the range of 0–150 μmol/L and to construct a point-to-point calibration curve. Analyses were carried out in triplicate on a Berthold Mithras microplate reader equipped with 3 injectors and entailed this sequence: (*a*) addition of choline oxidase solution to the sample (10 μL, 10 units/mL, pH 8, 0.2 mol/L sodium phosphate, 0.1% sodium cholate); (*b*) after 2 s, addition of an aqueous acridinium chemiluminescent indicator (10 μL, 4 μmol/L, 0.1% sodium cholate); (*c*) addition of aqueous sodium hydroxide (30 μL, 0.25 mol/L); and (*d*) recording of the light signal for 2 s.

The results show (Fig. 1) that in the normal blood donor samples, the median (25th–75th percentiles) choline concentration of 11.28 (9.73–13.13) μmol/L was in agreement with the literature *(6)*. In the troponin-positive plasma samples, the median (25th–75th percentiles) choline concentration was 20.6 (14.60–26.80) μmol/L. Nonparametric analysis of the 2 sample populations gave $P < 0.0001$, indicating that the nearly 2-fold difference in the median concentration of choline in the 2 sample populations was statistically significant. Furthermore, 75% of the troponin-positive samples exceeded 14.51 μmol/L, the value at the 90th percentile of the normal blood donor population, and 60% exceeded the 97.5th percentile (18.42 μmol/L).

Increased cardiac troponin-I is indicative of myocardial cell death attributable to prolonged ischemia and is used in the diagnosis of acute myocardial infarction; however, this biomarker can be increased in other conditions such as myocarditis *(7)* or myocardial ischemia-reperfusion injury *(8, 9)*. The cardiac troponin-I–positive samples tested here were collected without regard to diagnosis. Nevertheless, the overall increase in plasma choline concentrations measured in these troponin-positive samples was consistent with experimentally induced global ischemia in animal models in which both choline and troponin concentrations are increased *(2)*. The overlap between the normal blood donor and troponin-positive sample populations indicates the need to study more closely the time course for the rise and fall of plasma choline after a cardiac event and in other cardiac pathologies. A rapid assay for choline in human plasma, such as that used here, will facilitate further prospective studies in establishing the clinical value of choline.

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Laboratory Reporting of 25-Hydroxyvitamin D Results: Potential for Clinical Misinterpretation

To the Editor:

Vitamin D deficiency is a common health problem, and healthcare providers frequently measure circulating 25-hyroxyvitamin D concentration, the accepted standard for clinical assessment of vitamin D status *(1)*. In the past, assay of this analyte was mainly performed by RIA in which the sum of 25(OH)D_3 and 25(OH)D_2 was measured *(2)*. Newer HPLC and liquid chromatography mass spectroscopy (LC-MS) assays can measure the 2 forms separately *(3,4)*. Although the number of laboratories using HPLC or LC-MS for clinical 25(OH)D determination is not known, 17 of 153 laboratories participating in a recent QC survey used these methods, and some large commercial laboratories, which may analyze as many as 30 000 clinical specimens per month, use these methods. It has recently come to our attention that reporting both 25(OH)D_2 and 25(OH)D_3 may be confusing to physicians-in-training, who might consider a low 25(OH)D_2 or 25(OH)D_3 concentration to indicate vitamin D deficiency even if the total 25(OH)D result is within the reference interval.

To investigate whether the possibility of misinterpretation extends to healthcare providers who have completed training and are in clinical practice, we e-mailed an internet survey to 341 healthcare providers. The survey presented a hypothetical patient history [an 82-year-old person with a hip fracture in whom 25(OH)D was measured] with 3 potential 25(OH)D laboratory results with, for each set of results, the same 4 possible interpretations (vitamin D deficiency, vitamin D_2 deficiency, vitamin D_3 deficiency, or optimal vita-

Table 1. Potential for clinical misinterpretation when both $25(OH)D_2$ and $25(OH)D_3$ are reported.

Scenario	Vitamin D deficiency, treated with vitamin D, n (%)	Vitamin D_2 deficiency treated with vitamin D, n (%)	Vitamin D_3 deficiency treated with vitamin D, n (%)	Optimum vitamin D concentrations, no vitamin D treatment necessary, n (%)
$25(OH)D_3$ = 32 ng/mL $25(OH)D_2$ <5 ng/mL	1 (2)	7 (12)	0 (0)	49 (86)
$25(OH)D_3$ = 16 ng/mL $25(OH)D_2$ <5 ng/mL	52 (93)	2 (4)	2 (4)	0 (0)
$25(OH)D_3$ <5 ng/mL $25(OH)D_2$ = 40 ng/mL	2 (4)	0 (0)	11 (19)	44 (77)

Data are presented as n (%)—number of health care providers responding and percentage of total responding. This electronic survey depicted a hypothetical 82-year-old patient with a hip fracture for whom 3 scenarios of serum 25(OH)D results were reported. Four possible interpretations and treatment plans were presented for each scenario.

For all 3 scenarios, the following text accompanied the result: Vitamin D, 25-OH clinical reference values, measured as (*a*) <10 ng/mL, severe deficiency; (*b*) 10–24 ng/mL, mild to moderate deficiency; (*c*) 25–80 ng/mL, optimum concentrations; and (*d*) >80 ng/mL, toxicity possible

min D status). The clinical decision values for circulating 25(OH)D (see Table 1) were provided. The survey asked which patients should receive treatment with vitamin D. We e-mailed the survey to providers who were selected because they had ordered 1 or more 25(OH)D determinations in the preceding year. Only practicing healthcare providers received this survey; physicians-in-training were excluded.

In one scenario, the total 25(OH)D result was 16 ng/mL; all responding healthcare providers (45 physicians, 12 physicians assistants and nurse practitioners) correctly identified this as vitamin D deficiency in need of vitamin D treatment (Table 1). However, for the scenario reporting $25(OH)D_3$ <5 ng/mL and $25(OH)D_2$ 40 ng/mL, 13 (23%) interpreted these results to indicate either vitamin D deficiency or vitamin D_3 deficiency requiring vitamin D treatment (Table 1).

The important objective in assessing vitamin D status is to obtain a measure of the total circulating 25(OH)D, whether from a validated RIA, HPLC, or LC-MS assay. To achieve this objective, there is no advantage to reporting circulating $25(OH)D_2$ and $25(OH)D_3$ separately. Moreover, the results of this survey suggest that reporting $25(OH)D_2$, $25(OH)D_3$, and total 25(OH)D can confuse practicing healthcare providers, thereby leading to incorrect clinical decisions. We believe that, until a specific advantage for separate reporting of these metabolites is identified, laboratory reports should be limited to total circulating 25(OH)D or, if individual metabolites are listed, such reports must indicate clearly that the total circulating 25(OH)D is the measurement that should be used in clinical decision-making about vitamin D status.

The above recommendation does not diminish the important observation of D_2 and D_3 differing effects on maintenance of circulating 25(OH)D. Moreover, we acknowledge that there may be physiologic differences between D_2 and D_3. However, such theoretical differences have not been established and current dogma is that circulating $25(OH)D_2$ and $25(OH)D_3$ have equal clinical efficacy.

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Falsely Low LDL Cholesterol Results and Cholestasis

To the Editor:

LDL cholesterol (LDL-C) is commonly measured for evaluation and management of hypercholesterolemia. The Friedewald formula [LDL-C = (total cholesterol)—(HDL cholesterol)—triglycerides/2.2 for mmol/L] is commonly used to determine LDL-C, but this method has some well-established shortcomings and may not meet the National Cholesterol Education Program criteria of total error <12% *(1–3)*. Another approach is the use of homogeneous methods for direct quantification of LDL-C. In our laboratory, we use the