Glycerol Blanking in Triglyceride Assays: Is It Necessary?

As evidenced by the article by Jessen et al. in this issue, the question of whether to blank triglyceride analyses for glycerol is still alive and well and, as ever, controversial. The value of glycerol blanking has been debated in several such articles over the years, and, because of complexities involved, will probably be a source of disagreement in the foreseeable future. A laboratory director wishing to make a rational decision about incorporating a glycerol blank in a triglyceride analysis must consider well several issues.

Essentially all commonly used clinical methods for determining triglyceride concentration measure the glycerol that has been chemically or enzymatically hydrolyzed from triglyceride, rather than the triglyceride itself. Overestimation of triglyceride occurs when glycerol from sources other than from hydrolyzed triglyceride is also measured. Increased concentrations of interfering glycerol in plasma may arise from a wide variety of sources: metabolic disorders, stress, parenteral nutrition, and use of glycerol-containing intravenous medications or of blood-collection tubes with glycerol-coated stoppers. In some situations, activated lipases in the blood continue to hydrolyze triglyceride to glycerol and free fatty acids after the blood sample has been taken from the patient, so that the glycerol concentration varies with time in vitro (1). Given these possible sources of error, the routine inclusion of a glycerol blank for all triglyceride analyses seems prudent. However, is such action always necessary?

Suitable reagent systems for glycerol-blanked triglyceride measurements are available for most clinical analyzers. Why then are not all triglyceride measurements glycerol-blanked as a matter of course? The answer is simple: time and money. The available glycerol-blanking systems function in one of two ways. The "internal blanking" method uses a two-part reagent formulation in such a way that the free glycerol in the sample in a single cuvette is consumed before the addition of the second reagent, which contains lipase to hydrolyze the triglyceride. The liberated glycerol is then measured as the "true triglyceride concentration." The major drawback of this method is that it requires an analyzer capable of multiple reagent additions. In addition, the magnitude of the glycerol blank is not determined, although this value may be of some clinical interest. The "external blanking" method requires analyses on duplicate samples, one determined with the lipase component and one lacking it; the difference between the two measurements is the "true triglyceride concentration." This method can be run on any analyzer, but requires two separate reagents and the analysis of two samples, essentially doubling the cost and time required for analysis of a single plasma sample. However, this method does provide a quantitative measure of the glycerol blank.

In most samples, particularly from free-living, healthy subjects, the amount of free glycerol is small, generally less than that from a 0.23 mmol/L (200 mg/L) concentration of triglyceride. Some researchers have suggested that, because it represents only a small and generally inconsequen-

tial error in the measurement of triglyceride, the glycerol from nontriglyceride sources can be compensated for effectively by subtracting a calculated portion of the measured total triglyceride concentration. Although this method is simple and suffices in most situations, it seems more appropriate to report the measured value and to indicate that it contains an unmeasured amount of glycerol. Otherwise, the recipient of the information may put unwarranted trust in the validity of the value reported.

Purists may argue that if triglyceride concentration is to be measured at all, it should be done with the greatest accuracy possible; pragmatists invoke the point of diminishing returns. In the real world, the pragmatists seem to win more often, as evidenced by the incredibly small number of laboratories that perform glycerol-blanked triglyceride analysis. But what may appear to be a denial of good laboratory practice is complicated by the fact that, for the vast majority of triglyceride analyses, the blanked value has no more clinical importance than the nonblanked value, owing to the small amount of glycerol present in most samples. Therefore, from an economic standpoint, blanked measurements may validly be reserved for only those situations in which the glycerol blank is truly required. In such cases, a request for a glycerol-blanked analysis must be made by the ordering physician, or a "reflex" system should be instituted for retesting samples with abnormally high triglyceride concentrations. Although ideally the laboratory would always include a glycerol blank for each triglyceride measurement, such may not be economically feasible, as noted above.

When all samples are not routinely blanked for glycerol, the laboratory must determine why the sample was submitted for analysis and must know the population from which it was drawn, if it is to make the correct decision whether to include a glycerol blank. Because such information is generally not readily available to the laboratory, inconsistent application of glycerol blanking will undoubtedly ensue. However, if the information is available, the laboratory director should consider the following points in the decision-making process:

• Screening for triglyceride status. The current guidelines for the assessment of triglyceride status define normotriglyceridemia as a triglyceride concentration of ≤2.82 mmol/L in fasting plasma, whereas in definite hypertriglyceridemia the triglyceride concentration exceeds 5.65 mmol/L (2, 3). Triglyceride concentrations between 2.82 and 5.65 mmol/L are considered borderline and require further monitoring of the subject. If the purpose of an ordered test is to assess triglyceride status, a result of ≤2.82 mmol/L would correctly classify the subject as normotriglyceridemic, even if the endogenous glycerol content of the sample was very high. Of course, values >2.82 mmol/L would require glycerol blanking for proper diagnosis of abnormalities. In such situations, "reflex" glycerol blanking would be a suitable alternative to routine glycerol blanking for all samples.
• Assessment of cardiovascular risk. An important use of triglyceride measurements is for estimating the concentration of low-density lipoprotein (LDL) cholesterol, a major risk factor for cardiovascular disease. Given the labor and expense involved in measuring LDL cholesterol directly, the most common method for approximating LDL cholesterol involves an equation in which the very-low-density lipoprotein (VLDL) cholesterol is estimated from the total triglyceride concentration in plasma (e.g., 4). Several factors suggest that non-glycerol-blanked triglyceride analyses are suitable for estimation of LDL cholesterol: (a) Absolute errors in the measurement of plasma triglyceride concentration translate into smaller errors in LDL cholesterol concentration, because the triglyceride value is most commonly divided by 2.29 (based on the molecular mass of triolein); or divided by 5 when triglyceride concentrations are in units of mg/L (4). (b) The Friedewald equation (4) was developed with use of triglyceride concentrations that were not glycerol-blanked; however, a recent reassessment of the equation shows that the divisor of 2.29 is also suitable if triglyceride measurements are glycerol-blanked (5). (c) The formula method for determining LDL cholesterol concentration is only an estimate and depends critically on the assumption that the lipid composition of VLDL is constant in all individuals, which it is not. (d) Cardiovascular risk assessment should be considered only in individuals who are in a stable metabolic condition that reflects their normal everyday lifestyle; generally, the glycerol concentration under these conditions is <0.23 mmol/L, which would translate into an overestimation of LDL cholesterol of about 0.10 mmol/L, if the glycerol blank is not taken into account. (e) The Friedewald equation is valid only for triglyceride concentrations <4.52 mmol/L. In view of all these factors, the expenditure of more time and energy to obtain extremely precise and accurate triglyceride measurements does not seem warranted to attain an assessment of cardiovascular risk by estimating LDL cholesterol concentrations.

• Population. Although the amount of free glycerol in the plasma of free-living people is generally small, the amount in hospitalized patients can be quite large, depending on the cause of the hospitalization and the treatment given. Therefore, for the hospital population it is most prudent to include the glycerol blank and, if possible, to report the glycerol concentration, thereby allowing the physician to better evaluate the results.

Ideally, convenient and economical glycerol-blanking methods will be developed for all analytical systems; however, in the interim I have the following recommendations:

1. All laboratories should offer a glycerol-blanked triglyceride analysis, even though it may be performed only when requested, or when the reflex testing criterion is exceeded. A reflex "trigger" of 2.82 mmol/L for total triglyceride concentration is reasonable.

2. Reports from the laboratory should clearly state whether the triglyceride analysis was glycerol-blanked, e.g., designated as "Blanked Triglyceride" or "Unblanked Triglyceride." Physicians need to be educated as to how the inclusion of a glycerol blank may alter the meaning of the results. With this information, the physician, not the laboratory, will be responsible for interpreting the results in light of the medical status of the patient and of the treatment the patient has received.

3. Glycerol blanking of triglyceride measurements should be mandatory in laboratories that specialize in assessment of lipid status, have large populations of hyperlipidemic subjects, or participate in clinical or basic research. Glycerol blanking is a requirement for participation in the Lipid Standardization Program conducted by the Centers for Disease Control.

4. Glycerol blanking of triglyceride analyses need not be routinely conducted on outpatients' samples, unless economically feasible. However, because of the potential for higher glycerol concentrations in inpatients, all inpatients' samples should be routinely glycerol-blanked.

5. Instrument manufacturers and reagent suppliers should be encouraged to provide systems in which glycerol blanks can be economically incorporated into all triglyceride analyses. In the event of the broader availability of economical glycerol-blanking methods, all laboratories can be encouraged to glycerol-blank all triglyceride measurements and, if possible, report the value of the glycerol blank along with the triglyceride concentration. In the interim, however, judicious use of the glycerol blank is acceptable.

References

Thomas G. Cole
Chair, Ad Hoc Triglyceride Review Committee
Lipids & Lipoprotein Division, AACC
Lipid Research Center
Dept. of Medicine, Box 8046
Washington Univ. Sch. of Medicine
St. Louis, MO 63110