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What Information Should Manufacturers Provide on Their Procedures?

To the Editor:

Recently, our laboratory received a bulletin (Technical Bulletin CC06-001, Calcium C.f.a.s. Set Point and PNU/PPU Target Value Reassignments: All Clinical Chemistry Systems, January, 2006) from Roche Diagnostics Australia indicating that the calcium value of their calibrator for automated systems (C.f.a.s.) had been reassigned. According to the bulletin, the calibration was performed against atomic absorption spectroscopy (AAS). The bulletin also stated that Roche’s quality assurance system did not reveal any substantial lot-to-lot changes over the 5 years since the last reference standardization. No data were provided on the quality assurance system results or on the standardization.

The change for the assigned value for the C.f.a.s. lot being used in our laboratory (lot 166275) was from 2.04 mmol/L to 1.93 mmol/L. We were concerned that the magnitude of this change (5.4%) was greater than the desirable total error for calcium, 2.4% (1), and we felt that such a change could have a substantial effect on patient data. Thus, we asked Roche Diagnostics to provide further information regarding the reassignment.

We were informed that the Roche Diagnostics in-house standardization procedure included the assays of 10 patient pools on either the Roche AAS or the Hitachi Modular instruments (personal communication, Dr. George Koumantakis, Roche Diagnostics Australia). The results were provided to us as medians, and we assumed that these represented the results from several different determinations (data not provided). Fig. 1 shows the results for these 10 patient pools measured on the Hitachi Modular instrument plotted against the AAS results. Over this range, the Hitachi results provided by Roche Diagnostics Australia for the 10 patient pools were 5% higher than the AAS results, also provided by Roche Diagnostics. We were informed that Roche Diagnostics used this mean to reassign the values for the C.f.a.s. master lot, from which the calcium values for their various commercial lots of C.f.a.s. are derived. The effect of reducing the Hitachi result for the patient pools by 5% is shown as the dashed line in Fig. 1.

This method of reassigning was inappropriate for 2 reasons. First, the restandardization was carried out with patient pools with calcium values between 2.16 and 2.46 mmol/L to correct the assigned calcium concentration in C.f.a.s, which is ~2.0 mmol/L, well outside the range over which the restandardization was done. Second, the results also showed a proportional bias between the Hitachi method and AAS. We were not sure what effect these 2 issues would have on the reassignment of values outside the range covered by the standardization.

To investigate this further, Roche Diagnostics (Australia) kindly provided us with Standard Reference Material 956b (SRM 956b), obtained from the NIST, which are human serum-based materials containing 3 concentrations of calcium, with values determined by inductively coupled plasma mass spectroscopy (ICP-MS). We prepared these materials according to the protocol provided by NIST and assayed them immediately on the Hitachi Modular, using the o-cresolphthalein complexone method with the original assigned value used for the calcium value of the C.f.a.s. Aliquots were also prepared, stored at ~30 °C, and subsequently assayed on the Hitachi Modular over 5 days (twice per day) using thawed aliquots. The results are shown in Fig. 1. The error bars represent the errors provided by NIST and those obtained from our assays. The regression line has been fitted by use of linear least-square regression, but other regression methods gave the same results.

As with the standardization carried out by Roche Diagnostics, these results show a proportional bias between the results obtained with

![Fig. 1. Calcium concentrations measured on the Hitachi Modular plotted against the values obtained by the ICP-MS method for SRM956b or by AAS for C.f.a.s. The lines indicate the following: □, results provided by Roche Diagnostics for 10 patient pools assayed by AAS and on the Hitachi system; ◼—◼, Hitachi values reset by 5%, as indicated by Roche for resetting the calcium C.f.a.s. value (Technical Bulletin CC06-001); ◼—○—◼, results of measuring calcium in SRM926b on the Hitachi Modular, plotted against the values obtained by a reference laboratory using ICP-MS (n = 10 for Hitachi and 8 for ICP-MS).](image-url)
ICP-MS and those from the Hitachi Modular. However, at the calcium concentration in C.f.a.s., the difference between the 2 methods (mean difference = 0.017) was not statistically significant (P > 0.1). Our result of 1.974 mmol/L would indicate that the assigned calcium value for the concentrations in C.f.a.s. should have been changed by only ~0.02 mmol/L, not the 5%-6% change suggested by Roche.

This investigation highlights several issues. The first is that, for many assays, laboratories depend on manufacturers to provide accurate calibrators. If there is a significant reassignment of a calibrator, manufacturers need to provide supporting evidence for laboratories to consider. Second, it is usual laboratory practice for routine assays to be calibrated within the typical measuring range to minimize the need to extrapolate instrument data. It would seem logical for manufacturers to adopt the same procedure when assigning values to their calibrators. Third, Roche Diagnostics have indicated to us that they did not measure SRM 956b or a similar reference material in their standardization, and it would seem important that manufacturers use traceable materials wherever possible, rather than rely on in-house traceability.

A recent report by NIST estimated that an analytical bias of 0.1 mmol/L in the calcium value would affect 3.5 million people in the United States and would cost the healthcare system approximately $150 million per year (2). This study raises the following questions: Should manufacturers take part in quality assurance schemes and make their results available for public scrutiny? Should they provide detailed information on their standardization procedures? And should they be more open generally about the way they ensure the quality of their products? To all of these questions, this author believes the answer is yes.

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References

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A Mannose-Binding Lectin-Defective Haplotype Is a Risk Factor for Gastric Cancer

To the Editor:
El-Omar et al. (1) reported that interleukin (IL)-1 gene cluster variants that enhance the production of IL-1β (a powerful inhibitor of gastric acid secretion) increase the risk of gastric cancer in Helicobacter pylori (HP)-infected patients. IL-1β production is down-regulated by mannose-binding lectin (MBL) (2), an acute-phase glycoprotein that has a high affinity for gram-negative lipopolysaccharide and exerts immunological activity (3, 4). Variants in the promoter, the 5′UTR, and exon 1 of the MBL2 gene reduce the synthesis and activity of MBL (5).

To assess the relationships between MBL2 gene variants and HP-related gastric cancer, we analyzed the whole coding region and the 5′UTR of the MBL2 gene in DNA extracted (QIAamp, Qiagen) from neoplastic cells embedded in paraffin sections (used for histological diagnosis) from 145 unrelated patients (90 males) affected by noncardia gastric cancer. Eighty-seven (60.0%) had intestinal-type; 47 (32.4%), diffuse-type; and 11 (7.6%), mixed-type adenocarcinoma. All patients had HP-positive serology. For 75 patients, we also analyzed DNA extracted from blood or from nonneoplastic surrounding tissue. All participants gave informed consent.

We examined DNA extracted from blood of 553 (240 males) unselected, unrelated, healthy adults from Southern Italy. For each DNA sample, we sequenced the promoter (−550 to −221), 5′UTR (−464 to +104), and exon 1 (−97 to +206) of the MBL2 gene in both directions. Primers, PCR mix, and conditions are available on request. MBL2 mutations in exon 1 at codons 54, 52, and 57 are called B, D, and C, respectively (5). The wild-type allele is “allele A”. The 3 polymorphisms in the promoter region are called H/L (−550), X/Y (−221), and P/Q (+4 in the 5′UTR).

Analysis results for DNA from blood or from nonneoplastic tissue of 75 patients with gastric cancer invariably were the same as those for neoplastic tissue, excluding the possibility that MBL2 haplotypes were altered by somatic mutations.

The distribution of MBL2 haplotypes was in Hardy-Weinberg equilibrium in healthy individuals (χ^2 not significant). The haplotype distribution differed significantly between healthy individuals and gastric cancer patients (global χ^2: 23.4, P < 0.001). The multiple comparison of each haplotype frequency tested against the others pooled together (χ^2 or Fisher exact test as appropriate, with Bonferroni correction for multiple comparison) showed that only the HYPD haplotype was significantly different (Table 1, part A). It was present in 4.4% of alleles from controls and in 10.4% of alleles from gastric cancer patients (χ^2: 13.97; Bonferroni adjusted P = 0.00131). The distributions of the 6 other haplotypes were comparable in the 2 groups.

The R52C MBL2 mutation (allele D) alters the collagen-like domain of the MBL protein thereby limiting the formation of high-molecular-weight