Effects of Specimen Turbidity and Glycerol Concentration on Nine Enzymatic Methods for Triglyceride Determination

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We compared the effects of specimen turbidity and glycerol concentration on nine enzymatic methods for triglyceride measurement. We assayed 51 specimens with triglyceride concentrations of 0.85–8.21 mmol/L (75–727 mg/dL) and turbidity at 420 nm equivalent to ≥0.1 mmol/L (8.8 mg/dL) triglyceride (measured as part of our comparison method). The data were analyzed by multiple regression, which gave coefficients for the effects of glycerol concentration and the change in turbidity during the reaction. The effects of specimen turbidity and glycerol concentration were method-dependent and ranged from 6.20% to −15.67% of the measured result. The magnitude of the turbidity effect (in assays with a significant turbidity interference) was similar to that for glycerol (in assays with a significant glycerol interference). A triglyceride assay with a bichromatic measurement was less subject to interference from turbidity.

Indexing Terms: bichromatic analysis/analytical error/intermethod comparison

Accurate triglyceride test results are important in determining risk for coronary artery disease and in estimating low-density lipoprotein cholesterol (I, 2). Many factors may affect the accuracy of triglyceride measurements (3). Enzymatic methods to determine triglyceride may be affected by the glycerol concentration and turbidity of the specimen.

A recent publication, editorial, and subsequent letters to the editor have discussed the value of a glycerol blank for triglyceride determination (4–8). Certain clinical conditions are associated with an above-normal baseline concentration of glycerol and, consequently, a spurious increase of the triglyceride concentration measured in non-glycerol-blanked methods (5). Thus, Cole has recommended that all clinical chemistry laboratories be able to determine triglyceride with a glycerol blank for specimens from patients with certain diseases (5).

The turbidity of the specimen may also be a factor in determining triglyceride accurately (3). Specimens from patients with increased concentrations of triglycerides are frequently turbid (lipemic). All enzymatic methods for triglyceride determination include lipase, which catalyzes cleavage of the triglyceride molecule into glycerol and free fatty acids. This enzymatic reaction may reduce the turbidity of the specimen during the test reaction and can thus contribute to a change in absorbance that may introduce an error into the final result. The type (falsely high or low) and amount of error involved depend upon the chemical reaction and the type of blanking used (serum, reagent, or bichromatic). We evaluated these factors, glycerol concentration, and the effect of turbidity with nine enzymatic methods for the determination of triglyceride.

Materials and Methods

Instrumentation and Methods

Two of the nine methods were performed on the Hitachi 736 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN): their original glycerol phosphate oxidase ("GPO Trinder") reaction (HGP; cat. no. 1128027) and a newer, glycerol-blanked reaction (HGB; cat. no. 877557). ² We evaluated the Ektachem triglyceride assay with an Ektachem 700 analyzer (Eastman Kodak, Rochester, NY; cat. no. 1648088), using their dry-slide methodology, and the triglyceride assay on the Dimension analyzer (DuPont, Wilmington, DE; cat. no. DF69).

The rest of the methods were performed on a Cobas Fara analyzer (Roche Diagnostics, Belleville, NJ). These included reagent kits by Sigma Diagnostics (St. Louis, MO; cat. no. 337); Abbott Laboratories (North Chicago, IL; cat. no. 6097-03); Boehringer Mannheim (BMN; cat. no. 877557); and Behring (Behring Diagnostics, Somerville, NJ; cat. no. 869263) run as a one-step and a two-step reaction (B1S and B2S, respectively).

The Cobas Fara instrument permitted a bichromatic reading, which enabled us to customize the reaction wavelengths to determine the change in absorbance due to turbidity before and after the triglyceride assay. We chose the Behring kit for triglyceride as the "reference" comparison method because the reagents are supplied in three separate bottles, which permitted the assay to be blanked for both glycerol and turbidity. Bottle A contains pyruvate kinase (PK), lactate dehydrogenase (LDH), NADH, ATP, phosphoenolpyruvate (PEP), α-chymotrypsin, magnesium, phosphate buffer, and stabilizers; bottle B contains glycerol kinase (GK) and stabilizers; and bottle C contains lipase and stabilizers. The reactions are as follows:

² Nonstandard abbreviations: B1S, Behring's one-step triglyceride method; B2S, Behring's two-step triglyceride method; BMN, Boehringer Mannheim's triglyceride method; HGB, Boehringer Mannheim's glycerol-blanked triglyceride method; and HGP, Boehringer Mannheim's glycerol phosphate oxidase Trinder triglyceride method.
Triglyceride $\xrightarrow{\text{Lipase}}$ glycerol + free fatty acids (1)

Glycerol + ATP $\xrightarrow{\text{GK}}$ $\alpha$-glycerol phosphate + ADP (2)

ADP + PEP $\xrightarrow{\text{PK}}$ ATP + pyruvate (3)

Pyruvate + NADH $\xrightarrow{\text{LDH}}$ lactate + NAD$^+$ (4)

The B2S triglyceride assay is performed in a single cuvette with two steps. In the first step, the serum sample and reagents from bottles A and B are combined and incubated for 300 s, which consumes all the endogenous glycerol (one cuvette also contains water in place of serum for the reagent blank). These reactions (reactions 2–4) are an “internal blank” for glycerol; i.e., any endogenous glycerol is converted to lactate (9). Then, absorbance is measured at 340 nm and 420 nm; the 420-nm reading is used as the turbidity blank, and the 340-nm reading is the glycerol-free serum blank. Next, the contents of bottle C (containing lipase) are added, which convert triglyceride to glycerol and free fatty acids and reduce turbidity (reactions 1–4). After 500 s, absorbance is again measured at 340 nm and 420 nm. The change in absorbance ($\Delta$A) at 420 nm is multiplied by a factor of 1.736 (the difference in absorbance due to turbidity at 420 nm and 340 nm for our instrument) and subtracted from the $\Delta$A at 340 nm; the triglyceride result is thus blanked for both glycerol concentration and turbidity. Multiplying the $\Delta$A at 420 nm by the wavelength conversion factor (1.736) and the calibration constant ($\Delta$A per 1 mmol/L) from the calibration curve estimates turbidity concentration (triglyceride equivalent concentration in units of mmol/L). In a separate assay, we also used the Behring reagents to determine glycerol (measured at 340 nm without any lipase added; reactions 2–4). The glycerol content and change in turbidity due to lipase were analyzed by multiple regression to assess interference among the triglyceride methods. Given the complexities of measuring turbidity, the coefficients derived from the B2S method are only an estimate of the mean effect of turbidity on the method and are not intended as “correction factors.”

We chose 420 nm for the B2S turbidity blank, after performing a study that compared the effect of lipemia on the B2S reagents at different wavelengths. We measured the absorbance of three lipemic serum specimens at 340, 420, 500, 600, and 700 nm before and after incubation with lipase (bottle C, reaction 1) for 500 s. The $\Delta$A at each wavelength was compared with the $\Delta$A at 340 nm (the measurement wavelength affected by the turbidity) and gave the following percentages: 420 nm, 57.6%; 500 nm, 34.6%; 600 nm, 21.5%; and 700 nm, 16.1%. We used the inverse of each percentage as the factor to adjust the measured $\Delta$A at the other wavelengths to equal the $\Delta$A at 340 nm. We selected the $\Delta$A at 420 nm to estimate the turbidity adjustment because this wavelength gave the greatest change after the addition of lipase and was not affected by the absorbance of NADH/NAD$^+$.

Standards and Reagents

We used an aqueous-based Sigma triglyceride calibrator for those methods run on the Cobas Fara (Sigma, Abbott, B1S, B2S, BMN). The two methods run on the Hitachi 736 (HGP and HGB) were calibrated with the serum-based Precical (Boehringer Mannheim Diagnostics). The Ektachem method used serum-based calibrator at three concentrations. The Dimension method had three concentrations of aqueous-based calibrators. We tested all calibrators for glycerol and for triglyceride with the B2S reagent. Only the Sigma calibrator was almost all triglyceride with minimal glycerol (0.01 mmol/L). The Dimension calibrators were glycerol alone, and both the Ektachem and the HGB and HGP calibrators included some glycerol. To eliminate differences due to the different instrument calibrations, we adjusted the results for these last four methods for the difference from the Sigma calibrator by using the values in Table 1: (assigned value/measured value) × result.

We reconstituted and used all reagents as instructed in the manufacturer’s package inserts, except for B2S, where we used a second wavelength to estimate turbidity. Table 2 lists the blanking method, the reaction characteristics, and the wavelengths for each assay. All reagents for the Abbott and B1S methods were combined into one solution each. The following two blanks were determined for these two methods: a reagent blank and a serum blank (initial absorbance reading taken immediately after addition of serum sample and all reagents). To evaluate the effects of a reagent blank vs a serum blank, we recalculated the $\Delta$A values from these two methods, using only the reagent blank, and compared these results with the serum-blanked results and with those of the comparison method (B2S).

Procedures

We determined the serum triglyceride concentration in specimens from 51 patients [range, 0.85 to 8.17 mmol/L] by measuring the absorbance at 420 nm and adjusting for turbidity.

Table 1. Concentration (mmol/L) of triglyceride and glycerol in calibration material.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Assigned value*</th>
<th>Glycerol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precical</td>
<td>1.57 (HGB)</td>
<td>0.05</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>1.65 (HGP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ektachem 1</td>
<td>0.74</td>
<td>0.16</td>
<td>0.55</td>
</tr>
<tr>
<td>Ektachem 2</td>
<td>2.63</td>
<td>0.10</td>
<td>1.98</td>
</tr>
<tr>
<td>Ektachem 3</td>
<td>5.49</td>
<td>0.19</td>
<td>4.08</td>
</tr>
<tr>
<td>Sigma</td>
<td>5.85</td>
<td>0.01</td>
<td>5.67</td>
</tr>
<tr>
<td>Dimension 1</td>
<td>1.36</td>
<td>1.37</td>
<td>0.06</td>
</tr>
<tr>
<td>Dimension 2</td>
<td>2.71</td>
<td>2.78</td>
<td>0.01</td>
</tr>
<tr>
<td>Dimension 3</td>
<td>5.48</td>
<td>5.68</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Assigned by the manufacturer.
* Measured by the Cobas Fara with the Sigma calibrator.
* Ektachem results may be falsely lowered because of the presence of anticoagulating agents in the formula, which prevented the reaction from going to completion.
mmol/L (75 to 723 mg/dL) with the nine different methods. In addition, we determined the turbidity of all the specimens as part of the B2S triglyceride assay, and measured the glycerol concentrations in a separate assay. We primarily chose lipemic specimens from those that were submitted to our laboratory for routine chemistry testing, including some for whom ultracentrifugation was indicated. All specimens included in the study had a measured turbidity of ≥0.1 mmol/L (8.8 mg/dL) triglyceride equivalent concentration. Specimens too concentrated for the linear range for any particular method were diluted, and the assays were repeated according to the specifications of each manufacturer. This study was in progress when Boehringer Mannheim released the HGB. Therefore, the HGB method was used to assay 26 specimens, whereas at least 48 samples were analyzed by the other methods.

Statistics

We analyzed the results from the nine methods in several ways. First, we used the B2S triglyceride result, the glycerol result, and the turbidity result as the independent variables for multiple regression against each of the other eight methods. This regression gave us coefficients that show the magnitude of the glycerol effect and the turbidity effect, and a slope and intercept correlating the comparison method with the evaluated method adjusted for both effects. Second, we performed linear least-squares regression, comparing results from the comparison method with the following four values for the evaluated methods: unadjusted results, and results adjusted for glycerol, adjusted for turbidity, and adjusted for both effects by use of the coefficients from the multiple regression. We compared the difference between a reagent blank only vs a serum blank (which includes the reagent blank) for the Abbott and B1S methods by using Deming debiased regression (9). Last, we compared the values from the two different blanks from the Abbott and B1S methods with the B2S by linear least-squares regression.

Results

The results of this study show variability among the eight methods evaluated for triglyceride determination (Tables 3 and 4 and Fig. 1). Table 3 presents the slope, intercept, correlation coefficient, and the coefficients (calculated by multiple regression) that indicate the magnitude of the effects of glycerol and turbidity. The mean unadjusted result, the mean adjustments for glycerol and turbidity, and the mean adjusted result for both glycerol and turbidity for each of the eight methods (based on the information in Table 3) are shown in Table 4. We chose absorbance as a readily available measure of turbidity; the coefficients derived are an estimate of the magnitude of the turbidity present. The adjustments made in Table 4 are to demonstrate the magnitude and direction of error and should not be used to change patients' results.

Fig. 1 demonstrates the magnitude of the effects by showing a linear least-squares regression line for the unadjusted result (Eq. 1), adjusted only for glycerol (Eq. 2), adjusted only for turbidity (Eq. 3), and adjusted for both interferences (Eq. 4). Only the HGB method had no interference by both effects, as shown by all four lines on the graph being essentially superimposed. The three glycerol-blanked methods (HGB, BMN, and Sigma) showed a nonsignificant glycerol effect and have the lines for Eqs. 1 and 2 superimposed. The three bichromatic methods (HGB, HGP, and Dimension) showed a nonsignificant turbidity effect and the lines for Eqs. 1

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>Slope</th>
<th>Intercept, mmol/L</th>
<th>Glycerol coefficient</th>
<th>Turbidity coefficient</th>
<th>P</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>26</td>
<td>0.904</td>
<td>-0.04</td>
<td>0.082</td>
<td>&gt;0.4</td>
<td>-0.030</td>
<td>&gt;0.8</td>
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<tr>
<td>BMN</td>
<td>50</td>
<td>1.026</td>
<td>-0.02</td>
<td>0.027</td>
<td>&gt;0.7</td>
<td>-0.190</td>
<td>&lt;0.002</td>
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<tr>
<td>HGP</td>
<td>51</td>
<td>0.917</td>
<td>0.01</td>
<td>0.639</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>DIM</td>
<td>48</td>
<td>0.913</td>
<td>0.03</td>
<td>0.582</td>
<td>&lt;0.001</td>
<td>-0.037</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>SIG</td>
<td>48</td>
<td>1.035</td>
<td>-0.27</td>
<td>0.120</td>
<td>&gt;0.3</td>
<td>-0.351</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EKT</td>
<td>50</td>
<td>1.070</td>
<td>0.01</td>
<td>0.448</td>
<td>&lt;0.009</td>
<td>-0.386</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>ABB</td>
<td>51</td>
<td>0.982</td>
<td>0.24</td>
<td>0.518</td>
<td>&lt;0.0002</td>
<td>0.699</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B1S</td>
<td>50</td>
<td>1.012</td>
<td>-0.15</td>
<td>0.292</td>
<td>&lt;0.02</td>
<td>0.461</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

These values, derived from multiple regression, compare the results from the comparison method (B2S, x) with those from each of the other tests (y). The glycerol and turbidity results are included in the comparison to generate coefficients that indicate the amount of effect that these interferences have on each method; further, the slope, intercept, and r have been corrected for glycerol and turbidity interference by the coefficients.
Table 4. Effect of adjustment for interference by glycerol and turbidity among eight triglyceride methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Unadjusted mean, mmol/L</th>
<th>Adjustment amount</th>
<th>Adjusted mean, mmol/L</th>
<th>B2S mean, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>26</td>
<td>4.17</td>
<td>0.06</td>
<td>4.14</td>
<td>3.70</td>
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<tr>
<td>BMN</td>
<td>50</td>
<td>4.02</td>
<td>0.01</td>
<td>4.17</td>
<td>3.61</td>
</tr>
<tr>
<td>HGP</td>
<td>51</td>
<td>4.06</td>
<td>0.31</td>
<td>3.74</td>
<td>3.89</td>
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<tr>
<td>DIM</td>
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<td>4.03</td>
<td>0.28</td>
<td>3.78</td>
<td>3.96</td>
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<tr>
<td>SIG</td>
<td>48</td>
<td>3.64</td>
<td>0.06</td>
<td>3.86</td>
<td>4.20</td>
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<td>EKT</td>
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<td>4.22</td>
<td>0.22</td>
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<td>4.20</td>
</tr>
<tr>
<td>ABB</td>
<td>51</td>
<td>5.08</td>
<td>0.25</td>
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<td>4.08</td>
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<tr>
<td>B1S</td>
<td>50</td>
<td>4.52</td>
<td>0.14</td>
<td>3.99</td>
<td>4.09</td>
</tr>
</tbody>
</table>

* a. Mean values from the patients' samples.
* b. Derived by multiplying the separate coefficients from the regression for the assay by the glycerol concentration and the triglyceride equivalent concentration for turbidity.
* c. The mean of this method for the n of the regression.
* d. Adjusted for calibrator differences listed in Table 1.

and 3 are superimposed. The Abbott and the B1S graphs are examples of methods affected by both interferences present and in the same direction; each adjustment takes the result closer to the line of identity. The Ektachem graph shows interferences in opposite directions; the glycerol adjustment is negative but the turbidity adjustment is positive. The mean coefficient for the glycerol effect, based on the five methods that had a significant glycerol coefficient (B1S, Abbott, Ektachem, Dimension, and HGP), is 0.496 (unitless). The mean coefficient for the turbidity effect, based on the five methods that had a significant turbidity coefficient (BMN, Sigma, B1S, Abbott, and Ektachem), is 0.417. Thus, the magnitude of the effect of turbidity interference is similar to but a little less than (84.1%) that for glycerol.

Only the HGB method shows no significant interference from either glycerol or turbidity. The slope of 0.904 (Table 3) comparing HGB and B2S is primarily due to the differences in the calibration materials. The BMN method (with a slope of 1.03 vs B2S) used the same reagents as HGB but was performed on the Cobas Fara with the same calibrator as B2S (Sigma calibrator). In a separate experiment, we compared the HGB method on the Hitachi with the BMN reagents on the Cobas Fara (using the Hitachi calibrator with each method); the slope of the regression for the comparison was 1.04.

The properties of the triglyceride methods listed in Table 2 summarize our findings and predict the presence and direction of glycerol and turbidity effects. Only those methods with a glycerol blank have no significant effect for glycerol, and only those methods that are bi-chromatically blanked have no significant effect for turbidity.

The regression data for comparing a reagent blank (y) with a serum blank (x) for the Abbott method (slope = 0.792, intercept = -0.528, r = 0.755, P < 0.0001) and for the B1S method (slope = 0.858, intercept = 0.223, r = 0.981, P < 0.0001) show that the triglyceride result when only a reagent blank was used was significantly lower (P < 0.001, by paired sample t-test) than the triglyceride result obtained with use of a serum blank. The regression data comparing the two types of blanking used with the Abbott and B1S methods vs the B2S method also show a lower result for the reagent blank only (Fig. 2).

A comparison of the final absorbance reading at 420 nm (in the B2S method) with the ΔA at 420 nm shows a significant correlation (r = 0.396, P < 0.04). This suggests the presence of residual absorbance due to turbidity after the addition of lipase. The final absorbance at 420 nm, which is on average one-third higher than the ΔA attributable to the clearing of lipase, is essentially that of the serum blank plus residual turbidity. The bias
The Abbott lipase blank coefficients samples reagent method. (y = x); () the line of regression and (•) the measured data for the serum blank (Eq. 1); () the line of regression and (•) the measured data for the reagent blank (Eq. 2).

Fig. 2. A comparison of a serum or reagent blank for the Behring 1 step (BIS) method and for the Abbott (ABB) method with the B2S method.

The number, regression equation, P value, correlation, and standard error of the regression are shown. All results are in mmol/L. ( . . . ) the line of identity (y = x); () the line of regression and (•) the measured data for the serum blank (Eq. 1); () the line of regression and (•) the measured data for the reagent blank (Eq. 2).

between serum-blanked samples and reagent-blanked samples indicates that some absorbance from the serum is not corrected by the reagent blank. The turbidity coefficients from multiple regression for the two different blanks were the following: Abbott method (38 samples) with a reagent blank only, -0.41 ± 0.35 (P > 0.2); Abbott method with serum blank, 0.63 ± 0.09 (P < 0.0001); B1S method (50 samples) with a reagent blank only, -0.07 ± 0.07 (P > 0.3); and B1S method with a serum blank, 0.46 ± 0.07 (P < 0.0001). Thus, the turbidity coefficient in the multiple regression was significant for the serum blank but not the reagent blank for both methods. The correlation and precision between the two blanking procedures were better for the B1S method (r = 0.98 and S_{pb} = 0.30) than for the Abbott method (r = 0.76 and S_{pb} = 1.15). Further, comparing the change in turbidity (after the addition of lipase) with the triglyceride concentration of the specimen showed a significant correlation (r = 0.53, P < 0.0005).

Lipase brings about a hydrolysis of triglyceride within 100 s after mixing. Fig. 3 shows the absorbances from several of the more turbid specimens and from the reagent blank that were obtained with the B2S triglyceride assay. The Cobas Fara makes an initial reading for the serum blank, and then adds lipase. A first reading is made at 0.5 s as the cuvette spins to mix the sample and reagents; the additional readings are made at 100-s intervals. As would be expected, the initial absorbance is directly related to the triglyceride concentration of the specimen. A large drop in absorbance occurs rapidly (within 0.5 s) after the lipase is mixed with the triglyceride; the reaction plateaus after 100 s of incubation. Any dilutional effect from the added lipase is minimal because of the design of the Cobas cuvette, which is longitudinal to the light path (10).

Discussion

Specimens from patients with hypertriglyceridemia are frequently turbid. The turbidity of the specimen may change when enzymatic methods are used to determine triglyceride because lipase hydrolyzes triglyceride to free fatty acids and glycerol. Our study indicates a significant correlation between the magnitude of the turbidity and the triglyceride concentration. We evaluated the effect of the change in turbidity during the reaction among eight triglyceride methods and found that two factors influenced the magnitude of the turbidity effect: the direction of change of absorbance for the assay, and the type of blank correction.

The direction of change in absorbance for the triglyceride method is inversely related to the error due to turbidity, independent of any blanking procedure. The turbidity blank will always decrease during the reaction because lipase hydrolyzes triglyceride. If the method shows an increase in absorbance directly related to the triglyceride concentration, the change in absorbance will be reduced by the turbidity effect, and the reported results will be spuriously low. The three methods with
increasing absorbance and significant turbidity effect (BMN, Sigma, and Ektachem) show that the unadjusted result increases when adjusted for the turbidity effect. The opposite is true for the two triglyceride methods (B1S and Abbott) in which the change in absorbance is inversely related to the triglyceride concentration.

Four different types of blanking may be used for triglyceride assays: serum, reagent, glycerol, and bichromatic (3, 11, 12). A serum-blanked test determines the absorbance of serum (including turbidity), and sometimes of reagents before the reaction, and subtracts this value from the final absorbance reading. The ΔA for the reaction includes a decrease in absorbance from the action of the lipase on triglyceride, which would bias the result—either positively or negatively, depending on the direction of the reaction, as mentioned above (3, 11, 12). Thus, a serum blank does not correct for the change in turbidity caused by the addition of lipase.

A reagent-blanked test adds the reagent before the serum sample, takes an absorbance reading, and subtracts this value from the final reading. The reagent blank is independent of turbidity. If lipase completely removes the turbidity of the specimen and a reagent blank is measured, then only the absorbance due to the serum blank would bias the assay (11). When only a reagent blank was used with two of our methods (Abbott and B1S) and the results were compared with the B2S method by multiple regression, the turbidity coefficient was not significant, indicating that the lipase in the reaction was effective in reducing turbidity. However, when we compared the use of only a reagent blank with the use of a serum blank for these two methods, we found a significantly higher result with the serum blank—because of the turbidity that is part of the serum blank. Further, we found an absorbance due to residual turbidity after the addition of lipase. Thus, the reagent blank failed to account for the absorbance due to the serum and the residual turbidity.

A glycerol-blanked test involves performing an initial reaction with the serum to eliminate endogenous glycerol and then reading the blank absorbance, which would include the absorbance due to turbidity but not glycerol. After the addition of lipase, the final absorbance reading would be spuriously low due to the clearing of the turbidity in comparison with the turbidity still included in the glycerol-cleared serum blank absorbance (3, 12). Thus, the glycerol blank has the same problem as the serum blank for turbidity; i.e., the change in turbidity during the reaction is not captured by the blank.

A bichromatically blanked test measures absorbance at two different wavelengths, one corresponding to the peak absorbance of the chromophore and another near the base of the peak to serve as a baseline value. Because the ΔA for the secondary wavelength (where only the change caused by turbidity is seen) is subtracted from the ΔA for the measuring wavelength, the assay would be corrected for turbidity (3). Three methods (HGB, HGP, and Dimension) with a bichromatic blank have the smallest turbidity effect and are the only assays with a nonsignificant coefficient for turbidity. Thus, the interference by turbidity on a triglyceride method would be better minimized by a bichromatic blank than by a reagent or serum blank.

The validity of the bichromatic blank is based on the assumption that the background absorbance of the specimen is the same at both wavelengths (13). As we documented in this study, however, the absorbance due to turbidity differed at 340 nm and 420 nm, so we used a correction factor to approximate the amount of absorbance due to turbidity at the reaction wavelength. The bichromatic blank is superior to the reagent blank or serum blank when the absorbance of a nonchromophore constituent that absorbs at the wavelength for the assay happens during the reaction. Thus, we recommend a bichromatic glycerol-blanked method to obtain accurate triglyceride results for turbid specimens.

We thank Ellen Emmerich for her assistance with the turbidity effects at different wavelengths utilized in the comparison method (B2S).

References