Determination of Amniotic Fluid Bilirubin Absorbance in Specimens Contaminated by Blood

To the Editor:
The careful study of Foster et al. (1) reminds us of the difficulty of assessing amniotic fluid bilirubin absorbance in specimens contaminated by blood. However, it is not correct, as Foster et al. conclude, that bilirubin absorbance cannot be appropriately assessed in specimens contaminated in this manner.

To eliminate interference from nonbilirubin pigments, my colleagues and I (2) exploited the fact that bilirubin is highly photolabile whereas interfering chromagens such as hemoglobin and methemoglobin are remarkably photostable. A few minutes of exposure to an intense light source (such as that from an ordinary slide projector) eliminates most of the bilirubin absorbance and allows the absorbance present before irradiation to be determined as described in the next paragraph. Slide projectors of a type similar to the one used here continue to be manufactured, e.g., the Kodak Carousel 4600 (Eastman Kodak Company) or the Vivitar 5000 AF (Vivitar USA). These and similar projectors and their replacement lamps can be purchased at local camera stores or via the internet at sources such as Amazon.com. They are also readily obtainable “used” at eBay.com. Furthermore, the light sources in modern high-intensity projectors commonly used to display computer files are similar, although such projectors and their replacement lamps are typically much more expensive.

Specifically, the work demonstrated that the photodegradation of bilirubin can be described by second-order kinetics:

$$\frac{dA}{dt} = -(k/A_0)A^2 \quad (1)$$

where \(A\) is the absorbance at 464 nm at time \(t\); \(A_0\) is the absorbance at 464 nm before irradiation; and \(k\) is a constant.

Integration of Eq. 1 yields:

$$\frac{1}{A} = \frac{1}{A_0} + (k/A_0)t \quad (2)$$

from which we see that the reciprocal of the absorbance varies linearly in time and the reciprocal of the intercept at zero time is \(A_0\), the value we seek. Because we actually measure the change in absorbance between the irradiated and the nonirradiated specimens as a function of time, we algebraically rearrange Eq. 2 as:

$$\frac{1}{(A_0 - A)} = \frac{1}{A_0} \quad (3)$$

$$+ \left[\frac{1}{1/(kA_0)}\right](1/t)$$

We thus see that the reciprocal of the intercept of a plot of the change in absorbance as a function of the reciprocal of the time (i.e., a so-called “double-reciprocal plot”) yields \(A_0\) the absorbance of the nonirradiated specimen. In practice, an irradiation time <5 min reduces the absorbance at 464 nm by more than 50%; thus, the extrapolation used in Eq. 3 to obtain the intercept, and hence \(A_0\), is not a distant one.

A dual-beam spectrophotometer allows comparison of the irradiated specimen (in the reference channel) and the nonirradiated specimen (in the measurement channel) in a straightforward manner and renders negligible any drift in the spectrophotometer between periods of irradiation. Moreover, modern spectrophotometers are sufficiently stable that even a single-beam instrument, in which the absorbances for irradiated and nonirradiated specimens for each time period are measured individually, will allow accurate determination of the absorbance difference.

Study of specimens covering the entire range of clinical interest showed that results obtained by the Liley method (3) were the same as those determined via photodegradation performed on the same specimens (2). We advocate measurements at 464 nm because our study (2) determined that this is the actual absorbance peak of bilirubin in an aqueous environment, even when measured by the Liley method. Moreover, we demonstrated that the photodegradation result obtained at 464 nm was within ~5% of that obtained by the Liley method when the latter was determined at any wavelength between 450 and 475 nm. Although my colleagues and I studied the full spectrum of amniotic fluid between 300 and 600 nm, it is necessary only to determine the absorbance at 464 nm to obtain by this method the bilirubin absorbance value used in the Liley nomogram. This determination of the bilirubin absorbance could be accomplished in the face of interfering pigments with absorbances at least 100 times greater than that of bilirubin itself. Thus, the Liley nomogram could be used even in the case of highly contaminated specimens.

In summary, use of photodegradation with a routinely available slide projector as the light source enables the absorbance attributable to bilirubin to be determined even in specimens containing high absorbances of the other pigments commonly seen in amniotic fluid contaminated with blood. The absorbance attributable to bilirubin is determined from a double-reciprocal plot of the change with time of the absorbance at 464 nm between irradiated and nonirradiated specimens. Modern single-beam instruments are sufficiently stable for this approach; the dual-beam instrument used in the original study simplifies the procedure but is not necessary. The results obtained by this method are the same as those obtained by the Liley method in contaminated fluids.

References


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CYP3A5 Genotype Does Not Influence the Blood Concentration of Tacrolimus Measured with the Abbott Immunoassay

To the Editor:

Therapeutic drug monitoring of immunosuppressants is well established as an aid to optimizing patient management after allograft transplantation. Most laboratories measure cyclosporin and tacrolimus by immunoassays. However, measured values may be affected by the presence of circulating metabolites because of the limited specificity of the antibodies used in these assays.

Cyclosporin is metabolized by the cytochrome P450 3A4 (CYP3A4) and cytochrome P450 3A5 (CYP3A5) enzymes. Studies on the biotransformation of cyclosporin by CYP3A4 and CYP3A5 have demonstrated disparate patterns of metabolite profiles between the 2 enzymes for this substrate. CYP3A4 catalyzes the formation of 3 primary cyclosporin metabolites; 2 are monohydroxylated (AM1 and AM9), and the third is demethylated (AM4N). CYP3A5 produces only the AM9 metabolite (1). This may have important implications for immunoassay measurements of cyclosporin, as polymorphic expression of the CYP3A5 gene leads to some individuals not producing the CYP3A5 enzyme (2). In addition, the CYP3A5 enzyme has a lower catalytic activity for cyclosporin compared with CYP3A4. However, the preferential catalysis exhibited by these enzymes is not the same for all substrates.

Preferential production of particular metabolites could occur with tacrolimus. Genetic studies have shown that expressors of CYP3A5 require significantly larger doses of tacrolimus to attain therapeutic blood concentrations of this drug (3). These studies have been based on blood concentrations determined with an immunoassay (MEIA tacrolimus II immunoassay for the IMx analyzer; Abbott Diagnostics). If CYP3A4 and CYP3A5 were to produce different metabolite profiles from tacrolimus, then the degree of interference in the immunoassay would differ for expressors and nonexpressors of CYP3A5.

To investigate this possibility, we compared tacrolimus results, obtained with the Abbott immunoassay, with those of an in-house HPLC–tandem mass spectrometry (MS/MS) assay, using whole blood samples taken from expressors (homozygous CYP3A5*1*1 and heterozygous CYP3A5*1*3) and nonexpressors (homozygous CYP3A5*3*3) of the CYP3A5 gene.

Predose blood samples collected from kidney transplant patients receiving tacrolimus were analyzed. The samples were collected at least 3 months posttransplantation. A total of 136 samples taken from 105 patients were grouped into expressors (n = 74) and nonexpressors (n = 73) of CYP3A5. The samples were analyzed by the Abbott immunoassay and by an in-house HPLC-MS/MS method. Tacrolimus analysis was performed with the Abbott immunoassay and by an in-house HPLC-MS/MS method. The HPLC-MS/MS assay was used as the comparison method. For direct comparison, the Abbott calibrators and controls were used for both methods to minimize any calibration bias. The DNA was ex-