Correspondence

Current Status of CDC Lipid Standardization and International Needs for Standardization in Epidemiological Studies and Clinical Trials in Japan

To the Editor:

The CDC (Centers for Disease Control and Prevention) consists of seven national research centers of the Department of Health and Human Services of the United States. Lipid standardization activities are provided by one of the centers, the National Center for Environmental Health. The CDC and the National Heart, Lung, and Blood Institute (NHLBI) collaborated for establishment of the CDC-NHLBI Lipid Standardization Program (LSP) in 1957. The current traceable system for lipid standardization is composed of two channels; one is the CDC-NHLBI LSP provided by the CDC itself and the other is the Cholesterol Reference Method Laboratory Network (CRMLN) of international lipid reference laboratories established in 1989. In 1962, the CDC lipid reference laboratory was designated as the WHO Collaborating Center for Reference and Research in Blood Lipids.

The US standard for accuracy in cholesterol measurement is the National Reference System for Cholesterol (NRS/CHOL) established by the National Committee for Clinical Laboratory Standards (NCCLS). The NRS/CHOL is a unique voluntary consensus standard endorsed by professional, industrial, and government organizations. The accuracy base is made up of the National Institute of Standards and Technology (NIST) primary reference method (definitive method), an isotope dilution-mass spectrometry procedure, and the CDC secondary reference method, a modification of the extraction procedure of Abell, Levy, Brodie, and Kendall. The accuracy of cholesterol established in the NIST and CDC is transferable through two channels to clinical laboratories throughout the world. This basis for reference measurements is widely accepted as the universal standard.

The CDC-NHLBI LSP is currently involved in the standardization of laboratories in 25 countries. The participants in this program are limited to epidemiological laboratories, lipid methodology research laboratories and reference laboratories. In Asia and Oceania, there are 17 laboratories in six countries, China (10 participating laboratories), Japan (1), Singapore (1), Thailand (1), Australia (3) and New Zealand (1). In Japan, there were six participants, including the Osaka Medical Center for Cancer and Cardiovascular Diseases (OMC, now Osaka Medical Center for Health Science and Promotion) in 1974 and 1975, however five laboratories except OMC resigned from the standardization program within a year. The Osaka Medical Center for Health Science and Promotion (OMC for HSP) has been the only participating Japanese laboratory in the CDC-NHLBI LSP since 1974. The CRMLN is an international network for lipid standardization for traceability which is working in the standardization of reagent manufacturers and clinical laboratories (as of March, 2004). These consist of 10 lipid reference laboratories, recognized as clones of CDC, from eight countries, which have certificate ability for total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. The lipid standardization is important not only to achieve the international comparability between studies, but also to eliminate long-term shifts of lipid values in population studies. The OMC for HSP has participated in the CRMLN since July 1992 and standardized 10 reagent manufacturers and over 1,200 clinical laboratories in Japan through CRMLN lipid standardization programs. Many laboratories involved in epidemiological studies and clinical trials in Japan met the CDC criteria through the OMC for HSP. Original articles are contributed to medical journals published in USA and Europe. Seventy to eighty percent of publications have passed the criteria of the CDC lipid standardization, and they all mention this fact of certification. The lipid standardization is crucial for sound epidemiological and clinical studies before publication. For the publication of studies in Japan, editors and readers prefer that the laboratories involved have met the CDC performance criteria by the OMC for HSP, which is a member of the CRMLN. When epidemiological and clinical studies are conducted in Japan, it will be now very hard to directly participate in the CDC-NHLBI LSP because the program is full to capacity. Therefore, we strongly recommend participation in the CRMLN lipid standardization programs through the OMC for HSP.

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Response:
Standardization of the measurement of serum lipids — Looking back at 30 years of experience in clinical laboratories

Before the development of an enzymatic assay about 30 years ago, the measurement of serum cholesterol had been performed with a colorimetric assay using a chemical reaction with sulfuric acid/acetic anhydride (Liebemann-Burchard reaction) or ferric chloride/acetic acid/sulfuric acid (Killiani reaction) as a reagent. Many modified methods have been developed to solve the problems presented by 1) the pressure of cholesterol in both free and esterified forms 2) the co-existence of interfering substances and 3) the necessity to stabilize the colored product and make the assay procedure more accurate and convenient. The major issue is that cholesterol is insoluble in water and is present in the form of lipoproteins. Analysis of free and esterified cholesterol requires a tedious process and often gives low values of esterified cholesterol due to incomplete hydrolysis. Therefore, a direct measurement of total cholesterol has been used in routine work in the laboratory. Since serum cholesterol was recognized as an important risk factor for atherosclerosis and also as a target of treatment, assays of cholesterol in the United States were brought under the control of the Centers for Disease Control (CDC), in consideration of problems due to the complexity of the assay procedure.

Since an enzymatic assay system was developed (Allain CC et al., 1974) using a combination of cholesterol oxidase and peroxidase after hydrolysis of cholesteryl esters using cholesterol ester hydrolase, the procedure spread rapidly and soon became the routine method for the measurement of serum cholesterol in clinical laboratories because they could avoid the use of strong acid as a reagent. However, there was great concern regarding accuracy and the need arose for control by a classical (standard) assay system. There were many problems at an early stage in the development of such new assay procedures. Standardization was especially important for collaborative work involving many institutions for epidemiological studies and chronological observations.

The Japan Atherosclerosis Society has been conducting a nationwide investigation of serum lipid levels every 10 years since 1960, when Dr. Kimura participated in the Seven Countries Study. In the 3rd survey, carried out in 1980 with Dr. Sekimoto as a principal investigator (Research Committee on Familial Hyperlipidemia in Japan, 1983), I was engaged in the study group at a center laboratory for the control of lipid assay, with my colleagues at the National Cardiovascular Center (T. Yamamura, H. Kushiro, Y Nakao, et al.). In this study, lipid measurement was carried out at each member institution because of a lack of funds for centralization of the lipid assay. We prepared several samples of blood serum with different cholesterol levels covering a wide range, between 100 and 500 mg/dl, and also those with different triglyceride levels, and distributed them to the member institutions for standardization. At that time, several kits for enzymatic assay were commercially available. We checked the accuracy of these assay kits for cholesterol by measuring the standard samples and comparing the data with those obtained with a standard chemical assay system. We found that one of the assay kits newly brought onto the market at that time gave values more than 10 mg/dl higher than the expected values through the testing range of the cholesterol concentration, probably due to the interference of some component(s) in the blood serum. Another kit failed to give a proper value for a sample with a very high cholesterol concentration because of a deficiency in the enzyme(s) in terms of quality and/or quantity. For the assays of triglyceride, we carefully checked if the methods or assay kits used in individual laboratories could exclude free glycerol. The control of HDL-cholesterol was the most difficult. Although the assay system was based on the same principle (polyanion-metal-complex precipitation method: Burststein, M and Scholnick, HR, 1973), classical assay systems using heparin produced errors when serum samples were once frozen, while some modified systems managed to tolerate the samples being treated by repeated freezing and thawing. We distributed an announcement of the results of our research and recommended researchers use proper assay kits or control their system according to our standard. Our efforts stimulated the development of more accurate assay systems in the chemical and fermentation industries in Japan.

In 1984–1985, our laboratory at the National Cardiovascular Center made contact with the Department of Epidemiology and Mass Examination for Cardiovascular Diseases (Chief: Dr. Iida M), the Center for Adult Diseases, Osaka (currently known as the Osaka Medical Center for Health Science and Promotion: OMC), which had been standardized for lipid assay by the CDC as clearly described by Dr. T. Shimamoto in his Letter to the Editors. We had been doing a survey on serum lipid levels in a small town near Osaka, and in that year, we were planning to enroll all school boys and girls in the town (Yamamoto A et al., 1988). In this study, cholesterol was measured with a Determiner TC kit supplied by Kyowa Hakko Co. using an enzymatic method. Quality control for the cholesterol test was maintained by monthly comparisons of the values obtained by enzymatic measurements with the values obtained by the Zurkowski method, a chemical assay procedure that gives results comparable to the Zak-Henly and Abell-Kendall methods routinely used for standardization by the CDC. Triglyceride was measured by a kit supplied from Kokusai-Shiyaku Co. using a bacterial lipase-glyc-
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sterol dehydrogenase-NAD system. HDL-cholesterol was measured by a kit supplied from Nippon Shoji Co. using a heparin calcium precipitation method and the validity was checked in our laboratory (Kajikawa T et al., 1981) by electrophoretic analysis of lipoproteins in the precipitate and supernatant, and also by comparing the data with those obtained by standard heparin-manganese precipitation. Quality control for total cholesterol and triglyceride was carried out by measuring standard samples supplied from the laboratory of the OMC, and the estimated values of the 60 samples fell in the range of 99.2 ± 3.0% of the acceptable means for total cholesterol and 99.5 ± 8.6% for triglyceride.

Before we started the 4th survey on serum lipids in Japan in 1990, which was authorized by the Japan Atherosclerosis Society, we selected the Special Research Laboratory (SRL, Tokyo) as the core laboratory of our survey and advised them to accept the quality control of the CDC through the laboratory of the OMC. This was based on our experience in 1984-1985. We also checked the quality of their measurements by a sampling test, comparing their data with values we obtained ourselves.

As Dr. Shimamoto wrote in his Letter to the Editors, the OMC contributed a great deal to the standardization of serum lipids, especially since other Japanese laboratories resigned from the CDC Lipid Standardization Program, in which they had once participated. For evaluation of the quality of epidemiological studies and international collaborative work or chronological studies on cardiovascular risk factors, it is crucial to validate lipid measurements. The Japanese chemical and fermentation industries are eager to manufacture detection reagents for clinical diagnosis and many excellent and convenient assay kits and systems are now available for laboratory work. It seems important both for them and for clinical laboratories, either large companies or small scale laboratories in hospitals and lipid clinics, to show the validity of the accuracy and the tolerability of their assay systems for international studies. However, this requires both manpower and money. As now is a time of reductions in medicare costs, we have to seek a way of managing this kind of standardization as efficiently as possible.

We now have many more lipid parameters, including apolipoproteins, Lp(a), small dense LDL, etc. Direct measurement of LDL-cholesterol is now becoming popular, although the values used to be obtained by a calculation using the Friedewald equation. In such circumstances, collaborative work among people participating in laboratory medicine, lipid clinics, and epidemiology is an important factor in developing a proper system of evaluation and standardization for the measurement of such parameters. I strongly recommend that the Japan Atherosclerosis Society set up a committee to conduct such a project. However, it is also important not to suppress the opportunity to use new tools and to develop new areas for research and investigation.

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