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 manu facturers 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.

This work was supported in part by Roche Diagnostics (Australia).

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DOI: 10.1373/clinchem.2006.069773

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

Table 1. Number (and frequency) of mannose-binding lectin haplotypes (part A) and genotypes (part B) in patients affected by gastric cancer and in a population of healthy individuals.

A. Mannose-binding lectin haplotypes

	Alleles	HYPA	LXPA	LYQA	LYPB	HYPD	LYPA	LYQC
Control population, n (%)	1106	390 (35.26)	255 (23.06)	198 (17.90)	144 (13.02)	49 (4.43)	52 (4.70)	18 (1.63)
Gastric cancer patients, n (%)	290	87 (30.0)	62 (21.34)	55 (18.96)	36 (12.41)	30 (10.39)	20 (6.89)	0
P level		NS	NS	NS	NS	0.00131	NS	NS
Odds ratio		0.79	0.91	1.07	0.95	2.49	1.5	0
95% confidence intervals		0.6–1.04	0.66–1.24	0.77–1.5	0.64–1.4	1.55–4.0	0.88–2.56	

B. Genotypes, n (healthy individuals shown in parentheses)

	HYPA	LXPA	LYQA	LYPB	HYPD	LYPA	LYQC
HYPA	13 (45)	21 (102)	16 (92)	10 (62)	9 (26)	5 (14)	0 (4)
LXPA		4 (32)	14 (47)	13 (20)	0 (5)	4 (12)	0 (5)
LYQA			3 (16)	0 (11)	0 (4)	3 (7)	0 (5)
LYPB				0 (15)	0 (11)	0 (10)	0 (0)
HYPD					3 (1)	0 (1)	0 (0)
LYPA						0 (2)	0 (4)
LYQC							0 (0)

P values <0.05 were adjusted for multiple comparison bias by Bonferroni's method. NS = not significant.

complexes and reducing the immunological activity of the protein *(4)*. Participants bearing the D allele in heterozygosis (9 gastric cancer patients in the present study; Table 1, part B) have a 10-fold lower serum MBL concentration, and the immunological activity of the residual protein is very low because of impaired polymerization. Homozygotes for the D allele (3 gastric cancer patients in the present study; Table 1, part B) have no residual activity of the protein *(5)*.

We suggest that the low MBL activity in patients bearing the HYPD haplotype may allow enhanced bacterial colonization of the gastric mucosa and reduce down-regulation of IL-1β production, thereby increasing the risk of gastric cancer (odds ratio: 2.5; Table 1, part A). Indeed, IL-1β production is up-regulated by HP. IL-1β induces chronic hypochlorhydria, which promotes the spread of HP-induced inflammation and leads to gastric atrophy, a precursor of gastric cancer *(1)*. Hypochlorhydria also promotes the colonization of other bacteria that foster mucosal damage and the production of carcinogenic compounds *(1)*, adding to the scarce antibacterial activity of MBL caused by the HYPD haplotype. IL-1 gene polymorphisms that enhance IL-1β production are associated with an increased risk of gastric cancer in HP-infected patients *(1)*. Furthermore, the addition of sera containing unaffected MBL concentrations to monocytes stimulated by *N. meningitidis* down-regulates the production of IL-1β, whereas the addition of sera from MBL-deficient individuals enhances the production of the cytokine *(4)*. Otherwise, *MBL2* HYPD haplotype may be in linkage disequilibrium with variants of other genes responsible for the increased risk of gastric cancer.

To conclude, the MBL2-defective HYPD haplotype may be a novel genetic marker of risk for gastric cancer in HP-infected patients.

This study was supported by Ministero dell'Istruzione, dell'Università e della Ricerca, Italy (PRIN 2004) and by Regione Campania (L. 229/99). We are indebted to Jean Gilder for editing the text.

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DOI: 10.1373/clinchem.2006.071696

Assay Interferences from Blood Collection Tubes: A Cautionary Note

To the Editor:

Recent publications *(1, 2)* have reported an association between the surfactant present in BD Vacutainer® blood collection tubes and interference in select immunoassays on certain instrument platforms. Blood collection tubes contain not only surfactants but multiple additives that contribute to the optimal recovery of serum or plasma for laboratory analysis. For plastic tubes, which are largely replacing glass tubes because of safety considerations, several suppliers have added silica particles to promote blood clotting *(3–5)* and polyvinylpyrrolidone to aid the adherence of silica particles to the tube walls and to facilitate rapid dissolution of the silica in the blood specimen. Moreover, silicone-based surfactants or polypropylene oxide are present as coatings for the interior tube wall *(6, 7)* to act as hemorepellent agents and improve blood flow. Stoppers of tubes are also coated with lubricant to facilitate their removal and to maintain the lower pressure inside the evacuated tubes *(4)*. Separator gels are present in some tubes to serve as a barrier between the serum (or plasma) and the clot after centrifugation of the tubes *(8)*. Plastic blood collection tubes have been widely shown to be suitable for routine clinical chemistry analytes, hormone analysis, and therapeutic drug monitoring *(9–11)*.

Surfactants are also a common component of many immunoassays. They are used to decrease or eliminate nonspecific adsorption, improve stability of the reagents, or modify the solid-phase surface to render it less hydrophobic and thus minimize loss of noncovalently bound antibody. Inclusion of surfactants in immunoassay reagents requires careful selection and optimization. High concentrations of surfactants may lead to direct loss of passively adsorbed antibody from the solid phase, among other nonspecific effects *(12–13)*.

The reports by Bowen et al. *(1, 2)* show that a high concentration of a silicone-based surfactant is a potential source of the immunoassay interference in the DPC IMMULITE® 2500 Total Triiodothyronine (TT_3) assay. The authors indicate that one of the possible mechanisms of interference is desorption of the antibodies from the solid phase by the surfactant for the TT_3 assay *(1)*, resulting in a falsely increased estimate of the TT_3 concentration. As described above, this phenomenon is not unknown in immunoassays in which the antibodies are passively adsorbed onto the solid phase *(12–13)*. Other types of assay formats with more robust antibody binding schemes do not show this problem, even at high concentrations of surfactant as demonstrated on the AxSym™ analyzer. The concentration of surfactant at which Bowen et al. demonstrated the desorption of antibody in the TT_3 assay was 2- to 24-fold higher than the concentration of surfactant per milliliter of blood present in the BD Vacutainer tubes that exhibited the interference in the TT_3 assay. The current adjusted BD Vacutainer tubes have been shown to produce no clinically significant differences for a variety of assays across many instrument platforms when compared with competitive products [see "Note added in proof" in reference *(2)*]. The concentration of the surfactant has been adjusted to decrease any known assay interferences and yield clinically equivalent results compared with glass tubes *(14)*.

Assay interferences from blood collection tubes can present challenges to clinical laboratories because they are not easily detected by the daily quality control or even by proficiency testing programs because the samples for such testing are not exposed to the additives in the blood collection tubes *(15)*. However, it is always good practice for laboratories to monitor their reference intervals and population trends and report deviations to the device manufacturers. All laboratorians should be vigilant for potential effects on laboratory assays and work together in partnership with tube manufacturers and diagnostic companies to prevent and minimize problems.

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