that all 3 vials used by Magni et al. had a higher dry mass content than the 22 vials used by us. The reason for this discrepancy is unclear to us. However, because the accuracy validation of this method was based only on one type of SRM, the pronounced dry mass deviation of the vials used by Magni et al. raises the question whether those vials were suitable for demonstrating method inaccuracy <1%, as expected for Definitive Methods. Regardless, in their original work, they should have compared the measured glucose concentration of 6.226 mmol/L with the NIST target concentration based on dry mass, which is 6.304 mmol/L—calculated with the mean dry mass of 0.8542 g in Table 1 and the certified value of 7.38 mmol/(L·g). The result on a dry mass basis, therefore, would have been 1.2% too low in their original work because they did not discuss glucose breakdown at that time. If one took glucose degradation into account, the target would be 6.304 – 2.5 x 0.044 = 6.194 mmol/L. In this case the measured value of 6.226 mmol/L would be 0.52% too high.

The authors also state that comparison of values from different laboratories for a SRM are valid only when the same sample vial is used in a split measurement protocol. In our opinion, results for SRMs obtained in different laboratories with different vials must be comparable. Precautions in interpreting the results may sometimes have to be taken, depending on the SRM used. In the case of SRM 909, there is the possibility of analyte breakdown, and this has to be considered. If it is possible to correct for variability when the vials are being filled before lyophilization, this should be done particularly when a candidate Definitive Method is proposed.

In another point, Magni et al. said they had experienced no mass spectrometric problems in using the aldonitrile pentaacetate as derivative for determination of glucose. However, those of us at the laboratory in Gent sometimes encountered precision and accuracy problems during transfer of the glucose method from the INSTAND laboratory to our laboratory (2). We suspected that our injector was causing these problems. Indeed, these problems have not recurred since we installed a programmable-temperature vaporizing injection system on our combined GC-MS 5970 B Mass Selective Detector System (Hewlett-Packard, Palo Alto, CA). Therefore, we still advocate caution when using [153C6]glucose as internal standard and the aldonitrile pentaacetate deriva-
tive for mass spectrometric determination of serum glucose.

General note added: Both we and Magni et al. may have wrongly calculated mmol/(L·g) values from measured mmol/L values by dividing them by the dry mass content of the respective vial (glucose content given in ref. 1, or row 3 in Table 1, above). Although the resulting conclusions about bias are mathematically correct, this way of calculation is logically incorrect (the greater the dry mass, the lower the glucose values obtained). Different glucose values per gram of dry mass result only from different moisture content, glucose degradation, or inhomogeneity of the filling solution, but not from differences in filling volume. Corrections to compensate for variations in filling volume can only be made by multiplying the dry mass content with the mmol/(L·g) target value.

References

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Serum Concentrations of Basic Fibroblast Growth Factor in Breast Cancer

To the Editor:
Basic fibroblast growth factor (bFGF) is produced by a wide variety of normal and malignant cells (1). Although bFGF was originally reported to stimulate proliferation of cells of only mesodermal or neuroectodermal origin (1), this polypeptide has since been shown to stimulate the growth of rat mammary myoepithelial and stromal cell lines (2), human breast cancer MCF-7 and T47D cell lines (3, 4), and cultured epithelial cells derived from both normal and malignant breast biopsies (5). Stimulation of incorporation of [3H]thymidine into COMMA-D mouse mammary epithelial cells has been shown to be specifically inhibited by an immobilized anti-bFGF antibody (8).

We previously found increased serum bFGF in patients with a variety of malignant tumors, including breast cancer (7), by a sensitive and specific two-site enzyme immunoassay (EIA) method (8). To investigate whether bFGF concentrations in serum could reflect the malignancy of the tumors, we classified the patients with breast cancer according to the TNM classification of malignant tumors (9) and measured the bFGF concentration in their serum. We measured immunoreactive bFGF in sera by using anti-bFGF MAbs (Mab 98) IgG-coated polystyrene beads and anti-bFGF MAb (MAB 12) Fab'-linked horseradish peroxidase (HRP, EC 1.11.1.7; from Sigma Chemical Co., St. Louis, MO). The detection limit of our EIA system was 30 ng/L (3 pg/assay tube). The two MAbs (10) against recombinant human bFGF and recombinant human bFGF (II) used for the standards were generously provided by Takeda Chemical Ind. (Osaka, Japan).

Details of the EIA system were reported previously (8). In brief, 100 µL of samples and 150 µL of Buffer A (0.1 mol/L phosphate buffer, pH 7.5, containing 1 mol/L NaCl, 1% v/v Na2CO3, 1 g of NaNO3, and 1 mmol of MgCl2 per liter) were added to each tube containing one MAb IgG-coated bead. After incubation at 4°C overnight, the aqueous solution was removed by aspiration and the beads were extensively washed with 500 µL of Buffer B (0.1 mol/L phosphate buffer, pH 7.0, containing bovine serum albumin, 1 g/L). The beads thus treated were incubated at 4°C overnight with HRP-linked anti-MAb Fab' conjugate (300-fold dilution of the stock conjugate to 0.25 µL of Buffer B. After extensive washing with Buffer B, the beads were transferred to clean tubes. Finally, the HRP activity bound to the beads was fluorophotometrically measured with use of 3-(p-hydroxyphenyl)propionic acid (HPFP; Sigma) as the coupling reagent.

Sera from patients with primary breast cancer were obtained from pa-
tients who had undergone neither chemotherapy nor radiation therapy prior to collection of the samples, and who were later diagnosed by pathology as having breast adenocarcinoma. The age distribution at the time of diagnosis was 30-65 years. All the resected tumor specimens were histologically staged based on the TNM classification (9).

The patients examined in this study included no patients in stages Tia (noninvasive tumor), 0, or IV, and none was found either macroscopically or histopathologically to have any distant metastases. We also measured immunoreactive bFGF in sera from 23 breast cancer patients one week after surgical resection of the tumor region of the mammary gland and axillary lymph nodes to assess a possible effect of tumor resection on the concentration of immunoreactive bFGF in their serum.

As shown in Fig. 1, serum concentrations of bFGF in 25 of 35 (71%) stage I patients with primary breast cancer before surgery were higher than those of normal subjects. Ten of 13 (77%) stage II patients and all 5 (100%) stage III-a patients also showed increased serum immunoreactive bFGF before surgery. On the other hand, only 3 of the 12 (25%) stage I patients who had had surgical resection of the tumor region of the mammary gland and the axillary lymph nodes showed increased bFGF in their serum. Similarly, values for only two of the eight patients at stage II and one of the three patients at stage III-a were above normal. Combined, before surgery 40 of the 53 patients (76%) showed an increased concentration of immunoreactive bFGF in their serum, whereas only 6 of the 23 patients (26%) who had surgical resections did. By Student's t-test, the serum bFGF concentration of the patients after surgical resection of the tumor was significantly less than that of the patients before surgery in all stages examined (stage I, P <0.001; stage II, P <0.02; stage III-a, P <0.03), which suggests that breast cancer cells produce and secrete immunoreactive bFGF.

bFGF is one of the more potent inducers of angiogenesis in vivo as well as in vitro (1). Evidence suggests that the bFGF-related genes hst/K-fgf, int-2, FGF-5, FGF-6, and KGF, all of which cause transformation in vitro, may also play a role in malignancy in vivo (1) and that the bFGF exported into the extracellular milieu stimulates migration of the same cell that secretes it via a true autocrine mechanism (12). In addition, Theillet et al. (13) found that the hst and int-2 oncogenes are expressed in 17% of breast cancers in situ hybridization of the presence of these genes in the tumor cells themselves. Rowe et al. (4) purified a bFGF-like growth factor from primary breast tumors that was mitogenic for T47D cells, and Gomm et al. (14) immunohistochemically demonstrated the presence of bFGF in benign human breast tissue.

In conclusion, the results from this study and others support the view that bFGF has an important role in mammary gland tumorigenesis. The present results demonstrate that the serum concentration of bFGF is increased in most patients with breast carcinoma, even at the early stage, and may be a useful marker for breast cancer.

References
3. Karey KP, Sirbasku DA. A differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors

Fig. 1. Concentrations of immunoactive bFGF in sera from normal subjects and 53 patients with breast cancer of various stages (classified according to TNM system). (p<0.001) Concentration of serum bFGF in normal subjects and in patients with breast cancer at various stages before surgery and (p<0.03) at various stages after surgical resection of the tumor region. Each point represents the means of duplicate assays. (---) Cutoff value (mean + 2SD of normal value for positive findings: 254 ng/mL). Serum bFGF was measured in six patients before and after surgery (p<0.01). Data shown include results for 24 breast cancer patients previously reported (7).

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