Clinical Chemistry

Reference Measurement Procedure for Total Glycerides by Isotope Dilution GC-MS

Bob Barrett: This is the podcast from *'Clinical Chemistry'*. I am Bob Barrett. Measurement of Serum Lipid Concentrations is an important component in assessing and managing cardiovascular disease risk. Triglycerides are an independent risk factor for cardiovascular disease and a therapeutic target in hypertriglyceridemic patients. In addition with the use of the Friedewald Equation, triglyceride concentration is often used in estimating LDL-cholesterol, a major therapeutic target for treatment.

That's why a need persists for well-characterized reference measurement procedures to guarantee the accuracy and traceability of serum triglycerides obtained from routine procedures. Recently the CDC developed an Isotope Dilution Gas Chromatography Mass Spectrometry Measurement Procedure for Total Glycerides and Serum.

Joining us in this podcast is Selvin Edwards. A Research Chemist currently with the Division of Laboratory Sciences in the National Center of Environmental Health, at the Centers for Disease Control and Prevention, who along with colleagues from the CDC, published these new Research Measurement Procedures in the April 2012 issue of '*Clinical Chemistry*.'

So tell us why has CDC developed a new Reference Measurement Procedure for Total Glycerides?

Dr. Selvin Edwards: The Chromotropic Acid Method and the standard that CDC previously used to assure accurate clinical lab measurements became obsolete. It's been replaced by the Mass Spectrometry Method that we described in our paper. When we combine this Isotope Dilution, Mass Spectrometry Method with appropriate referenced materials, it provides what we need to assure the quality of the Total Glyceride Measurements used in everyday patient care. This is an important step forward because a patient's Total Glyceride or TG level is one part of the traditional panel of biomarkers, physicians use to assess the risk of cardiovascular disease.

And the panel of biomarkers also includes HDL, LDL and Total Cholesterol. Many consider TG by itself to be an independent marker of cardiovascular related diseases, such as Type II Diabetes, Childhood Obesity and Metabolic Syndrome. In clinical lab practice, Total Glyceride concentration in a patient's serum also used to calculate LDL-cholesterol from the concentrations of HDL and Total Cholesterol by the Friedewald Equation. Also Enzymatic Methods that are routinely used in clinical labs to measure TG, don't measure just triglyceride.

These assays also measure mono and diglycerides as well as free glycerol. So the measure is in fact a Total Glyceride

measurement. The Total Glyceride reference method assures standardization of actual measurements that obtained from assays used in clinical labs. So accurately measuring the concentration of a patient's TG is critical for assessing risk for cardiovascular disease, and it contributes to better diagnosis treatment and prevention of this disease.

Bob Barrett: Doctor what are the advantages of this new method?

Dr. Selvis Edwards: You know there are several advantages. For example, the method has been optimized to operate with the highest level of accuracy and the lowest level of imprecision using only a few micro liters of serum. With this procedure, we consistently get reliable results with minimum variability for materials that are analyzed. In addition, the method facilitates high extraction efficiency of the analyte, from the sample matrix with minimal losses during sample cleanup.

Another advantage is that the method is highly specific as a result of chromatographic separation from potentially interfering compounds and mass selective analysis of the analyte. These advantages support its use of a referenced measurement procedure for Total Glyceride measurement.

Bob Barrett: Why is there a need to standardize Total Glyceride Measurements?

Dr. Selvis Edwards: Well, the primary challenge for physicians and researchers concerning Total Glycerides in patient care is the high and sometimes extreme discordance between test results obtained from different labs and test methods. This high variability for TG measurements between labs and assays makes it difficult to accurately assess patient risk and ensure proper care. Considered that the American Heart Association reports that the median variability for Total Glyceride measurement exceeds 23%.

By comparison, the median variability for Total Cholesterol which is also biomarker for cardiovascular disease is only 4.9%. Even though not all of the variability is assay dependent, having a standard for measurement minimizes assay variability.

Bob Barrett: So how does the CDC use this method to minimize the measurement variability?

Dr. Selvis Edwards: The Mass Spectrometry Reference Method is used to assign Total Glyceride reference values to serum materials that are prepared according to a standardized procedure. These materials are used in CDC's Lipid Standardization Program and made available to clinical labs, so they can assess the accuracy of their assays. The results from each participating

labs are evaluated against predefined bias and imprecision tolerances established at the CDC.

Labs that pass our evaluation receive certificates as an indication of achievement. Those that don't pass the evaluation are asked to find the cause of their performance problem and make corrections at will, enable them to meet the accuracy goals of the Lipid Standardization Program, still help participating labs by providing technical assistance when necessary and sharing our experiences with our methods.

For example, we provide training for reference labs that want to set up mass spectrometry based assays for Total Glycerides. Another way that we assure the quality of Total Glyceride Measurements with our method is through collaborative partnership with organizations such as the College of American Pathologists. We assign reference values for Total Glycerides, to serum materials that these organizations use to assess labs and to test systems for accuracy.

Also, we use this reference method to collaborate with outside partners on studies to assess potential sources of measurement problems. These activities help ensure their reliability of clinical lab measurements.

Bob Barrett: Is there a similar program that enables manufacturers to assure the accuracy of their assays for Total Glycerides?

Dr. Selvis Edwards: Thanks for asking. The Cholesterol Reference Method Laboratory Network provides a certification program for manufacturers. Right now however, Total Glycerides are not part of that program. It currently provides certification for HDL, LDL and Total Cholesterol assays.

CDC's reference lab will work with assay manufacturers who want to ensure the accuracy of their methods or to ensure the recovery of their test materials for Total Glyceride. CDC has long-standing experience with such collaboration.

Bob Barrett: Well, Dr. Edwards there's so much information about this topic, is there anything else you would like to share and include in this segment?

Dr. Selvis Edwards: I just like to say that over the years, CDC consistently has assured the reliability of lab test by creating an administering programs such as the Lipid Standardization Program and their Cholesterol Reference Method Laboratory Network. These programs have enhanced the detection and management of cardiovascular disease risk in the general population, and we expect that they will continue to do so.

Bob Barrett: Selvin Edwards is a Research Chemist with the division of Laboratory Sciences in the National Center of Environmental Health at the CDC in Atlanta. He's been our guest in this podcast from '*Clinical Chemistry*.'

I'm Bob Barrett, thanks for listening!

Total Duration: 9 Minutes