Improved Specificity of a New Homogeneous Assay for LDL-Cholesterol in Serum with Abnormal Lipoproteins, Yasumasa Iwasaki, Hirohiko Matsu, and Nobuo Nakashima (Department of Clinical Laboratory, Nagoya University Hospital, Nagoya, Japan; * address correspondence to this author at: Department of Endocrinology, Metabolism, and Nephrology, Kochi Medical School, Kochi University, Kohasu, Oko-cho, Nankoku 783-8505, Japan; fax 81-88-880-2344, e-mail iwasaki@med.kochi-u.ac.jp)

**Background:** Although a homogeneous assay for serum LDL-cholesterol (LDL-C) has become a routine clinical procedure, problems remain in assay performance characteristics.

**Methods:** We examined the performance of a recently developed automated homogeneous assay (New-Daiichi assay) for serum LDL-C and compared the results with those obtained by the current homogeneous method (Denka-Seiken assay) or by ultracentrifugation as a control.

**Results:** The New-Daiichi assay showed satisfactory basic performance characteristics such as reproducibility, linearity, and stability. There was no interference in the assay by various substances examined. The LDL-C values obtained with this method correlated well with those obtained by ultracentrifugation. In samples from patients with obstructive jaundice, both methods detected cholesterol from abnormal lipoproteins (such as lipoprotein-X and -Y), but the New-Daiichi assay was less reactive and more specific for LDL-C.

**Conclusion:** The new method has improved performance for the accurate measurement of LDL-C in clinical practice.

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LDL-cholesterol (LDL-C) is generally recognized as an atherogenic form of cholesterol (1–3), and its precise measurement is important in lipid-lowering therapy for preventing coronary heart disease and/or stroke (4, 5). Ultracentrifugation is still the gold standard for determining plasma LDL concentration, but the technical complexity of this method makes it inappropriate for routine use. Estimation with the Friedewald equation is widely used (6), although the calculated values can be inaccurate for plasma triglyceride (TG) concentrations >4000 mg/L or in the presence of chyomicrons or type III hyperlipoproteinemia.

Homogeneous assays based on chemical methods for the direct determination of plasma LDL-C have been developed in Japan and are now widely used for routine testing (7–9). These direct homogeneous assays are easily adapted for use with clinical chemistry analyzers, but they do present some chemical problems. In particular, LDL-C specificity can be inadequate. LDL-C values may be overestimated when VLDL-cholesterol is measured as LDL-C, or they may be underestimated when all LDL-C subfractions are not detected as LDL-C (9). In this study, we evaluated a recently developed homogeneous assay for the determination of LDL-C (New-Daiichi assay; Daiichi Chemical Co). The principle of the assay is shown in Fig. 1A. Newly developed detergents used in the assay are expected to improve specificity for LDL-C detection in samples with abnormal lipoproteins.

We first evaluated the basic performance of the New-Daiichi assay and observed excellent within-run reproducibility, with CVs of 0.6%–1.2% and 0.9%–1.2% for clinical samples (a pooled serum obtained from 10 patients without obvious biochemical abnormality) and a commercially available control serum, respectively. Between-run reproducibility for the same assay reagent also showed a narrow range (1.0%–1.6%) for both the pooled and control serum tested for up to 35 days. Furthermore, the New-Daiichi assay was linear up to 1000 mg/L (not shown). No interference with the assay was observed in the presence of bilirubin F [316 μmol/L (18.5 mg/dL)], bilirubin C [333 μmol/L (19.5 mg/dL)], hemoglobin (4.85 g/L), turbidity (1840 formadine turbidity), ascorbic acid [1.1 mmol/L (20 mg/dL)], or Intralipid (1.0%), as determined by a commercially available reagent set (Interference-Check A plus; Sysmex) (10). We compared the New-Daiichi assay with the LDL-Ex homogeneous LDL-C assay (Denka-Seiken) (7), which is currently in routine use at our hospital laboratory. We tested 270 clinical samples (both fasting and nonfasting), including samples from patients with hyperlipidemia (n = 70), hyper gammaglobulinemia (n = 28), and monoclonal gammapathy (n = 24). There was a good correlation between the New-Daiichi assay (y) and the Denka-Seiken assay (x) for samples without hyperbilirubinemia (n = 270; y = 0.970x + 1.77; r = 0.99), and no discordant values were obtained (see Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol52/issue5).

For 38 clinical samples, we also investigated the relationship of the New-Daiichi assay with ultracentrifugation. An aliquot (1 mL) of each serum sample was subjected to ultracentrifugation (d = 1.006 kg/L; 368 000g for 3 h at 4 °C; Hitachi CS120GX with AT2-0168 rotor). We then measured total and HDL-cholesterol in the bottom fraction (HDL + LDL) with a Determiner I TCII reagent set (Kyowa-Medex) and a heparin-manganese precipitation method (Daiichi), respectively. Finally, we calculated the LDL-C concentration by subtraction of the 2 fractions. The New-Daiichi assay (y) and ultracentrifugation (x) showed a strong correlation (n = 38); y = 0.947x + 5.49; r = 0.99; see Fig. 1 in the online Data Supplement). In addition, there was a good correlation between New-Daiichi assay values (y) and those calculated with the Friedewald equation (x) for 494 randomly collected (both fasting and nonfasting) control and hyperlipidemic serum samples (TGs >4000 mg/L; y = 0.953x + 5.89; r = 0.978), although minor discrepancies (Friedewald > New-Daiichi) were observed for a few samples with moderately high TG concentrations (1500 < TG < 4000 mg/L; see Fig. 1 in the online Data Supplement). The reason for this discrepancy was unclear and may have been related to differential estimation of some abnormal lipoproteins.
such as intermediate-density lipoprotein remnants, or small, dense LDL.

For samples from patients with hyperbilirubinemia [mean total bilirubin concentration = 212 μmol/L (12.4 mg/dL)], regression analysis with 95% confidence limits revealed a discrepancy between the New-Daiichi and Denka-Seiken assays for 4 of 28 samples. The 4 samples also had substantially higher mean (SD) serum total bile acids [31.8 (12.7) and 140.3 (64.2) μmol/L in samples without and with discrepancy, respectively; P < 0.0005]. In these samples, the values obtained by the New-Daiichi assay were lower than those obtained by the Denka-Seiken assay in all 4 cases (see Fig. 2 in the online Data Supplement). As expected, in all 4 cases, abnormal lipoproteins such as lipoprotein-X (Lp-X) and Lp-Y were detected by agarose gel electrophoresis (1 μL of serum subjected to electrophoresis on agarose gel at 90 V for 46 min; gel then stained with Fat Red 7B or stained for cholesterol and TGs), suggesting that differential reactivity to these abnormal lipoproteins may have caused the difference between the 2 assays.

All samples showing marked discrepancies between the New-Daiichi and the Denka-Seiken assay were from patients with cholestasis caused by various diseases (hepatic metastasis of colon cancer, biliary obstruction by pancreatic cancer, biliary obstruction by hepatocellular carcinoma, and cholestasis resulting from congenital biliary obstruction). Obstructive jaundice was present in all cases, and agarose electrophoresis showed Lp-X or Lp-Y in all samples (11). Abnormal lipoproteins such as Lp-X and Lp-Y are frequently observed in patients with cholestatic liver disease and are reported to influence homogeneous LDL-C assays (12).

To further investigate the cause of the discrepancy, we chose 2 patients with obstructive jaundice for whom we had obtained sufficient serum samples and had deter-

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Fig. 1. Principle of the new homogeneous assay for the determination of LDL-C (A), and results of agarose gel electrophoresis and gel-filtration chromatography of 2 samples with hyperbilirubinemia (B).

(A), lipoproteins other than LDL are degraded by detergent 1 in the first reaction. Cholesterol released from these lipoproteins generates H₂O₂, which is eliminated by peroxidase and 4-aminoantipyrine. In the second reaction, LDL is degraded by detergent 2 and produces H₂O₂, which is visualized by reaction with peroxidase, 4-aminoantipyrine, and N,N-bis(4-sulfobutyl)-nitroblue tetrazolium disodium salt (DSBmT). CM, chylomicrons. (B), serum total lipids, cholesterol, and TGs were stained after agarose gel electrophoresis. LDL-C was determined by either the New-Daiichi assay or the Denka-Seiken assay in each fraction obtained by gel-filtration chromatography. T-Cho, total cholesterol.
determined the total cholesterol and LDL-C values after fractionation of their samples by gel-filtration chromatography. In brief, 200 μL of serum was subjected to gel-filtration chromatography with Superose 6HR 10/30 (Pharmacia) and eluted with phosphate-buffered saline (pH 7.4) at 300 μL/min. Serial fractions (300 μL) were obtained for the LDL assay with a fraction collector.

For patient 1, agarose gel electrophoresis revealed Lp-Y (Fig. 1B). Comparison of LDL-C values for each fraction obtained by gel-filtration chromatography revealed that the New-Daiichi assay had a reactivity to Lp-Y of ~70% of that of the Denka-Seiken assay (Fig. 1B). We observed no difference between the 2 assays for the LDL fraction, and there was no reaction with the HDL fraction.

For patient 2, Lp-X was detected by agarose gel electrophoresis with staining for cholesterol (Fig. 1B). Comparison of LDL-C values determined by the 2 assays showed a difference for the Lp-X fraction, for which the New-Daiichi assay yielded a value ~50% of that obtained with the Denka-Seiken assay. This abnormal lipoprotein disappeared after 3 weeks when the patient’s jaundice improved, and there was no discrepancy between the 2 assays at that time (not shown). Thus, we assume that the primary cause of the discrepancy was differential reactivity for these abnormal lipoproteins and that the New-Daiichi assay was more specific for LDL-C.

In conclusion, we found that the New-Daiichi assay has excellent basic performance characteristics and shows good reproducibility. Furthermore, there was no interference with the assay by any of the coexisting substances tested, and reagent stability was excellent, allowing measurement for 35 consecutive days without calibration. We therefore conclude that this new homogeneous LDL-C assay has a satisfactory basic performance and can be used in clinical chemistry analyzers for routine laboratory testing. In regular samples (including those from patients with hyperlipidemia), results showed an excellent correlation with those obtained by the Denka-Seiken assay and by ultracentrifugation. On the other hand, in samples from patients with obstructive jaundice, both assays detected cholesterol from abnormal lipoproteins (Lp-X and Lp-Y), but the New-Daiichi assay was less reactive and therefore may be more specific for LDL-C. Although determination of LDL-C in patients with cholestasis is of limited clinical significance, improving the specificity of a homogeneous LDL-C assay is still an important technical advance from an analytical point of view.

References


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