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Modified Assay for Total Iron-Binding Capacity Unaffected by Change in pH of Lyophilized Control Sera

To the Editor:

The Letters of Devgun (1) and Vernet-Nyssen (2) concerning inconsistent and falsely high values for total iron-binding capacity (TIBC) with lyophilized control sera prompt us to report our minor modification of Ramsay's method (3). We add extra HCl in the Fe(III) saturating reagent to compensate for the loss of carbon dioxide from either control sera or patients' samples during lyophilization or after prolonged standing.

The effect of this modification was evident in experiments with old pooled human sera and in repeat experiments of trials of the Netherlands National External Quality Control Program. About 150 laboratories participated in the trials, which involved control sera from different manufacturers and of different origin. Our TIBC values by the unmodified method were consistently higher than the overall mean values. In 16 trials with mean values ranging from 41 to 128 pmol/L, our results deviated from the mean for each trial by +2 to +53 pmol/L (mean, +27%; range, +3% to +60%). To try to improve our performance, we determined TIBC eight times in each of two pooled human serum specimens and in the control sera from 14 trials, after adding an extra increment of HCl to the Fe(III) reagent (final range, 0 to 40 mmol of HCl per liter of serum). In seven of these sera, with a pH after reconstitution of less than 8.5, the TIBC remained constant over the whole HCl concentration range. This possibly also indicates that the manufacturers have corrected those sera for pH beforehand. In the other nine sera, with pH values after reconstitution >8.5, the TIBC values decreased with increasing amounts of added HCl, until reaching constant values, which were in accord with the overall trial's mean values. The total decrease varied from 10 pmol/L (in the pooled sera) to 65 pmol/L in a control serum. Adding more HCl than 15 mmol/L serum caused no further decrease in TIBC. After modification of our method by including this amount (15 pmol per liter of serum) of HCl in the Fe(III) reagent, we find excellent agreement in all trials between our results and the overall mean values. In 14 further trials with TIBC values ranging from 39 to 133 pmol/L, the results of our modified method deviated by no more than −1 to +9 pmol/L; the mean deviation was +4 pmol/L (+7%; range, −3% to +19%). Moreover, the within-series (n = 10) CV was decreased from 4.2% to 0.9% after modification.

In summary, for accurate determination of TIBC in sera by Ramsay's method, extra HCl has to be added to the Fe(III) reagent. This modification is suitable for determination in lyophilized control materials and human serum specimens.

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Effect of Macro Creatine Kinase on Results of Hybritech CK-MB Kit

To the Editor:

Immunoinhibition methods for measuring the MB isoenzyme of creatine kinase (CK; EC 2.7.3.2) are well established and frequently used routinely in investigating myocardial infarction. These methods rely on inhibition of the CK-M subunit and measurement of residual CK-B activity, which is assumed to result from CK-MB (1, 2). However, increased CK subunit activity without increased CK-MB may result from an increase in CK-BB (3, 4), or from macro CK (Types 1 and 2) (5) due to immunoglobulin-bound CK-BB, or from a CK resistant to immunoinhibition. Type 1 is not associated with any specific disease. Macro CK Type 2 and increased CK-BB have been found in the sera of patients with tumors.

We investigated the effectiveness of the Hybritech CK-MB kit (Hybritech U.K. branch, Nottingham, U.K.) in a group of patients with Type 1 macro CK and in patients with increased CK-BB as determined by electrophoresis (Table 1). All of these patients were suspected of having had a myocardial infarction. Both total CK and CK-BB were routinely measured with the Hitachi 705 analyzer and kits from Boehringer Mannheim, the CK-MB method being an enzyme immunoinhibition assay.

Of the four patients with a CK-BB band in this study, two had a known primary tumor, one had suspected metastatic disease, and the fourth polycythemia. All four patients had undetectable CK-MB as measured by the Hybritech kit, although according to the enzyme immunoinhibition assay they had increased CK-BB subunit activity, which could have been interpreted as increased CK-MB. Similar observations were seen for the four patients with macro CK, only one of whom had a detectable CK-MB band (Hybritech...