To the Editor:
We have addressed the efficiency of Schiff base removal in the Performance Characteristics section of the current Variant Hemoglobin A1c Instruction Manual (March, 1997). Under conditions that simulate routine (batch) analysis, the reduction rate is 80–100%. The decrease in Schiff base occurs not only during the incubation time but also during the priming and calibration sequence, when the sample gradually cools from ambient temperature to the Variant’s sample storage temperature (8 °C). Much of the data reported in this letter was produced by using a limited incubation (10 min) and “stat” analysis. Under the conditions described by the authors, Schiff base removal will be less effective. The Instruction Manual currently recommends a 30-min incubation for extremely hyperglycemic individuals; this approach will yield the most accurate results in tertiary-care centers where patients with type I diabetes are treated.

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The authors of the Letter respond:
To the Editor:
We welcome these comments, but are still unclear how Bio-Rad intends the instrument to be used in tertiary-care centers (or other laboratories). Must the technologist make a decision on whether the sample is from an “extremely hyperglycemic” patient, before deciding whether to incubate the hemolysate for 15 or 30 min? How is “extreme” hyperglycemia defined? Alternatively, does Bio-Rad now recommend a 30-min approach for all samples analyzed in tertiary-care centers, in case they are from extremely hyperglycemic individuals? As we have shown, even if analyzed “stat,” samples after a 30-min incubation give results that are, on average, only 2.9% higher than the plateau values. The Variant is a very precise instrument and we see no merit in adding analytical imprecision by incomplete removal of the labile fraction.

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Analytical Agreement and Clinical Correlates of Plasma Brain Natriuretic Peptide Measured by Three Immunoassays in Patients with Heart Failure

To the Editor:
Brain natriuretic peptide (BNP) is a 32-amino acid peptide structurally related to atrial natriuretic peptide and predominantly secreted by myocardial ventricles. Interest in this peptide has recently increased because its concentration carries prognostic value in patients with myocardial infarction [1], congestive heart failure (CHF) [2], or cardiac hypertrophy [3]. The circulating concentration of BNP is also a predictor of mortality, independently of cardiovascular disease [4]. Three commercially available immunoassay methods are designed to measure the plasma concentration of human BNP: two recent nonextraction assays and an older extraction RIA. Here, we compared these three immunoassays and correlated them to cardiac function in healthy volunteers and patients with CHF.

We studied 26 patients (15 men, 11 women; ages 51–84) with depressed left ventricular function and a wide range of ejection fractions (22–57%) measured by bidimensional echo-Doppler technique. Six volunteers without evidence of cardiovascular disease were also included in the evaluation. Blood samples (14–21 mL) were collected in chilled tubes containing EDTA-sodium and aproitin (500 kallikrein inhibitor units/mL), and the plasmas obtained were immediately separated and stored in aliquots at −80 °C until assay. Each plasma sample was assayed for BNP with a nonextraction RIA from Peninsula Labs. (cat. no. RIAS9086), a nonextraction IRMA from Shionogi (Shionoria® BNP), and an extraction RIA from Phoenix Pharmaceuticals (cat. no. RK-011-03). For the last assay, plasma was extracted on C18 Sep-Pak (Waters) cartridges. Protocols and manufacturers’ directions were followed for all immunoassays. Plasma volumes for the determinations by the three methods were 0.1, 0.1, and 1 mL, respectively. The procedures followed were in accordance with the current revision of the Helsinki Declaration of 1975.

Figure 1 displays the agreement between each of the three immunoassays evaluated, according to the representation of Bland and Altman [5]. There was a good agreement between the two nonextraction immunoassays, though with a zero-bias (difference = −97 ± 128 ng/L, mean ± 2 SD). Conversely, there was a significant divergence between either nonextraction immunoassay and the assay utilizing extraction, the difference increasing with the average BNP concentration. The extraction immunoassay gave lower BNP concentrations, on average, than either nonextraction method.

All three immunoassays showed a good clinical correlation with left ventricular ejection fraction for the patients with CHF. After log-transformation of BNP concentrations (y values), the regression analysis resulted in the following respective parameters for the Shionogi, Peninsula, and Phoenix immunoassays: slope −0.042 ± 0.01, −0.026 ± 0.01, and −0.024 ± 0.01; intercept 3.91 ± 0.22,
Increased Plasma Endothelin-1 After Nicotine Consumption in Nonsmokers

To the Editor:
Endothelin-1 (ET-1) is a potent vasoconstrictive peptide originally isolated from the supernatant of cultured porcine endothelial cells [1]. ET-1 has not only contractile effects but also growth effects on both smooth muscle and heart muscle cells in vitro [2]. Studies have shown that some substances [1, 3], e.g., vasopressin [4], stimulate the release of immunoreactive ET-1 from cultured bovine carotid endothelial cells.

Smoking is known to induce a variety of effects in the cardiovascular and hormonal systems in humans [5–10]. Administration of nicotine causes the release of some hormones [9, 10] and produces increases in blood pressure, heart rate, cardiac output, and oxygen consumption [5–8]. Recently, Yildiz et al. [11] reported that heavy cigarette smokers had higher plasma ET-1 concentrations than either light smokers or controls. In another study, Haak et al. [12] stated that after a short-term tobacco consumption the plasma concentrations of ET-1 significantly increased within 10 min of smoking.

Although tobacco smoke contains other compounds, besides nicotine and carbon monoxide, we decided to test in 10 healthy nonsmoker volunteers (all medical students, 5 men and 5 women, ages 21–24 years) the acute effect of nicotine chewing gum (Nicorette, supplied by Pierrel Pharmaceutical) on plasma ET-1 concentrations. Each piece of chewing gum contained 2 mg of nicotine. We also measured the plasma concentrations of the vasoconstrictor peptide vasopressin, also known to act on the cardiovascular system.

On the day of the study, fasting subjects arrived between 0800 and 0900. At the time of collecting venous samples, all subjects had been kept in supine position for at least 30 min to stabilize their physical condition. Before and 5, 15, 30, and 60 min after the subjects chewed the nicotine gum, venous blood samples were collected in ice-chilled EDTA-coated tubes and centrifuged at 3000 g for 30 min; the plasma obtained was frozen and stored at −70 °C until assayed. At the same intervals, the subjects’ blood pressure and pulse rate were monitored. After 1 week, all volunteers repeated the experimental study but with a placebo gum that looked the same as the nicotine gum.

Plasma ET-1 was assayed with a specific RIA (RIC-6901; Peninsula Lab., Belmont, CA), as previously described [13]. Vasopressin was also measured with an RIA (RK-065–07 Phoenix Pharmaceuticals, Mountain View, CA). Briefly, vasopressin was extracted from samples with a C18 Sep-Pak column after acidification with 5 mL of 1 g/L trifluoroacetic acid.