chloroacetic acid/sulfosalicylic acid as a fixative, as judged by the sharpness and intensity of the fixed bands.

The silver-stain procedure discussed here is a simple, inexpensive, and relatively quick method for detecting BJP in urine. Only a small sample volume is required, 10 μL of urine or even less serum, compared with the 5 mL of urine required when the sample must be first concentrated. This procedure also is more economical because diluted antisera are used for immunofixation.

References

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Discrepancies in Carcinoembryonic Antigen Measurements: Survey and Control Values vs Values for Patients

George G. Klein, Larry A. Dodge, and Gustavo Reynoso

We analyzed the carcinoembryonic antigen (CEA) test results reported in the College of American Pathologists' (CAP) surveys to determine the relationship between the source of CEA used to manufacture the survey specimens and the discrepancies among analytical methods. With the 1983 survey specimens, which were prepared from metastatic colon carcinoma, laboratories using Roche RIA with Clinetics columns reported results that were only one-half the values reported by laboratories using the Abbott polyclonal enzyme immunoassay. With the 1984 specimens, prepared from a different metastatic colon carcinoma, and the 1985 specimens, prepared from a tissue-culture source of CEA, the Roche results were about one-sixth as large as the Abbott results. These differences are larger than the reported assay differences for patients' specimens. In addition, twofold proportional differences were found when survey and control specimens were tested with different lots of Abbott polyclonal reagent, whereas only random differences were found with 102 patients' specimens. Evidently, assay systems perform differently with proficiency-testing and control specimens than with patients' specimens.

Additional Keyphrases: radioimmunoassay, enzyme immunoassay compared · proficiency testing · control materials · analytical error

Methodologic differences among assays of carcinoembryonic antigen (CEA) have been well documented (1–7). These discrepancies may be caused by differences in antibody specificity, reference standards, extraction procedures, separation methods, data-reduction methods, or numerous other possibilities. French investigators showed that four reference preparations of CEA responded differently in various European assay systems (2), and Davidson et al. showed that differences in three British assays were due to the characteristics of the assay systems rather than differences in standards (5). The Roche polyclonal RIA procedure has been shown to agree on the average with Abbott polyclonal enzyme immunoassay (EIA), with discordant results (both larger and smaller) being found for 16 to 21% of the patients (4–7). However, cross-comparison of the standards used in these assays shows larger discrepancies (8).

Major interassay differences have been presented in the test results for the proficiency-testing specimens for CEA distributed by the College of American Pathologists (CAP). These discrepancies are larger than would be expected from reported comparisons of the analytical methods. Because we wondered if these differences were related to the different sources of CEA material used to prepare the specimens for the surveys, we divided the CAP surveys according to the source of CEA used for making the test specimens and compared the test results reported for various analytical procedures. In addition, we compared the large proportional differences found when proficiency samples and control material were measured with different lots of Abbott polyclonal reagent and the smaller random differences encountered when the same reagents were used to measure fresh samples.

Materials and Methods

The summary statistics for test results from participants in the 1983, 1984, and 1985 Ligand Assay Proficiency Surveys were obtained from the CAP. The specimens for these surveys were manufactured by the Diagnostic Division of CooperBiomedical, Malvern, PA, by adding purified CEA to serum that had been converted from plasma by the addition of calcium and thrombin, followed by separation of the coagulum, dialysis to remove calcium, and reforification of salts to physiological limits. Three different sources of
CEA were used to make the proficiency specimens. For the 1983 specimens, the CEA was an extract of a liver carcinoma metastasized from the colon; this material was obtained from Roche Diagnostics, Nutley, NJ. The 1984 specimens also were based on an extract of liver carcinoma, but obtained from the Clinical Investigation Association. For the 1985 specimens a tissue-culture CEA, obtained from Abbott Laboratories, N. Chicago, IL, was used. All proficiency specimens were mixed, lyophilized, packaged, and shipped by Diagnostic Division of CooperBiomedical.

We compared the CAP survey participants' results for the major methods reported. For the 1983 series and specimens K1 through K9 of the 1984 series, we compared the Abbott-EIA, Abbott-RIA, and the Roche Diagnostics/Indirect Assay (with a Clinetics columns) because these were the procedures most commonly used by the survey participants. Beginning in the latter quarter of 1984, we included two additional assay systems in our analysis: the Abbott-EIA Monoclonal and the Hybritech-R assay (Hybritech, La Jolla, CA). We compared the average reported survey values from various combinations of these assay systems by analyzing the slopes and intercepts calculated from least squares linear regression. Student's $t$-tests were used to test if the slopes of the regression lines differed from unity.

In our second investigation of CEA methodological discrepancies, we examined lot-to-lot differences in the Abbott-EIA polyclonal reagent supplied in the spring of 1984. The concentrations of CEA in the 1983 CAP survey specimens, as measured with lot no. 62-689Hz reagent, were compared with the concentrations measured with prior lots of reagent. In addition, we compared values obtained by using these different lots of Abbott EIA reagents for measuring both commercial and locally prepared quality control pools and 102 patients' specimens. The commercial control pools (Hyland Diagnostics, Costa Mesa, CA) were: Omega I (lot no. 4814F001B), Omega II (lot no. 4824F001B), and Omega III (lot no. 4834F001B). The in-house control pools were made by adding a perchloric acid extract of a liver metastasis of colon carcinoma into blood bank plasma that had been converted to serum by the addition of calcium and thrombin. The control pools, proficiency samples, and patients' specimens were stored frozen at $-20^\circ$C for one to 12 months between analyses.

**Results**

We found that the differences in the measurements of CEA varied systematically with the analytical method and the source of the CEA material used in the surveys. Figure 1 compares the results by the Roche RIA indirect assay with use of Clinetics columns and the results by the Abbott polyclonal procedure. With the 1983 CEA preparation, the former results were about one-half as great (slope = 0.48, $p < 0.001$); with both the 1984 and 1985 CEA preparations, the former results were about one-sixth as great (slope = 0.16 and 0.14, $p < 0.001$). Even though the 1983 and 1984 CEA preparations were purified from liver metastasis, they gave substantially different results. In contrast, the 1985 survey specimens, prepared from cell culture, and the 1984 survey specimens, prepared from metastatic colon carcinoma, gave similar within-assay results. The Abbott EIA and Abbott RIA procedures gave approximately the same results with all three CEA preparations (slope = 1.06 for 1983, 0.86 for 1984, and 0.91 for 1985).

In the 1985 survey, the new methods showed further discrepancies. As Figure 2 shows, Abbott EIA polyclonal results were 30% greater than the Hybritech-R results ($p < 0.001$), the Abbott EIA monoclonal results were 11% greater than the Hybritech-R ($p < 0.1$), and the Roche RIA results with Clinetics columns were only 22% as great as the Hybritech-R ($p < 0.001$).

We found proportional differences between measurements obtained with different lots of reagent from the Abbott EIA polyclonal assay for control specimens made from partly purified CEA preparations, but not for patients' specimens. Measurements made with lot no. 62-689Hz and subsequent lots produced values for the 1983 CAP survey material, the commercial control specimens, and the in-house control specimens about twice as great as the values measured with prior lots of reagents (slopes = 2.09, 1.95, and 1.86, respectively; $p < 0.001$ for each). This proportional difference was not noted with 102 patients' specimens, which showed random variations but no systematic differences between reagent systems (see Figure 3). Linear regression of the logarithms of the results for the patients' samples yielded a slope of 0.99 (not significantly different from unity, $p > 0.4$); five patients had values that differed by more than 40% between results obtained with the new reagent vs. the values obtained with the previous reagent, and two had results approximately twice those obtained with the previous reagent. In the double logarithmic plot of Figure 4 the lot-to-lot proportional discrepancy found with the controls is depicted.
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Because soluble sulfate 1 mol/L perchloric acid and in half-saturated ammonium sulfate (9, 10). It gives a homogeneous band of β mobility on standard electrophoretic systems, but is heterogeneous by isoelectric focusing, with a major band at pH 4.5. The charge heterogeneity of various CEA preparations has been attributed, in part, to variations in sialic acid content of the molecule. The amino acid sequence of CEA is relative-

ly constant, but the carbohydrate moieties are variable (11, 12). Studies with monoclonal antibodies indicate that CEA contains no more than five or six antigenic sites (13–18). However, many antisera to CEA cross react with glycoproteins other than CEA (19), and glycosaminoglycans can interfere with some assay systems (20).

The quantitative differences between the Roche and Abbott polyclonal assays demonstrated with the CAP survey specimens may be related to differences in the assay standards. When the Schandl assay by the Abbott polyclonal method the Roche standard equivalent to 125 µg/L, only 37 µg/L (28%) was recovered (8). Conversely, the Abbott 10 and 20 µg/L standards were measured as 1.0 and 2.3 µg/L, respectively, in the Roche assay. In our study, most of the CAP survey specimens produced higher values by the Abbott assay than by the Roche assay. According to the Schandl's data, the Abbott results would have been even higher if they had been based on the Roche standard. On the other hand, the values obtained with the Roche assay would have been higher, coming closer to the Abbott values, if they had been based on the Abbott standards. This implies that the CAP survey specimens are more like the Abbott standards than the Roche standards. This is not unreasonable, considering that the 1985 CEA source material was from Abbott, but it is interesting that the 1983 CEA material obtained from Roche also produced higher results in the Abbott assay. However, the difference in standards probably is not the major explanation for the differences between these assays because both sets of standards produce similar average CEA measurements for patients' specimens (4–7).

In general, the quantitative differences among assay systems are greater for standard or control preparations than for patients' specimens. The differences for patients' results appear to be randomly distributed, with both over- and underestimation (4–7), whereas the standard reference preparations have more systematic, proportional between-assay differences. The differences found for specimens prepared from the purified CEA could represent a random selection process, in which the particular tumors selected for making the CEA preparations correspond to the extreme values in the patient-comparison studies. This, however, is unlikely because purified CEA from two vastly different sources showed the similar systematic differences among the assay systems. More likely, these differences may be related to changes in CEA molecules caused by the processes used to produce the control specimens. Perhaps the purification procedures used to isolate the CEA from the tissue sources select out certain antigenic subclasses of CEA, thereby magnifying the methodologic assay differences. We do not now know the immunochemical causes of these differences, but perhaps future investigations with highly specific monoclonal antibodies will help resolve or reconcile these assay differences.

G.G.K. and G.R. are members of the CAP Ligand Assay Resource Committee, which designs and evaluates the CEA survey for CAP.

References

Discussion
Because CEA is defined in terms of its physical chemistry and immunological properties, a variety of different substances may be included under that definition; this heterogeneity may account for the differences we have noted in this study. CEA, characterized as a glycoprotein with a molecular mass of 186 000 Da and 45 to 57% carbohydrate, is soluble in 1 mol/L perchloric acid and in half-saturated ammonium sulfate (9, 10). It gives a homogeneous band of β mobility on standard electrophoretic systems, but is heterogeneous by isoelectric focusing, with a major band at pH 4.5. The charge heterogeneity of various CEA preparations has been attributed, in part, to variations in sialic acid content of the molecule. The amino acid sequence of CEA is relative-

Fig. 3. Regression line comparison of results obtained with different lots of Abbott EIA polyclonal reagent
For the 12 1983 CAP "K" control results, the results are correlated by a slope of 2.09 (r = 0.996); for the commercial controls, the slope is 1.95; and for the in-house prepared controls, the slope is 1.95. Regression analysis of logarithms of the results for 102 patients' specimens yielded a slope of 0.99 (r = 0.992)

Fig. 4. Log-log comparison of 102 patients' results (C3) obtained with different lots of Abbott EIA polyclonal reagent
The lower line represents the regression line for the logarithms of the patients' results (slope = 0.99, r = 0.992). The upper line represents the regression line for the 1983 CAP specimens. Only two patients' specimens showed differences as large as those found with the survey specimens

as an upward bias of the regression line. The scatter of the patients' values about their regression line suggests that the two patients' values lying on the regression line for the controls probably represents assay variation rather than results for patients with a different form of CEA. Unfortunately, we could not confirm these discrepant patients' values by retesting owing to the unavailability of the previous set of reagents.

G.G.K. and G.R. are members of the CAP Ligand Assay Resource Committee, which designs and evaluates the CEA survey for CAP.

References
Clinical Value of Immunoradiometric Assay of Thyrotropin for Patients with Nonthyroidal Illness and Taking Various Drugs

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Using a two-site immunoradiometric assay, we measured concentrations of thyrotropin (TSH) in serum of 134 clinically euthyroid subjects, 93 patients with nonthyroidal illness, and 80 patients who were being treated with various drugs. Abnormal concentrations of TSH, free thyroxin, and free triiodothyronine, respectively, were recorded in serum of three (3.2%), 19 (20.4%), and 37 (39.8%) of the patients with nonthyroidal illness and in three (3.8%), five (6.3%), and 10 (12.5%) of the patients taking drugs. TSH could be detected in all patients' serum samples. We conclude that, for most patients without thyroid disease, a basal (i.e., unstimulated) measurement of their TSH concentration in serum will indicate their thyroid status more reliably than will assay of free thyroxin or free triiodothyronine.

Additional Keyphrases: thyroid status • screening

Recent developments in monoclonal antibody production have brought about a rapid change: competitive-binding assays are being replaced by immunometric assays to measure hormones. Especially, assays of thyrotropin (TSH) are now predominantly based on immunometric principles and show improved sensitivity (1–4). With assays involving total incubation times of less than 3 h, the concentrations of thyrotropin circulating in euthyroid subjects can be differentiated from the lesser concentrations circulating in thyrotoxic patients. With these assays, measurement of the basal (unstimulated) concentration of TSH will predict the response of a thyroliberin-stimulation test (5); consequently, a single measurement of TSH, supplemented with assays of free thyroxin (FT₄) and free triiodothyronine (FT₃), has been proposed as the preferred strategy for testing thyroid function (6). However, this approach will be valid only if the thyrotropin concentration in most hospital patients without thyroid disease is within the same range of values found in healthy, euthyroid subjects.

We have previously reported the development of a two-site assay for TSH that differentiates thyrotoxic patients from normal subjects (7). We describe here our experience with this assay with a larger population of normal subjects, patients with nonthyroidal illness, and a group of patients taking various drugs.

Subjects and Methods
Subjects. We studied patients admitted to the University Hospital of Wales with severe illness, either chronic liver disease, chronic renal failure, myocardial infarction, or cerebrovascular accident. They were not admitted for investigation of any thyroid disorder and were selected only if they had no pre-existing thyroid disease or family history of thyroid disease. All patients were examined by one of us (P.E.E.) and were judged to have no goiter and to be

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Nonstandard abbreviations: TSH, thyrotropin (thyroid stimulating hormone); FT₄, free thyroxin; FT₃, free triiodothyronine.

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