recognize them, and these bands are not included in our abnormal SPE results. False-positive M-spikes are more common in urine than serum and can be caused by clearance of some drugs, antibiotics, radiographic dyes, myoglobin, and lysosome (5). These false-positive M-spikes are reminders of the need for IFE confirmation.

The more subtle abnormalities present a dilemma for laboratories. The reflex criteria for non–M-spike SPE and UPE abnormalities had specificities of 28% and 48%, respectively. The 85 patients with hypogammaglobulinemia yielded 10 monoclonal gammapathies, half of which were monoclonal free light chains. These results are similar to those of Lakshminarayanan et al. (4), who found that 8% of 380 hypogammaglobulinemic sera contained a monoclonal protein and that almost one-third were free light chains. The identification of quantitative abnormalities such as hypogammaglobulinemia or increased β or α fractions depends on the definition of appropriate trigger points. If, for example, the trigger for reflexing samples with increased α2 fractions were raised from 14 g/L to 15 g/L, the number of reflexed samples with increased α2 fractions would have decreased by 75%. The identification of fuzzy γ bands, however, is a qualitative judgment that is difficult to standardize. Our data indicate that reflexing samples with these subtle abnormalities has merit for identifying potentially serious disease. It may be useful also to incorporate the γ-globulin concentration as well as the soluble free light chain ratio and clinical data, if available.

Because we do not always know the reason for ordering SPE, one strategy for the laboratory is to alert the physician and suggest a follow-up IFE if clinically indicated. The reflexing of abnormal protein electrophoresis results represents the laboratory’s effort to provide diagnostic information efficiently to clinicians and patients. These data help define guidelines for reflex triggers and emphasize the importance of subtle protein electrophoresis abnormalities. It should be stressed to clinicians, however, that the most efficient way to diagnose a suspected monoclonal gammopathy is through the use of test panels that contain SPE, IFE, and free light chain quantification.

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References

4. Lakshminarayanan R, Yueju L, Janatpour K, Beckett L, Jialal I. Detection by immunofixation of M proteins in hypogammaglobulinemic pa-
5. Katzmann JA, Kyle RA. Immunohistochemical charac-

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Improved GC-MS Procedure for Simultaneous Measurement of Ethylene Glycol and Glycolic Acid

To the Editor:

Our laboratory developed procedures for the simultaneous measurement of ethylene glycol and glycolic acid by gas chromatography–flame ionization detection (GC-FID)1 (1) and by GC-MS (2). The

1 Nonstandard abbreviations: GC-FID, gas chromatography–flame ionization detection; 1,3-PD, 1,3-propanediol; DMF, N,N-dimethylformamide; HIBA, α-hydroxyisobutyric acid.
selection of a suitable structurally similar internal standard for glycolic acid proved problematic because of either coeluting interference during GC-FID or ion overlap of deuterated and nondeuterated di-tert-butyldimethylsilyl derivatives during GC-MS analysis. As a compromise, 1,3-propanediol (1,3-PD), an acceptable internal standard for ethylene glycol, was also selected as the internal standard for glycolic acid in the GC-MS procedure.

Considerable experience with the procedure has revealed a limitation of this structurally dissimilar internal standard for measuring glycolic acid. In the procedure, serum proteins are precipitated with acetonitrile containing 1,3-PD, the supernatant is dehydrated with a water scavenger, and the volume is reduced to <100 µL in a N,N-dimethylformamide (DMF)-trapping solvent (but not to dryness). Evaporation of DMF causes some presumed evaporative loss of ethylene glycol and a somewhat lesser loss of 1,3-PD (evaporation to dryness leads to severe loss of both and should be avoided). Thus, the use of 1,3-PD compensates reasonably well for any loss of ethylene glycol, so that the relative recovery and imprecision of the analysis are acceptable (CV < 7%). In contrast, there is little to no loss of glycolic acid during DMF evaporation. Consequently, a progressively greater glycolic acid response relative to 1,3-PD is observed as the DMF evaporates. For the method to be reasonably robust, some care is thus required during volume reduction to avoid excessively uneven DMF loss between the calibrator and sample tubes. Between-port variance in the air flow rate when a multiport air manifold is used may especially produce such uneven evaporation. With close attention to this limitation, a typical CV for glycolic acid analysis is <7%; however, when such analyses are performed by multiple technologists, who multitask on all shifts, the CV for glycolic acid increases to between <10% and 12%.

We now incorporate α-hydroxyisobutyric acid (HIBA) [0.144 mmol/L (15 mg/dL) in 90% acetonitrile/10% glacial acetic acid] as the internal standard for glycolic acid. The di-tert-butyldimethylsilyl derivative of HIBA elutes about 0.05 min later than glycolic acid and is monitored with qualifier ions 147 and 189 and with target ion 275. This structurally similar compound is recovered in an amount equivalent to glycolic acid during the volume-reduction step. Thus, there is less concern about variation in the final volume, and the procedure is more robust. As an illustration, we processed serum samples fortified with a glycolic acid concentration of 2.4 mmol/L (18 mg/dL) or 10.5 mmol/L (80 mg/dL) and containing both 1,3-PD and HIBA, with little care taken to control sample volume reduction; in some cases, samples were purposely reduced to volumes greater than typical. As shown in Table 1, under these lax conditions of volume reduction, CVs are substantially lower, and recovery is improved when HIBA is used as the internal standard instead of 1,3-PD.

The original GC-MS procedure incorporates a 15-min incubation at 60 °C with N-methyl-Ν(tert-butylidimethylsilyl)trifluoroacetamide to derivatize the analytes and internal standard. We have now determined that flash derivatization (no incubation) produces equivalent recovery and imprecision. Moreover, 3 calibrators were used in the original procedure. Table 1 shows that single-point calibration [13.1 mmol/L (100 mg/dL)] provides equivalent values and near-equivalent imprecision compared with a 3-point calibration. With these 2 modifications, the analysis time for a single sample is reduced by about 30 min to a typical time of ≤1.5 h.

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References

1. Yao HH, Porter WH. Simultaneous determination of ethylene glycol and its major toxic metabolite, glycolic acid, in serum by gas
Increasing Cardiac Troponin Changes Measured by a Research High-Sensitivity Troponin I Assay: Absolute vs Percentage Changes and Long-Term Outcomes in a Chest Pain Cohort

To the Editor:

With novel high-sensitivity cardiac troponin assays, there is great interest in determining the change needed between successive cardiac troponin measurements to inform the diagnosis of acute myocardial infarction (MI). Recent publications have reported both short- and long-term reference change values (RCVs) in healthy populations for both high-sensitivity cardiac troponin I (hs-cTnI) and high-sensitivity cardiac troponin T (hs-cTnT); thus, the RCV perhaps could be the metric used for determining the optimal clinical change value. The RCVs for the hs-cTnT and hs-cTnI assays, however, are markedly different, possibly because of assay characteristics, differences in the biology of the 2 isoforms, or the healthy populations studied. The optimal clinical change for detecting patients with acute MI might be greater than the published RCVs. Two recent studies that used ROC curve analyses suggest exactly that. Giannitsis et al. determined the optimal δ to be ≥243% for hs-cTnI, whereas we identified an optimal δ of ≥235% on the basis of MI or death at 30 days for a research hs-cTnI assay. Percentages may work well when values are low but may become impractical when more substantial increases occur. The 2 studies neither reported δ in terms of absolute concentration nor defined its role in long-term risk stratification. In this report, we calculate δ for hs-cTnI, both in absolute concentration and as a percentage, and we relate these values to long-term outcomes.

The study population has previously been described. In brief, individuals presenting with chest pain to the emergency department had blood collected into heparinized tubes hourly until 6 h after symptom onset and then at 9, 12, 24, and 48 h, or until the patient was discharged, declined participation, or was removed from the study by those responsible for care. The present study included only the individuals (n = 223) with ≥2 hs-cTnI measurements (research assay, Beckman Coulter) and with peak hs-cTnI concentrations occurring after the earliest-collected sample. The absolute δ was obtained by subtracting the earliest hs-cTnI concentration from the peak concentration, and the percentage value for δ was obtained by dividing the absolute δ by the earliest hs-cTnI concentration and multiplying by 100.

Health outcomes (death or MI) were obtained via linkage to the Registered Persons Database for mortality outcomes and with the Canadian Institute for Health Information Discharge Abstract Database for Ontario hospital discharges associated with MI over a 10-year period after study presentation. Kaplan–Meier and Cox proportional hazard analyses were performed with tertiles for both the absolute δ and the δ percentage, with tertile 1 (i.e., the group with the smallest changes) used as the referent for the Cox models (adjusted for age and sex). ROC curve analysis and logistic models for death or MI at 1 year were also performed to find the optimal δ. The statistical analyses were performed with SAS software (SAS Institute). P values <0.05 were considered statistically significant. The study received prior ethics approval.

Of the 223 study participants, 60% were male, and female participants were older (mean, 67 years, vs 62 years for males; P = 0.006), with 136 events (i.e., death or MI) occurring within the 10-year period. The median number of hs-cTnI measurements per individual was 5 (interquartile range, 3–6), and the median time to the peak hs-cTnI value was 9 h from symptom onset (interquartile range, 6–13 h). The Kaplan–Meier analysis indicated different probabilities of survival among the tertiles of change, with both the absolute δ group and the δ percentage group manifesting significant differences at 1 year [P < 0.001, and P = 0.032 (log-rank tests), respectively]. Only the absolute δ value was statistically predictive at 10 years [P < 0.001, and P = 0.070 (log-rank tests), respectively] (Fig. 1). Individuals in the highest δ tertile (>104 ng/L for absolute δ and

1 Nonstandard abbreviations: MI, myocardial infarction; RCV, reference change value; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.