A Scorecard Doesn’t Help When the Players Keep Changing Shirts

To the Editor:

Killingsworth describes two recent regulatory initiatives by the US Food and Drug Administration (FDA) and urges the clinical laboratory community to lobby against them (1). Although I agree with many of his points, I suggest a different perspective.

In late summer of 1992, the FDA issued a “Draft Guideline for Compliance” to manufacturers of unapproved test kits being sold “For Research Use Only” in the US, yet being widely used for the diagnosis and monitoring of patients. Many of these tests are for the newer tumor markers and are considered Class III Medical Devices, which require a long and expensive premarket approval process overseen by the FDA’s Center for Devices and Radiological Health (CDRH) before they can be used legally in a clinical laboratory. The FDA’s jurisdiction here is generally not questioned, although the agency seemed to look the other way for several years, during which time the tests in question gained acceptance among users. The medical community purchased and offered the tests and used the results with little apparent regard for their unapproved status.

If the draft document is enforced unchanged, the FDA will come down hard on those manufacturers of unapproved test kits. Also, some of the same reagents incorporated into stains for histochemistry or flow cytometry will be targeted for sanctions unless submitted for approval. Included among the analyzers threatened with sanctions are a few that are Class II Medical Devices (and thus require only a 510(k) clearance before clinical use, in general a much shorter and less expensive process), e.g., luteinizing hormone and human chorionic gonadotropin. The implication is that when the reagents include monoclonal antibodies and (or) the analyte is being used as a tumor marker, the test kit falls into a different regulatory category. Now there is a monster can of worms.

A single paragraph of the compliance document, quoted in full by Killingsworth, has erupted into the “home brew” controversy. As a potential piece of rulemaking, the paragraph is poorly written, unclear, out of context, and probably unenforceable. It certainly should disappear in its entirety, and one can only hope and lobby to make that happen. In some early, unofficial follow-up statements, some sources at the FDA have indicated that the paragraph may not remain as written. The FDA needs to be encouraged in that direction. There is overlap with the Clinical Laboratory Improvement Act (CLIA) quality-control (QC) regulations, as Killingsworth indicates, and, in subpart K of the regulations, CLIA outlines the design of appropriate method evaluation and QC programs for modified or in-house tests.

Urinary testing for the human immunodeficiency virus (HIV), the specific example that Killingsworth uses of a threat to laboratory research and development under the “home brew” initiative, actually represents a different, and probably unrelated, FDA action. In the summer of 1991, the FDA ordered laboratories to stop testing urine with kits licensed for testing serum for antibodies to HIV. The key word here is “licensed.” The majority of tests for antibodies to HIV, hepatitis B and C markers, and several other analytes are used not by clinical laboratories, but by the blood-banking community to screen the blood supply. These tests are regulated by a separate section of the FDA, the Center for Biologics Evaluation and Research (CBER). Blood banks themselves, including their testing functions, are regulated by the FDA, not by the Health Care Financing Administration, although agreement has been reached between the two agencies that FDA will regulate blood bank laboratories with CLIA criteria. Tests falling into CBER’s jurisdiction are licensed, rather than approved, and the licensure process, unlike the premarket approval and 510(k) processes, specifically requires that the test be used only according to the manufacturer’s directions. Further, manufacturers of licensed test kits are required to submit samples of every lot of kits to the FDA for testing prior to release of that lot for sale, another major difference from CDRH-regulated kits. “Off-label” usage of these tests has never been allowed, a fact that may have escaped many clinical laboratories. The blood banks accept and support this approach to testing, because the fact that they use test kits exactly as the manufacturers and the regulations require should not only ensure maximum consistency of test results, but also protect them in legal situations. Thus, they work with the FDA and use its authorizations as a shield.

Is the FDA a metastasizing organism to fight against, or a filter to assure at least a minimum quality of the test kits that we use? Contrary to the old expression, you probably can have it both ways, but that costs time, money, energy, and lots of ink.

Reference

Patricia E. Garrett
Boston Biomedica, Inc.
West Bridgewater, MA 02379

Conjugated, but Not Unconjugated, Bilirubin Negatively Interferes in Hitachi 747 Assay of Inorganic Phosphorus

To the Editor:

The most common assays of inorganic phosphorus (P) in serum or plasma are the phosphomolybdate methods: P, complexes with ammonium molybdate in strong acid, resulting in a complex that can be quantified directly by measuring absorption at 340 nm; alternatively, the complex can be reduced to form a blue complex of unknown structure with a maximum absorption at 680 nm. Our laboratory measures P, both ways—by direct measurement at 340 nm (Hitachi 747; Boehringer Mannheim, Indianapolis, IN) and at 680 nm after reduction (Ektachem E700; Eastman Kodak, Rochester, NY).

During the first 2 months of routine operation with the 747, we noted that many icteric samples produced P, results 4-10 mg/L lower than those produced by the E700. However, we found no evidence for an interference on either method when samples were supplemented with a bilirubin standard (Instrumentation Laboratory, Lexington, MA). Furthermore, the manufacturers’ documentation states that both of these P, methods are free from bilirubin interference. All icteric samples that produced discrepant P, contained

CLINICAL CHEMISTRY, Vol. 39, No. 11, 1993 2345
conjugated bilirubin (CBIL) > 50 mg/L. However, the bilirubin standards used in most interference studies, including our own, are primarily unconjugated bilirubin. Therefore, we prepared several mixtures of a normal adult plasma and a plasma with high CBIL content (>120 mg/L). Plasmas were chosen so that the normal and icteric plasma had identical \( P_1 \) values by the Ektachem method.

Mixtures were analyzed by both \( P_1 \) methods. Regardless of CBIL concentrations in the mixtures, the \( P_1 \) value did not change on the Ektachem. In contrast, with the 747 we found a proportional decrease in \( P_1 \) values for samples with increasing CBIL concentrations. The negative bias on the 747 was as great as 10 mg/L when the CBIL was >150 mg/L. To confirm the presence of an interference, we compared values obtained from a well-accepted manual, protein-free, molybdate-based method (1). Assay of 14 samples with CBIL values from 36 to 102 mg/L produced \( P_1 \) values 2-9 mg/L lower on the 747 than those obtained from the manual comparison method (Figure 1). \( P_1 \) concentrations of these samples from the manual method were 290-570 mg/L (mean ± SD = 37.9 ± 7.1). Samples with total bilirubin <20 mg/L produced equivalent results by both methods. Ektachem \( P_1 \) values averaged 1.8 mg/L higher (range 0-4) than the manual method values, but these differences were independent of CBIL values (not shown).

Monitoring absorbances (340 nm primary/376 nm secondary) of the \( P_1 \) reaction on the 747 revealed a potential cause for the negative interference. The 747 makes initial absorbance readings after addition of reagent 1 (R1; acid and detergent) to obtain a sample blank and makes subsequent readings after addition of the ammonium molybdate reagent, R2. The difference in absorbance between the two readings (after correction for sample dilution) is used to calculate \( P_1 \) concentration. The initial R1 absorbance for all nonicteric samples was 0 or slightly positive (100-200 arbitrary absorbance units). In contrast, samples containing >40 mg/L CBIL had a negative initial absorbance, and the magnitude of the negative R1 absorbance was proportional to CBIL.

Preliminary data from our laboratory and from the manufacturer suggest that altering the secondary wavelength may eliminate this interference. Until this is corrected, we advise that \( P_1 \) not be determined for icteric samples on the Hitachi 747. Finally, this observation demonstrates that the use of commercial bilirubin standards for interference studies may not always mimic the interferences caused by in vivo forms of bilirubin.

Reference

Francisco Alvarez
Kenneth Whalen
Mitchell G. Scott

Diag. of Lab. Med.
Box 8118
Washington Univ. School of Med.
St. Louis, MO 63110

1 Author for correspondence.

Urinary Oligosaccharides in Pregnant or Lactating Women: Pitfall in Screening

To the Editor:

Urinary oligosaccharide screening is a useful first-line diagnostic analysis to delineate disorders of glycoprotein degradation (1). The method is not, however, without problems in interpretation, one of the commonest being the excretion pattern found in neonates, which may often cause confusion with that of mannosidosis. In cases of "adult-onset" glycoproteinoses, the oligosaccharide excretion patterns may be weak, and such patients may be overlooked. In the course of metabolic workup of a female adult patient suspected of having a glycoproteinosia, we found a urinary excretion pattern akin to that of mannosidosis, despite a normal activity of \( \alpha \)-mannosidase.

The 29-year-old patient was referred because of mental retardation and schizophrenia. She had bilateral malar hypoplasia, epicanthal folds, and no organomegaly. She was cooperative and verbal and understood simple commands. No facial asymmetry was apparent. Magnetic resonance imaging revealed hypoplasia of the corpus callosum and mild cortical atrophy. Her chromosomes, amino acids, organic acids, very-long-chain fatty acids, carnitine, and biotinidase were all normal. Urinary oligosaccharide screening revealed a pattern similar to that of neonates. \( \alpha \)-Mannosidase activity in leukocytes was normal, and a peripheral blood smear showed no vacuolated lymphocytes.

Because the patient had just delivered a baby, we decided to examine urines from pregnant or lactating women for abnormal urinary oligosaccharide excretion patterns. Urinary oligosaccharides were examined by thin-layer chromatography (1).

The patterns were consistent with that of neonates; i.e., many bands were present that stained violet with orcinol, suggesting the presence of glucose or galactose (Figure 1). Patients with mannosidosis have patterns that stain brown with orcinol reagent. Urines from the pregnant and lactating women we examined (n = 70) also gave a similar pattern. No attempt was made to correlate gestational or postpartum age with the degree of oligosaccharide excretion. In all cases this pattern had diminished by 6 months postpartum, as was the case in neonates.

Human milk contains a large number of oligosaccharides, some of which are excreted in the urine of pregnant and lactating women (2). Lactose and oligosaccharides with a lactose backbone are the most predominant (3).

---

Fig. 1. Thin-layer chromatographic pattern of urinary oligosaccharides

(A) Standard sugars (top to bottom): glucose, lactose, raffinose; (B) patient with \( \alpha \)-mannosidosis; (C) adult control urine; (D) patient (after delivery); (E) pregnant subject; (F) lactating subject; (G) breastfeeding subject; (H) child of subject G