potassium by flame photometry, chloride and total carbonates by a Beckman CO2 analyzer, urea nitrogen and glucose in blood by the Beckman Analyzers for each, total bilirubin by a manual Jendrassik–Gröf procedure, and creatinine by a kinetic Jaffe method with a Gilford 3500 spectrophotometer. Visual inspection for hemolysis was augmented by testing with Hemoccult slides (SmithKline Diagnostics), which were sensitive to as little as 7.8 mg of hemoglobin per liter.

To assess the results of the comparative chemistry analyses, we computed the means and standard deviations for each analyte for each container and tested the paired data for significant differences by the Student’s t-test (Table 1). Slopes, intercepts, and correlation coefficients were also computed for each analyte. Only differences in sodium and total CO2 were statistically significant (p < 0.05). The mean value for sodium in the Caraway samples was 138 mmol/L vs 136 mmol/L for the Microtainer samples, with a slope of 1.05 and a correlation coefficient (over the small range of sodium values measured) of 0.889. We judged that the statistically significant difference in sodium was not clinically significant. From additional experiments examining the cause of the differences in total CO2 (about 2 mmol/L), we concluded that the lower values obtained in Microtainer samples were due to greater loss of CO2 to the atmosphere, during the extended period (up to 6 h) of storage and analysis in this multi-analysis protocol. The disparity was not reproducible in subsequent experiments over shorter time periods (<4 h).

The incidence of semiquantitative (42% and 52%) and visible (18% and 20%) hemolysis in Microtainer and Caraway tubes, respectively (Table 2), was higher than reported elsewhere for samples of capillary blood (3). However,
er, most of the patients in the hemoly-
sis study were less than 13 days old, and the protocol required blood col-
collection for the study be from one skin
puncture—both factors known to in-
fluence the increase in hemolysis in
blood collection by skin puncture (3).
Samples from patients older than 13
days, in whom blood was easily ob-
tained from one skin puncture, had a
5% incidence of semiquantitative
hemolysis for both types of tubes. Plasma
yield averaged 51% with each contain-
er.

Handling time for samples obtained
with Microtainer Tubes was signifi-
cantly reduced as a result of (a) the
avoidance of the scoring and breaking
procedure required for Caraway-tube
specimens; (b) the larger volume of the
Microtainer Tube (equivalent to two
Caraway tubes), which eliminated
handling of extra tubes on many sam-
ples; and (c) the short centrifugation
time in the Microfuge to separate plas-
ma from cells in the Microtainer Tubes
[A brief study showed that normal
specimens in Microtainer tubes re-
quired as little as 1 min of centri-
fugation time for effective separation (data
not shown)].

In summary, we found Microtainer
Tubes to be generally as effective as
Caraway tubes in providing accurate
chemistry results, in allowing recovery
of plasma, and in preserving the cellu-
lar integrity of the samples. Many lab-
oratories may find the savings in han-
dling time and the decreased possibili-
ty of blood-borne infection with
Microtainer Tubes to be persuasive fac-
tors in selecting a capillary blood-col-
collecting container.

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Use of Magnetic Circular Dichroism
in the Diagnosis of Porphyria

To the Editor:

Detection and accurate diagnosis of
the porphyrias continue to be bedeviled
by confusion and controversy for many
reasons, including their comparative
rarity and their variability in both
clinical and biochemical expression.
Qualitative and semiquantitative
screening methods provide time-saving
support for the more nearly accurate
and informative porphyrin and enzyme
assays (1). Most current screening
methods, which have been developed
to investigate urines only (2–4), are inad-
equate if, as usually the case, they
are used alone. Failure to screen fecal
porphyrins may have serious, even fa-
tal, consequences (5). Thus the screen-
ing of fecal porphyrins is essential,
especially for detection of the large but
as-yet-unknown proportion of asym-
ptomatic cases. A recent paper on this
subject (6) contains serious flaws and,
in my opinion, merely adds to the
confusion. Although I collaborated in the
research project, I withdrew as coau-
thor of the article because of dis-
agreements with the draft submitted to
Clinical Chemistry, which I wish to
discuss here.

Some of the flaws are common to both the
urinary and fecal data, but I shall con-
centrate on the latter because the
fecal screening method, if valid,
would represent the most innovative
aspect of the article.

Firstly, no standard curves have been
included for mesoporphyrin and
deuterporphyrin, both of which fre-
quently constitute substantial frac-
tions of normal fecal porphyrins, or for
the isocoproporphyrin fractions, which
often predominate in the feces of sub-
jects with porphyria cutanea tarda,
arguably the most common of the
porphyrias (1). With this in mind, the
claim that "the technique involving
MCD [magnetic circular dichroism] is capa-
ble of identifying the major por-
phyrin in the urine or feces" is unsub-
stantiated, because λmax and λmin val-
ues for these other porphyrins are lack-
ing, and any shift in these wavelengths
produced by the various mixtures of
porphyrins commonly found in normal
and porphyrin excreta has not been
measured or adequately discussed.

It is stated that "Figure 5 shows that
about 65% of the porphyrins are ex-
tracted from Dean's solution . . . into
HCl. . . . " The word "about" may allude
to the substantial errors to be expected
when the extraction procedure de-
scribed is used, a procedure not de-
veloped for quantitative purposes, yet nei-
ther error bars nor any hard data are
provided in the Figure (as also is the
case in Figure 4) and no standard
deviations are provided in the text. The
"Procedures" section indicates that the
standards were made up by adding
porphyrins to fecal samples before ex-
traction, a poor substitute for measur-
ing the efficiency with which Dean's
solution extracts the porphyrins that are
naturally present in feces. In my
experience and that of others (5), this
may vary markedly, depending mainly
on the water, lipid, total porphyrin,
and bacterial content of the particular
fecal sample. Further, I question how
(whether) the original porphyrin content of the excreta to
which the standard porphyrins were
added was either measured or allowed
for. Without full insight into the sub-
stantial errors involved in the stan-
dard curves, it is questionable that
they can be used for even semiquanti-
tative measurements, especially for di-
agnostic purposes. Yet this is what the
authors recommend, albeit with the
suggestion that the acceptable upper
limit of the normal range should be
reduced by 25% to "<150 nmollg dry
weight of feces." The inadequacy of

Table 1. Comparison of Values for Eight Common Chemistry Tests in Samples Collected in Microtainer Tubes (MT) with Samples Collected in Caraway Tubes (CT)

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean ± SD, mmol/L</th>
<th>MT values vs CT values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>138 ± 5</td>
<td>136 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slope: 1.05 Intercep: -5</td>
</tr>
<tr>
<td>K</td>
<td>4.9 ± 0.9</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 0.80 p: 0.834</td>
</tr>
<tr>
<td>CO₂</td>
<td>22.5 ± 6.7</td>
<td>20.5 ± 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 0.98 p: 0.970</td>
</tr>
<tr>
<td>Cl</td>
<td>106 ± 9</td>
<td>106 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 0.98 p: 0.960</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.7 ± 0.9</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 0.96 p: 0.897</td>
</tr>
<tr>
<td>Urea N</td>
<td>3.5 ± 3.2</td>
<td>3.5 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 1.01 p: 0.996</td>
</tr>
<tr>
<td>Tot. bili.</td>
<td>0.16 ± 0.07</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 1.00 p: 0.995</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.053 ± 0.027</td>
<td>0.053 ± 0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r: 0.99 p: 0.920</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the Incidence of Hemolysis and Plasma Yield in Samples Collected in Microtainer Tubes (MT) with those Collected in Caraway Tubes (CT)*

<table>
<thead>
<tr>
<th>MT</th>
<th>CT</th>
<th>Differences not significant between the two kinds of tubes at p &gt; 0.7, &gt;0.1, and &gt;0.6, respectively.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible hemolysis 15% (11/60) 20% (12/60)</td>
<td>18% (11/60) 20% (12/60)</td>
<td></td>
</tr>
<tr>
<td>Test-positive 42% (25/60) 52% (31/60)</td>
<td>42% (25/60) 52% (31/60)</td>
<td></td>
</tr>
<tr>
<td>Plasma yield 51% ± 12% 51% ± 11%</td>
<td>51% ± 12% 51% ± 11%</td>
<td></td>
</tr>
</tbody>
</table>

* Differences not significant between the two kinds of tubes at p > 0.7, >0.1, and >0.6, respectively.