Erythrosine B, Tartrazine, Lissamine Green B, Pyronin G, Acetyl
Yellow G, Ponceau 6R, Ponceau SX, Food Orange #2, and
Food Yellow #3. The concentration of dye tested was 50 mg/
L of incubation mixture (25 μg/tube) in RIA systems for
human lutropin (hLH), its alpha and beta subunits, rat LH,
rat corticotropin (rACTH), rat insulin, and human thyroxin
(T4) and testosterone. (For T4 we used the MAGIC-T4 RIA-
kit from Corning, Medfield, MA 02052.) The testosterone
RIA involved 3H-tracer and dextran-coated charcoal separa-
tion; all others had 125I-labeled tracers and double-antibody
solid-phase separation.

All dyes gave strongly colored incubation mixtures at 50
mg/L; most were clearly visible at 10–20 mg/L.

For most of the RIA systems, most of dyes could be added
to tracer and (or) antibody without substantial effects.
Erythrosine B, however, affected not only the initial binding
but also the nonspecific binding in most of the systems. Also,
Lissamine Green B and Pyronin G decreased the initial
binding in the testosterone RIA, and Food Orange #2 did
the same in the RIA of insulin.

In our laboratory we successfully use Pyronin G (10 μg/
tube) as an additive for the tracers, in combination with
other dyes for the antisera.

Non-Effect of Uremia on Analytical Recovery of
Aluminum in Serum, Donald J. Mikkelsen and Elizabeth
A. Windleborn (Dept. of Biochem., Waikato Public
Hospital, Private Bag, Hamilton, New Zealand)

The difficulties imposed by the serum matrix on Al
determinations by graphite-furnace atomic absorption spec-
trophotometry are well known (1, 2). That components of
uremic serum might cause a difference in analytical recov-
er of Al from that obtained for normal serum is of great
concern.

Attempting to duplicate the effect described by Brown et
al. (3), we used a Model 560 atomic absorption spectropho-
tometer with a Model 400 heated graphite analyser, an
AS 40 autosampler, and a Model 024 chart recorder (all from
Perkin-Elmer Corp., Norwalk, CT 06856). Pyrolytically
coated tubes and L'vov platforms were used for all analyses.

Samples of non-uremic and uremic serum (500 μL) were
mixed with 250 μL of a solution containing, per liter, 1.4 g of
Mg(NO3)2 and 10 mL of Triton X-100 surfactant, and 250 μL of
various Al standard solutions. We assayed these under the
instrument conditions described by Brown et al., but
reduced the step 3 temperature to 130 °C to eliminate
spattering during the drying step.

There were no significant differences in the recovery of Al
from uremic serum samples at 25, 50, or 100 μg/L final
concentration of added Al, the mean analytical recovery
(and range, n = 8) being 97% (93–112%), 100% (96–103%),
and 97% (96–99%), respectively, expressed as a percentage
of the recovery obtained for normal serum assayed by the
same technique. The mean (and range) of endogenous Al
present in the normal and uremic sera was 8 (6–12) and 30
(18–41) μg/L, respectively. The uremic samples were all
obtained from hemodialysis patients.

The mean (and range) of urea values obtained for our
uremic sera, in mmol/L, was 32.0 (17.0–45.0), the reference
range 3.5–7.5.

We conclude that it is not necessary to precipitate pro-
teins (3) or to extract the sample (4) for assays of Al in
uremic serum to be reliable.

References
1. Fuch W, Cedergren A, Cederbery C, Vesserman J. Evaluation of
some critical factors affecting determination of aluminum in blood,
plasma, or serum by electrothermal atomic absorption spectroscopy.
2. Parkinson IS, Ward MK, Kerr DNS. A method for the routine
determination of aluminum in serum and water by flameless
atomic absorption spectrometric determination of aluminum in
serum with a new technique for protein precipitation. Clin Chem
aluminum in body fluids by solvent extraction and atomic
absorption spectrometry with electrothermal atomisation. Clin

Assessing the Automatic Diamat Liquid Chromatograph
for Glycated Hemoglobin, S. Brenna,1 L. Prencipe,1 N.
Montalbetti,1 V. Perlangeli,1 and R. Palpou21 (Laboratory
of Clinical Biochemistry and Hematology, Ospedale Ca
Granda-Niguarda, 20162 Milan, Italy; 2 Bio-Rad Laboratories, Segrate, Milan, Italy)

We have explored the performance of the Bio-Rad Diamat
HPLC chromatograph in the assay of glycated hemoglobin.
The completely automated instrument carries a weak cation-
exchange resin column; the support is silica with a
carboxymethyl functional group.

For HbA1c, imprecision in the series (n = 30), expressed as
CV%, was 1.3% on a sample from a normal subject, and 0.5%
on a sample from a diabetic patient; imprecision between
series (n = 34) for similar samples was 3.7% and 2.3%,
respectively. Accuracy was assessed in different series by
correlating HbA1c values (y) with those obtained with a Bio-
Rad ion-exchange column at 28 °C (x). The regression line
equation was y = 1.034x – 0.41 (r = 0.944; n = 49).

The interferent HbF can be separated and quantified; the
assay results vary linearly with HbF up to 50% of total Hb.
HbF in excess of 3%, however, produces an apparent
decrease of HbA1C results. The instrument also reveals the
presence of HbS and calculates its peak area, and can
therefore be used for screening both HbF and HbS.

Glycated hemoglobin is eliminated by incubating the
hemolysate for 45 min at room temperature. HbA1c and
HbA1 readings are not significantly altered if sample
hematocrit is artificially changed from an initial 75% all the
way down to 15%. The reference intervals, determined from
105 normally distributed healthy subjects, are 4.4 to 6.0%
for HbA1c and 5.5 to 7.1% for HbA1.

Erythrocyte Binding of Theophylline, Determined by
CHU Timone, 13385 Marseille Cedex 5, France)

We previously reported erythrocytic binding of drugs (Therapie
1982;37:601–2) and noticed the importance of such a "tissular index" in drug monitoring. Because we commonly use the Emt system (Syva Co.) for monitoring the
concentrations of theophylline in plasma, we tried to deter-