Lipid and Lipoprotein Testing in Resource-Limited Laboratories

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The role of total cholesterol (TC) and lipoproteins in the assessment of coronary heart disease (CHD) is firmly established from population and intervention studies. Total and low-density lipoprotein cholesterol (LDLC) levels are positively associated with CHD, and high-density lipoprotein cholesterol (HDLC) levels are negatively associated with CHD. Efforts to identify and treat people at increased risk based on cholesterol and lipoprotein levels have led to more lipid testing and the need for very reliable test results. Thus, quality laboratory services are an essential component of healthcare delivery and play a vital role in any strategy to reduce morbidity and mortality from CHD. In laboratories with limited resources, establishing laboratory capability to measure CHD risk markers may be a considerable challenge. Laboratories face problems in selecting proper techniques, difficulties in equipment availability and maintenance, and shortage of supplies, staffing, and supervision. The Centers for Disease Control and Prevention (CDC) has been providing technical assistance for more than 30 years to laboratories that measure lipids and lipoproteins and is willing to provide technical assistance as needed for other laboratories to develop this capability. CDC can provide technical assistance to establish lipid and lipoprotein testing capability to support a CHD public health program in areas with limited laboratory resources. This assistance includes: selecting a suitable testing instrument; providing training for laboratory technicians; establishing a simple quality control plan; and instructing staff on how to prepare frozen serum control materials suitable for assessing accuracy of lipid and lipoprotein testing. (*Ethn Dis*. 2003;13[suppl2]:S2-107–S2-109)

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Introduction

The role of total cholesterol (TC) and lipoproteins in the assessment of coronary heart disease (CHD) is firmly established from population and intervention studies.[1] Total and low-density lipoprotein cholesterol (LDLC) levels are positively associated with CHD, and high-density lipoprotein cholesterol (HDLC) levels are negatively associated with CHD. Efforts to identify and treat people at increased risk based on cholesterol and lipoprotein levels have led to more lipid testing and the need for very reliable test results. Thus, quality laboratory services are an essential component of healthcare delivery, and play a vital role in any strategy to reduce morbidity and mortality from CHD.

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Methods

For TC, triglyceride (TG), and lipoproteins, there exists sophisticated and technically demanding high-level analytical methods that serve as the references for establishing true values for these biochemical markers.[2] These sophisticated and technically demanding methods are beyond the ability of most routine clinical and hospital laboratories and are, therefore, performed in only a few highly specialized laboratories throughout the world, such as, the Centers for Disease Control and Prevention (CDC), the Cholesterol Reference Method Laboratory Network,[3] and the National Institute for Standards and Technology. For routine testing of TC and TG, almost all clinical laboratories use enzyme-based methods. High-density lipoprotein cholesterol (HDLC) is defined by the method used to isolate the lipoproteins and includes a family of similar particles that vary in size and composition. Until the recent introduction of direct assays to measure HDLC, the most widely used technique to determine HDLC was selective chemical precipitation. Selective precipitation of HDLC cholesterol occurs by mixing polyanions and divalent cations or other chemicals to precipitate very low-density lipoprotein cholesterol (VLDLC) and LDLC that are sedimented by low-speed centrifugation. High-density lipoprotein cholesterol (HDLC) in the supernate is quantitated by conventional enzymatic assays. The traditional approach to determining LDLC is by either of 2 methods. The first method combines ultracentrifugation and polyanion precipitation and is generally referred to as "beta quantification." This technique is very involved and serves as the reference method for LDLC but is not used for routine clinical measurements. The most commonly used method for routine clinical purposes is one in which LDLC is estimated from primary measurements of TC, TG, and HDLC using the Friedewald equation[4]:

([LDLC = TC − HDLC − TG/5];

where the factor TG/5 is an estimate of VLDL cholesterol).

Within the last few years, new methods have been developed that permit the direct measurement of both HDLC and LDLC, thus eliminating the need for a separation step. Both of these new methods can be fully automated and added to most routine clinical chemistry analyzer platforms. Although this is a significant advantage since it eliminates

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the separation step, the use of the new assays by laboratories in resource-limited countries may not be possible because of availability and cost of reagents.

Although most routine clinical laboratories in developed countries use moderate to large-scale chemistry analyzers with sophisticated electronics, computer control, and special utility requirements, the use and support of such instrumentation in resource-limited laboratories is probably not possible and in most cases not warranted. Hospitals in large urban areas may be able to support the use of sophisticated instrumentation, but inadequate staff training, potential lack of technical support, maintenance, and reagents will limit their effective use. A more reasonable approach for performing lipid and lipoprotein measurements in resource-limited laboratories is the use of a newer generation point-of-care or physician's office analyzer. These instruments are typically lower in cost, compact, portable, and easier to operate than the larger laboratory-based systems. One drawback is that the menu of tests on these instruments is fairly limited. To provide the capability of lipoprotein profile testing, the author recommends that a point-of-care device be used to measure TC, TG, and HDLC and that LDLC be calculated by the Friedewald equation. Maintenance of these point-of-care instruments may be easier, since it is possible to ship the instrument to the manufacturer for service or repair rather than having to depend on the availability of a technical service representative to come and service the instrument onsite. Operated properly, these smaller instruments can provide lipid and lipoprotein results consistent with larger analyzers and can meet the prescribed performance required for assessing CHD risk.

PRE-ANALYTICAL ISSUES

Collecting, handling, shipping, and storing of samples are potential sources for variation of lipid and lipoprotein measurements that laboratories must take into account. Effects from storage of serum on TC, TG, HDLC, and LDLC are minimal within 4 days if stored in the liquid state at 0°C, within 6 months if stored at −20°C, and for years if stored at −50°C to −80°C.[5] Unfortunately, in resource-limited laboratories, adequate refrigeration and freezer facilities for storage may not be available. Transport of samples from rural areas to a central facility may actually be the limiting step in obtaining a quality result. Care must be taken to insure the integrity of the sample during transit by using either ice or frozen coolant packs. A new technique that may have application in resource-limited countries involves using filter paper to collect a blood spot(s), which can then be shipped under ambient conditions to a laboratory for analysis. Another important advantage of using a more portable instrument is the ability to place the device in the field and thus eliminate the need for transporting samples.

QUALITY ASSURANCE

Regardless of the type of instrument a laboratory uses for measuring lipids and lipoproteins, a strong emphasis on quality assurance is important in obtaining the quality of results needed for disease detection and management. Maintaining a minimum standard of laboratory services begins with having adequately trained laboratory staff. Developing a complete laboratory manual that covers all aspects of laboratory functions is fundamental to operating a quality laboratory, irrespective of size. The laboratory manual should contain a thorough description of the methods used, safety considerations, procedures for sample collection, processing and storage, and rules for quality control. This manual will serve as a key resource of information for laboratory technicians performing routine lipid and lipoprotein testing. In addition to the laboratory manual, regular ongoing training of laboratory staff will be needed. Such training can be accomplished by onsite workshops and lectures, textbooks, and distance learning through the Internet where access is available. Minimizing the complexity of the analytical instrumentation, as previously mentioned, will simplify the level of operator training required. Laboratory technicians must also be properly trained in all aspects of laboratory safety, particularly in the proper collection and handling of blood products.

The most important aspect to providing quality results is to have in place both internal and external quality control procedures. Internal quality control indicates whether a laboratory method is adequately repeating itself to give the same performance over time. Control materials covering the diagnostic levels of importance must be tested daily in conjunction with patient sample testing. Lyophilized serum-based control materials would be the most appropriate type of materials for use in this environment because they are typically very stable and require only refrigeration for storage. Although these materials are suitable for evaluating precision, they are not appropriate for assessing the accuracy of an assay because of non-commutability issues. If quality control materials are not available from the instrument vendor, or if resources to purchase such controls are limited, an alternative approach is to collect blood from several

donors and prepare serum pools for use as control pools. Storage of these pools will require a minimum of −20°C storage capability. Quality control rules must be established and followed to insure the repeated quality of testing.

In the case of CHD, it is also very important to know an individual's accurate lipid and lipoprotein levels since risk assessment and intervention are now based on classification of actual lipid and lipoprotein levels and not just reference or normal range comparisons. To accomplish this, an external assessment of performance is also needed. Such assessment is usually done through participation in an external quality assessment program. Participation in an external quality assessment scheme serves several purposes. Perhaps more valuable for resource limited laboratories, the exercise provides a useful source of continuing education for the participating laboratory, especially if the materials can be stored and re-examined after the results are returned.

TECHNICAL ASSISTANCE FROM CDC

Although resource-limited laboratories face many obstacles, establishing public health and clinical laboratory capacity for measuring lipids and lipoproteins as part of a CHD prevention effort can be accomplished. The CDC has been providing technical assistance for more than 30 years to laboratories that measure lipids and lipoproteins and is willing to provide technical assistance, as needed, for other laboratories to develop this capability. The CDC can provide technical assistance to establish lipid and lipoprotein testing capability to support a CHD public health program in areas with limited laboratory resources. CDC assistance includes: selecting a suitable testing instrument; providing training for laboratory technicians; establishing a simple quality control plan; and instructing staff on how to prepare frozen serum control materials suitable for assessing accuracy of lipid and lipoprotein testing. For example, in recent years, CDC has collaborated with the Pan American Health Organization (PAHO) to establish lipid testing laboratories in Central and South America to implement CARMEN, a region-wide project to assess noncommunicable disease risk factors such as CHD. The CDC has assisted PAHO in selecting qualified laboratories, conducted site visits, trained laboratory staff, and standardized laboratory measurements. Where no local or regional external assessment program is available, CDC can provide assistance through its Lipid Standardization Program.[2] Typically, CDC assists in standardizing a central laboratory that can then work to standardize other laboratories in the area. Through this process, many laboratories can be standardized and establish links to CDC with a minimum of resources.

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