showed mean values close to the stated values (101, 105, and 95%, respectively), but the variability was very large with some control sera.

We compared results obtained for GGT, LD, AST, ALT, and alkaline phosphatase in patients' specimens with the Cobas-Bio (y), and with the CentrifiChem 400 (x). Respective correlations were: $y = 4.137 + 0.804x$ ($r = 0.997$), $y = 4.075 + 0.900x$ ($r = 0.988$), $y = -0.018 + 1.051x$ ($r = 0.998$), $y = 3.333 + 1.101x$ ($r = 0.999$), $y = -1.380 + 0.963x$ ($r = 0.998$).

For calcium and phosphorus results compared from the Cobas-Bio (y) and a Technician SMA 12/60 (x), the correlations were: $y = 0.496 + 0.809x$ ($r = 0.887$), $y = 0.035 + 1.020x$ ($r = 0.967$).

The results showed good agreement for all analytes except calcium and GGT. Correlations for calcium were poor. The slope is bad and the intercept unsatisfactory (0.5 mmol). The correlation coefficient is excellent for GGT, but the slope is such (0.8) that results are lower with the Cobas-Bio.

The engineering section of our laboratory evaluated mechanical and electrical safety and reliability of the Cobas-Bio. With the protective covers in position, there were no exposed electrical plugs or connections. Removal of the back covers requires a screwdriver, so no hazardous contact should occur accidentally. The only dangerous rotative device is the rotor, but it is fully protected by lowering a special cover whose accidental opening during a run immediately stops the motor. The sample and reagent probe arms and the sample turntable were considered good and safe features. The different mechanisms, controls, and electronics performed well and gave no trouble. However, we experienced some problems with the sampling needle, which frequently became obstructed and requires systematic cleaning as a normal maintenance procedure.

Overall we found the Cobas-Bio to be a practical analytical instrument. Points to be regularly checked are: the frequent clogging of the needle, and the optical quality and cleanness of the rotor cuvettes. We emphasize that, with a careful washing, the rotors can be reused, particularly for enzymic tests, largely improving the cost effectiveness.

We encountered problems in the phosphorus endpoint determinations, where the repeatability was not so good as provided by kinetic enzyme determinations; however, we obtained fine results for triglycerides, uric acid, and glucose (details not presented here).

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Minimizing Contamination of Specimens for Zinc Determination

To the Editor:

A blood-collection tube was recently marketed by Becton Dickinson (BD, no. 6527) especially for use in sampling blood for trace-element determination. We have used these tubes and conventional BD Vacutainer Tubes (no. 4710) to determine serum zinc concentrations in adults.

Blood was drawn from six healthy subjects into both types of tubes and stored at −20 °C. Samples were analyzed after 2 h, two days, and two weeks. We took care not to invert the tubes, because zinc in the rubber stopper has been shown to be the main source of contamination (*1*). Zinc was measured by flame atomic absorption spectrophotometry (Perkin-Elmer, Model 370).

Mean zinc concentration of samples drawn into conventional tubes (no. 4710) were respectively 1470, 1460, and 1460 μg/L after the three time intervals (Table 1). The mean zinc concentration of samples drawn into the special tubes (no. 6527) were 1060, 1040, and 1080 μg/L, which is significantly decreased ($p < 0.01$) in comparison to zinc concentrations in conventional tubes. In addition, blood zinc concentrations measured at two days and two weeks did not differ significantly from the 2-h values, indicating no further contamination occurred. Thus we confirm (*2*) that use of the special tubes for trace metal analysis does decrease the serum zinc contamination as compared to conventional stoppered tubes.

To determine if serum zinc concentration was correlated with contamination, we plotted zinc concentration in blood drawn into trace-element tubes (x) vs zinc concentrations in blood drawn into conventional tubes (y). A linear regression line ($y = -35.5 + 0.99x$) and a correlation coefficient of 0.92 ($p < 0.01$) was obtained. Evidently, zinc contamination does not vary with serum zinc concentration, but remains constant. Thus, when the extent of contamination is known and constant, a correction factor may perhaps be used to calculate actual serum zinc concentrations.

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Table 1.

Zinc Found in Blood from Six Persons, Drawn into Trace-Analysis Tubes (No. 6527) and Conventional Tubes (No. 4710)

	2-h storage		Two-day storage		Two-week storage	
	6527	4710	6527	4710	6527	4710
			μg/L			
	1070	1320	1070	1320	1130	1390
	1450	1760	1390	1830	1390	1760
	1010	1450	950	1450	950	1390
	1010	1510	1070	1510	1070	1450
	880	1320	820	1260	950	1320
	950	1450	940	1390	1010	1450
$\bar{x} \pm$ SEM	1060 ± 80	1470 ± 70	1040 ± 80	1460 ± 80	1080 ± 70	1460 ± 60
p value	<0.01		<0.01		<0.01	

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Fluorometric Determination of Tryptophan in Commercial Amino Acid Products

To the Editor:

We have modified and improved a fluorometric method (*1*) for determination of tryptophan and applied it to use with commercial products. In the original method trichloroacetic acid (TCA) was used, which is not highly desirable as an analytical reagent because of its instability on storage, difficulty in handling, cost, and its breakdown to yield carbon dioxide, which causes interference in automated methods (*2*). Tryptophan, by reaction with formaldehyde and ferric ions in acidic media, is converted to norharman, which fluoresces intensely at 445 nm.

The apparatus we used was an Amino-Bowman spectrofluorometer, Model J4-8860 (American Instrument Co., Silver Spring, MD 20910). The excitation and emission maxima of norharman are 370 and 445 nm, respectively. The excitation slit was set at 1 mm, the emission slit at 2 mm. All glassware was acid washed. All reagents used were analytical grade. L-Tryptophan (A grade; Calbiochem, San Diego, CA 92112) was used without further purification.

The following solutions were prepared: 2.0×10^{-4} mol/L tryptophan, 4.5×10^{-2} mol/L ferric chloride stock solution, 4.5×10^{-4} mol/L ferric chloride in 0.1 mol/L HCl, 4.5×10^{-4} mol/L ferric chloride in 120 g/L TCA, and 7.4 g/L formaldehyde. A tryptophan working solution was made by diluting the stock solution to 10^{-3} mol/L (~0.2 g/L). Solutions for the analytical working curve were prepared by using 0 to 175 μL of the working solution, in 25-μL increments.

To 0 to 175 μL of the tryptophan solution in a 10-mL volumetric flask, add 1.0 mL of the 7.4 g/L formaldehyde solution, and just before incubation add 3.0 mL of one of the ferric chloride solutions. Immediately place the flasks in either a 100 °C water bath or an oven at 100 °C for 1 h. (The reaction is temperature dependent and the temperature of the solution should be maintained as near 100 °C as possible.) Then dilute the samples to volume with distilled water, allow to cool to room temperature, and measure the fluorescence intensity at 445 nm, using an excitation wavelength of 370 nm.

Table 1 shows the relative fluorescence intensities of tryptophan and some related compounds after reaction to produce norharman. We determined that the optimal HCl concentration was 0.1 to 0.4 mol/L and that the emission and excitation maxima of norharman did not vary within this range. We tested several commercial amino acid products [Hepatamine® (experimental solution), FreAmine® II, and Nephramine® with claimed tryptophan concentrations of 0.66, 2.0 and 1.3 g/L, respectively; American McGraw, Irvine, CA 92714], using either HCl or TCA as the condensation agent. FreAmine and Hepatamine contain 15 amino acids, and Nephramine contains 10. With use of HCl, analytical recoveries were 98 to 103%, with TCA 91 to 103%. Coefficients of variation ranged from 1 to 2%. The linear range for the determination of tryptophan after reaction is from 5.23 pmol/L to 5.23 nmol/L ($r = 0.9996$). The use of HCl offers an advantage in reproducibility over TCA and obviates its disadvantages.

This method is more sensitive and reproducible than are other currently used methods of analysis for tryptophan. It can be used for analytical and stability analysis of tryptophan in a wide variety of solutions containing most other amino acids.

Table 1. Relative Fluorescence Intensity of Tryptophan and Some Possible Interferences

	Day	Condensation agent	Correlation (r^2)	Slope	y-intercept	Relative fluorescence intensity [a], %
Tryptophan	1	HCl	0.998	5.80	0.037	100
Tryptophan	1	TCA	0.998	5.49	−0.22	100
Tryptamine·HCl	1	HCl	0.994	2.76	0.25	48
Tryptamine·HCl	1	TCA	0.990	2.04	−0.049	37
Kynurenine	1	HCl	0.999	0.00887	0.014	1.5
Tryptophan	2	HCl	0.998	5.68	−0.17	100
N-Formylkynurenine	2	HCl	0.990	0.0878	0.019	1.5
5-Hydroxytryptophan	2	HCl	0.968	0.0701	−0.012	1.2
Phenylalanine	2	HCl	—	—	—	0 [b]

[a] Relative fluorescence intensity is defined as ($\text{slope}_{\text{interference}}/\text{slope}_{\text{tryptophan}}$) × 100, where both slopes were obtained on the same day with use of the same condensation agent.

[b] Phenylalanine gave a concentration-independent average fluorescence intensity of 0.0074, a typical blank value.

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Use of Qualitative Serum Beta-Chorlogonadotropin Test to Monitor Problem Pregnancies

To the Editor:

With use of an antiserum to the β-chain of human choriogonadotropin (HCG) pregnancy can be detected within a few days after conception. Adequate concentrations of HCG for the gestational age (*1*) are measured by quantitative radioligand assays performed under conditions suitable for binding equilibrium (18-h incubation with first antibody), to eliminate or minimize the interference of other peptide hormones that are structural relatives of HCG (*2*). The qualitative test in serum is usually reported as positive or negative, based on comparing the response of the patient's sample with that of a "calibrating" serum sample having an HCG concentration of 25 ± 5 int. units/L. We propose that the actual ratio of patient's serum response to calibrator response can be used to assess the progress of problem pregnancies in