Letters to the Editor should be typed double-spaced (including
references) with conventional mar-
gins. The overall length is limited to
five manuscript pages, including
not more than one figure or table.

Single-Reagent Automated Determination of Angiotensin
Converting Enzyme in Serum

To the Editor:

Increases of serum angiotensin con-
verting enzyme (ACE, EC 3.4.15.1; also
known as dipeptidyl carboxypeptidi-
dase, kininase II) have been associated
with active sarcoidosis and may reflect
stimulation of the monocyte–macrophage
system (1,2).

We adapted the method of Holm-
quist et al. (3) to a random access anal-
alyzer to obtain a simple, automated
assay for serum ACE that involves
only a single, stable reagent and 5 min of
analysis time.

3-[(2-Furylacryloyl)-l-phenylala-
nylglycyl-glycine (FAPGG) was pur-
chased from Bachem Feinchemica-
len AG, Bubendorf, Switzerland. "Lipo-
clean" (1,1,2-trichloro-1,2-trifluoroeth-
ane) was from Behringwerke AG, Mar-
burg, F.R.G. All other reagents were
analytical grade from Merck, Darm-
stadt, F.R.G.

Buffered substrate solution: In a
100.0-mL flask, mix the following (fi-
nal concentrations in parentheses):
1.192 g of 4-(2-hydroxyethyl)-l-pipera-
 sineethanesulfonate (HEPES) (50
mM/L), 1.753 g of NaCl (300
mM/L), and 50.0 mg of FAPGG (1.25
mM/L). Add distilled water and 250
μL of saturated NaOH solution. Bring
to pH 8.35 at room temperature and
store at 4 °C. Ronca-Testoni S (4), using
FAPGG with ACE, found a pH opti-
mum of 8.20 (pH measured at 37 °C,
identical to pH 8.35 at room tempera-
ture for a HEPES buffer solution).

The random access automated anal-
lyzer (RA-1000; Technicon Corp., Tarr-
rytown, NY) was used without modifi-
cation. The analyzer settings were:

<table>
<thead>
<tr>
<th>Percent sample vol, 60 (30 μL of sample)</th>
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<tbody>
<tr>
<td>Filter position, 340 nm</td>
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<tr>
<td>Delay, 60 s</td>
</tr>
<tr>
<td>Percent reagent vol, 54 (270 μL of reagent)</td>
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</tbody>
</table>

Using FAPGG as the chromophoric
monitor of substrate hydrolysis was a
simple way to measure ACE activity.
The λmax of FAPGG shifts from 305 nm
e = 25 000 mol⁻¹·L⁻¹·cm⁻¹ to 300 nm
upon hydrolysis, thus providing the
basis of this assay. At the instrument
settings listed above, the decrease in
absorbance is measured every 30 s
from 1 to 5 min. Measuring absorbance
at 340 nm, a wavelength readily avail-
able on automated analyzers, one can
work with high concentrations of sub-
strate, which is not possible at the
wavelengths of maximum absorptivity.

One unit of ACE activity is defined as
the amount of enzyme required to
hydrolyze 1 μmol of substrate per min-
ute. The activity concentration was
calculated as follows: U/L = (ΔA/min) × 20 400. The enzyme factor (20 400)
was derived from: 300/50 = the ratio of
total incubation volume to serum vol-
ume; 1/0.7 cm⁻¹ = 1/0 optical pathway;
1/(700 mol⁻¹·L⁻¹·cm⁻¹) = Δe¹, maxi-
imum ΔA (0.490) when hydrolysis was
allowed to go to completion; 10⁶ = factor
for converting mol/L (from
Beer’s law) into ACE activity units.
The maximum ΔA should be measured
for every new batch of substrate.

Serum samples were obtained from
97 bloodbank donors, ages 18 to 65
years. The blood was allowed to clot
at room temperature and centrifuged
within 2 h (2000 × g, 15 min). Lipemic
sera were emulsified with two volumes
of Lipoclean, then centrifuged at 2000
× g for 10 min. The mean activity
of these subjects was 55.5 U/L (SD 18.5
U/L).

To assess the reproducibility of the
method, we analyzed two serum sam-
ple—one with normal, one with
above-normal ACE activity. The re-
sults are summarized in Table 1.

The linearity of the enzymatic re-
action extended to >200 U/L, as dem-
onstrated by serial dilution of an ex-
tremely active serum sample with iso-
tonic saline: serum diluted eight-

time.

<table>
<thead>
<tr>
<th>Table 1. Precision Data</th>
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<tbody>
<tr>
<td>ACE acty, U/L</td>
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<tr>
<td>Mean</td>
</tr>
<tr>
<td>Within-day</td>
</tr>
<tr>
<td>55.8</td>
</tr>
<tr>
<td>200.7</td>
</tr>
<tr>
<td>Between day</td>
</tr>
<tr>
<td>54.1</td>
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<td>198.3</td>
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</table>

To the Editor:

α-Tocopherol acetate in the circula-
tion can decrease the vitamin E con-

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oidosis and Other Granulomatous Disorders, WB Saunders, Philadelphia, PA, 1985, pp
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Dirk Hendriks
Simon Scharpé
Marc van Sande
Jean-Pierre Vingerhoed

Units of Clin, Biochem. and Urology
Faculty of Medicine
University of Antwerp
B-2610 Wilrijk, Belgium
centration as calculated from "high-performance" liquid-chromatographic (HPLC) methods in which α-tocopheryl acetate is used as an internal standard.

We measured α-tocopheryl and α-tocopheryl acetate in the sera of five children who were receiving, for total parenteral nutrition, infusions containing supplemental α-tocopheryl acetate (1 g/L; MVI concentrate; Armour Pharmaceutical Co., Kankakee, IL) by the AACC Proposed Selected Method (1). We used retinyl acetate as the internal standard. Two of these five who had been receiving the vitamin supplement for several days had significant amounts of α-tocopheryl acetate in their sera. When α-tocopheryl acetate was used as internal standard, its combination with circulating α-tocopheryl acetate resulted in calculated values for α-tocopheryl in serum being as much as 50% lower than the actual concentration.

Thus we advise that, e.g., retinyl acetate (2) be used as an internal standard for α-tocopheryl assay when intravenous α-tocopheryl acetate is being administered.

We point out two additional sources of exogenous α-tocopheryl acetate: "MVI Pediatrics" (Armour), containing 1.4 g/L, and "E-Peral" (O'Neal, Jones and Feldman, St. Louis, MO), containing 24 g/L. Both are preparations for intravenous use. The latter was recalled recently after it was associated with infant mortality (3).

References

Charles P. Turley
M. A. Brewster
Arkansas Children's Hosp. and
Univ. for Med. Sciences
Little Rock, AR

George L. Catignani
North Carolina State Univ.
Raleigh, NC

Antibodies Interfering in Immunoassays

To the Editor:

In a recent Letter, Bock et al. (1) described an apparent false-positive result in an immunoenzymometric assay for human choriongonadotropin (Tandem-E HCG; Hybritech, San Diego, CA 92121), with positive units by addition of mouse serum.

We have found similar interferences in the Tandem-E thyrotropin (TSH) assay and in an in-house immunoassay for TSH (IRMA). A woman, aged 57 years, who was receiving therapy with lithium was referred for investigation of her thyroid function. Initial biochemical investigations showed a value for total thyroxin (T₄) in serum of 134 nmol/L (normal range 65–145 nmol/L), and a TSH value as measured by the Tandem-E assay of 46 milli-int. units/L (normal range 0.4–4.8 milli-int. units/L). After addition of non-immune mouse serum to give a final concentration of 20 mL/L re-assay, the TSH was 18.7 milli-int. units/L. Serum from this patient, pretreated with Sepharose-Protein A (Sigma Chemical Co., Poole, U.K.) to remove IgG, gave on re-assay a TSH value of 18.2 milli-int. units/L. We repeated these experiments, using an IRMA that includes two mouse monoclonal antibodies (2), and these findings were confirmed. These results suggest the presence in the patient’s serum of an antibody against mouse immunoglobulin as the cause of the interference.

In contrast, two other patients showed a similar interference in the IRMA but no effect in the Tandem-E assay. The first of these patients, a 76-year-old woman with no clear history or symptoms of thyroid disease, no detectable thyroid autoantibodies, and a normal value for total T₄, 114 nmol/L, was given a thyroliiberin test. The IRMA gave TSH values as follows: basal = 70 milli-int. units/L, 20 min = 80 milli-int. units/L, 60 min = 60 milli-int. units/L. On re-assay after addition of non-immune mouse serum these respective TSH values were 3.0, 7.0, and 8.0 milli-int. units/L. With the Tandem-E assay TSH values were: basal = 1.5 milli-int. units/L and 60 min = 6.3 milli-int. units/L. The second patient showed a similar interference. With the IRMA the initial TSH value was 25.8 milli-int. units/L; after addition of non-immune mouse serum the value was 1.5 milli-int. units/L (total T₄ = 122 nmol/L), but the Tandem-E TSH assay gave a normal value for TSH, 1.2 milli-int. units/L, with no change on addition of non-immune mouse serum.

There are several reports of antibodies in patients’ serum interfering in immunoassays, both radioimunoassays and immunoassays. Cusick et al. (3) described an antibody interference in an immunoassay for TSH involving one monoclonal and one polyclonal antibody; a similar interference in an alpha-fetoprotein immuno-

metric assay has been reported (4). Circulating antibodies to ruminant immunoglobulins of sufficient titer to cause positive bias in immunoassays in 7% of blood donors have been reported (4), although other studies suggest that these antibodies may not be so common (5). More recently, antibodies reacting with mouse monoclonal antibodies have been described (6).

There are few data available describing the specificity of these antibodies, but one might expect a considerable variation in the degree of interference, depending on the population and titer of antibodies in the patients’ serum and those used in the assay. Evidently the presence of such antibodies should be considered when measured TSH concentrations are not compatible with either the clinical history or other thyroid-function tests. The routine addition of non-immune mouse serum to immunoassay reagents would seem to be advisable.

References

P. M. Clark
P. R. Raggatt
C. P. Price
Dept. of Clin. Biochem.
Addenbrooke's Hospital
Cambridge, U.K.

Estimation of Low-Density Lipoprotein by the Friedewald Formula and by Electrophoresis Compared

To the Editor:

Low-density lipoprotein (LDL) may be the culprit responsible for deposi-