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Survey of Total Error of Precipitation and Homogeneous HDL-Cholesterol Methods and Simultaneous Evaluation of Lyophilized Saccharose-containing Candidate Reference Materials for HDL-Cholesterol

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Background: Standardization of HDL-cholesterol is needed for risk assessment. We assessed for the first time the accuracy of HDL-cholesterol testing in The Netherlands and evaluated 11 candidate reference materials (CRMs).

Methods: The total error (TE) of HDL-cholesterol measurements was assessed in native human sera by 25 Dutch clinical chemistry laboratories. Concomitantly, the suitability of lyophilized, saccharose-containing CRMs (n = 11) for HDL-cholesterol was evaluated.

Results: In the precipitation method group, which included 25 laboratories and four methods, the mean (minimum–maximum) TE was 11.5% (2.7–25.2%), signifying that 18 of 25 laboratories satisfied the TE goal of ≤13% issued by the National Cholesterol Education Program (NCEP). In the homogeneous HDL-cholesterol method group, which included five laboratories, each performing two different methods, the mean (minimum–maximum) TE was 9.5% (6.0–17.3%) for the Boehringer assay and 15.7% (3.3–30.7%) for the Genzyme assay. For the Boehringer homogeneous assay, one of five laboratories did not meet the TE criterion, whereas

for the Genzyme homogeneous assay, three of five laboratories exceeded the 13% criterion. The biases on the HDL-cholesterol values found by various precipitation methods were highly variable in all CRMs, irrespective of the quality, whereas the biases found by the homogeneous method from Boehringer were far less than ±5% for the highest-quality CRMs (CRMs 4–6).

Conclusions: The NCEP goal was met by 24 of 35 laboratories assessed by use of native human sera. Selectively pooled, lyophilized CRMs that are cryoprotected with 200 g/L saccharose have ample potential for use in the standardization of homogeneous HDL-cholesterol methods.

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Several prospective epidemiological studies and clinical trials have demonstrated that low HDL-cholesterol (HDL-chol)⁷ concentrations are an independent risk factor for coronary heart disease (CHD); this inverse relationship is maintained over a wide range of HDL-chol concentrations (1, 2). Accordingly, HDL-chol determinations are included in most national CHD prevention programs to predict an individual's risk and to guide treatment (1, 3, 4). As an example, the latest revision of the Dutch Cholesterol Consensus takes into account the cholesterol/HDL-chol ratio for determining the absolute risk of individuals for developing CHD within the next decade, besides classical risk factors as hypertension, diabetes

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⁷ Nonstandard abbreviations: HDL-chol, HDL-cholesterol; CHD, coronary heart disease; NCEP, National Cholesterol Education Program; TE, total error; apoB, apolipoprotein B; PEG, polyethylene glycol; DS, dextran sulfate; PTA, phosphotungstic acid; LRL, Lipid Reference Laboratory; CRM, candidate reference material; DCM, Designated Comparison Method; and UC, ultracentrifugation.

mellitus, and family history (4). Analogously, the US National Cholesterol Education Program (NCEP) Adult Treatment Panel II has identified an HDL-cholesterol concentration <0.91 mmol/L as a major risk factor for CHD, and considers HDL-cholesterol concentrations >1.55 mmol/L as a negative risk factor (3). Because of the enhanced demand for measurement of HDL-cholesterol in medical practice, precise and accurate assays are warranted. To this end, the NCEP Working Group on Lipoprotein Measurement issued performance guidelines for HDL-cholesterol analyses that all clinical laboratories should achieve by 1998 (5). One goal is that clinical laboratories should measure HDL-cholesterol with a total error (TE) $\leq 13\%$, which is achieved, for example, if clinical laboratories have a mean bias of no more than $\pm 5\%$ and perform with a CV $\leq 4\%$ at ≥ 1.09 mmol/L.

To date, clinical chemistry laboratories frequently have measured HDL-cholesterol by chemical precipitation of the apolipoprotein B (apoB)-containing lipoproteins with either polyethylene glycol (PEG) 6000, dextran sulfate (DS)/MgCl₂ or phosphotungstic acid (PTA)/MgCl₂, followed by quantification of the cholesterol content in the HDL-containing supernate (6). Standardization of these HDL-cholesterol assays is challenging because, in addition to matrix effects, there is documented variability between the commonly used precipitation reagents; these differences in precipitation efficiency and recovery become obvious especially in lipemic sera (7–9). Therefore, homogeneous HDL-cholesterol assays that no longer require sample pretreatment have recently become attractive (10–14).

In The Netherlands, the accuracy of HDL-cholesterol measurements has not been investigated thus far. Consequently, a pilot HDL-cholesterol survey encompassing a representative sample of 25 (14%) Dutch clinical laboratories was conducted. The project was a joint project of both the Lipid Reference Laboratory (LRL) Rotterdam and the Dutch National External Quality Assurance Society, named the SKZL. The survey aimed at documenting the state-of-the-art accuracy, imprecision, and TE of the four most commonly used second-generation methods and of two recently introduced third-generation HDL-cholesterol assays, using fresh human sera. Because periodical monitoring of the accuracy of HDL-cholesterol measurements in the Dutch proficiency testing program awaits the availability of commutable reference materials, 11 candidate reference materials (CRMs) with different concentrations of HDL-cholesterol and triglycerides were developed and investigated for their suitability. The HDL-cholesterol Designated Comparison Method (DCM) and the HDL-cholesterol Reference Method of the CDC were used as the reference methods (15, 16).

Materials and Methods

STUDY DESIGN

Of 180 Dutch clinical chemistry laboratories participating regularly in the National External Quality Assurance Society scheme of the SKZL, 25 laboratories took part in a pilot survey that aimed to assess the accuracy and imprecision of currently used routine HDL-cholesterol methods. Apart

from the controlled inclusion of the type of method used to measure HDL (see below), laboratory inclusion was random, but known to be representative for the quality of the Dutch laboratory healthcare system. Four academic hospitals and 21 peripheral hospitals participated.

In the 25 laboratories, assessment of accuracy and imprecision was performed using a standardized protocol that was essentially based upon the cholesterol protocol for certification of clinical laboratories developed by the CDC [Mulder and Cobbaert, submitted for publication; Refs. (15, 16)]. In brief, accuracy was assessed using a split-sample comparison with fresh human specimens and performing duplicate measurements during 3 consecutive days. Between assays, native specimens were stored at 4 °C. The overall imprecision of the field HDL-cholesterol methods was calculated from the repeated measurements of the native sera. In parallel and in all three assays, the suitability (or lack thereof) of the HDL-cholesterol CRMs was investigated. To this end, the HDL-cholesterol CRMs were freshly reconstituted at each occasion, i.e., for each assay.

HDL-cholesterol value assignment was done centrally by the LRL Rotterdam, using the CDC DCM for the native sera, and using the CDC Reference Method or a modification of that method for the CRMs.

NATIVE HUMAN SERA

Because the traditional approaches for assessing accuracy are complicated by matrix effects, the CDC strategy for transferring accuracy from reference laboratories to clinical laboratories was used (15, 16). To this end, 6 to 10 fresh, normotriglyceridemic (triglycerides <2.26 mmol/L) specimens from hospital workers, spanning the clinically relevant HDL-cholesterol range, were selected by each contributing laboratory (Mulder and Cobbaert, submitted for publication). All sera were measured in duplicate in a single assay during 3 consecutive days by each participant. Each day a new calibration was performed. Between assays, sera were stored at 4 °C. These field HDL-cholesterol data were all gathered within 72 h after sample collection. In addition, on the day of sample collection, aliquots of each serum sample were stored at -20 °C or lower for future value assignment by the LRL Rotterdam (15, 16).

CRMs FOR HDL-cholesterol

CRMs intended to closely mimic fresh patient sera were developed by the SKZL. Pools were prepared as described previously (17). In total, 11 serum pools were made, containing various concentrations of HDL-cholesterol, triglycerides, and cryo- and lyoprotectant (saccharose). Each pool consisted of material originating from at least 150 different patients. Before individual patient sera were frozen at or below -20 °C, either 50 or 200 g/L saccharose was added to each individual serum aliquot that was intended for use in future pool preparation. By the time that a few liters of serum pool were gathered per stratum, individual patient specimens were thawed and mixed or supplemented as follows.

CRMs 1, 2, and 3, with low, medium, and high HDL-

chol concentrations, were prepared by mixing the appropriate HDL-chol concentrations. The final saccharose concentration in CRMs 1–3 was maintained at 50 g/L. CRMs 4, 5, and 6, with low, medium, and high HDL-chol concentrations, were prepared from the same mother serum pools as those used for preparing CRMs 1, 2, and 3, respectively. Before CRMs 4–6 were aliquoted and lyophilized, their final saccharose concentration was increased to 200 g/L with additional saccharose. The addition of saccharose caused volume expansion. To achieve nearly equal HDL concentrations in the reconstituted CRMs, the volumes before lyophilization were adjusted to 1 and 1.4 mL for specimens containing 50 and 200 g/L saccharose, respectively. In the end, upon reconstitution in 1 mL of distilled water, the final HDL-chol concentrations in CRMs 4, 5, and 6 were somewhat higher than in CRMs 1, 2, and 3, respectively.

CRMs 7, 8, and 9, with low, medium, and high HDL-chol concentrations, respectively, and a final saccharose concentration of 200 g/L, were prepared from randomly gathered serum aliquots that were initially frozen with 200 g/L saccharose. In contrast to the preparation of CRM pairs 1 and 4 and 3 and 6, the low and high HDL-chol concentrations in CRMs 7 and 9 were created by, respectively, diluting or concentrating the randomly gathered serum pool. Notably, total triglycerides were <2.6 mmol/L in CRMs 1–9. Finally, CRMs 10 and 11, with medium (4.85 mmol/L triglycerides, mainly VLDL) and high concentrations of triglycerides (15.8 mmol/L triglycerides, mainly chylomicrons), respectively, and a final saccharose concentration of 200 g/L, were prepared from appropriate serum aliquots that were initially frozen with 50 g/L saccharose. Sample preparation and subsequent lyophilization of all CRMs was performed as described previously (17).

SERA USED FOR THE VALIDATION OF THE MODIFIED CDC REFERENCE METHOD PROCEDURE

A frozen serum pool from the CDC, i.e., AQ15, which is usually used for internal and external quality-control assessment of the HDL-chol Reference Method and the HDL-chol DCM, was used for verifying the HDL-chol accuracy in the 2-mL procedure compared with the 5-mL procedure. In addition, precision was checked with two freshly frozen single donor sera from hospital workers. All pools were stored at -80°C , and none contained saccharose.

EVALUATION OF LIPOPROTEIN INTEGRITY IN THE HDL-CHOL CRMS

Reconstituted CRMs were first checked macroscopically for turbidity. Lipoprotein integrity was then studied by means of density gradient ultracentrifugation (UC) (17). To this end, serum samples were stained with Coomassie Brilliant Blue before UC, which yielded blue-colored lipoprotein bands within the density gradient after UC. The HDL-chol CRMs were then characterized by lipoprotein

electrophoresis with the Paragon system (Beckman Instruments), with staining performed with Sudan Black (17). Finally, because the CRMs were targeted with the CDC Reference Method, the infranatants obtained after UC at serum density were inspected visually and judged on the basis of homogeneity, i.e., the absence or presence of flakes.

DESCRIPTION OF THE VARIOUS HDL-CHOL METHODS USED IN THIS STUDY PROTOCOL

CDC DCM used for accuracy assessment of normotriglyceridemic human sera. HDL-chol value assignment in both the freshly frozen human specimens and the CRMs was carried out by the LRL of the Academic Hospital Rotterdam, The Netherlands. The LRL Rotterdam is a permanent, international member of the CDC Cholesterol Reference Method Laboratory Network (15, 16, 18). The accuracy base for HDL-chol reportedly consists of the CDC DCM (for normotriglyceridemic sera) and the CDC Reference Method (for hypertriglyceridemic sera) (19).

The CDC DCM, a two-step procedure encompassing M_r 50 000 DS/MgCl₂ precipitation of apoB-containing lipoproteins, and subsequent Abell-Kendall analysis (18) was used to determine HDL-chol concentrations in the frozen native specimens sent by each participating laboratory to the LRL. To this end, duplicate determinations were performed in a single assay.

CDC Reference Method and modified CDC Reference Method for value assignment of the CRMs for HDL-chol. The CDC Reference Method, a three-step procedure consisting of an UC step at serum density, heparin/MnCl₂ precipitation of apoB-containing lipoproteins, and subsequent Abell-Kendall analysis, was used for targeting the CRMs. CRMs 1–3 were assigned values with the original CDC Reference Method on the basis of quadruplicate determinations in four independent assays.

In the presence of 200 g/L saccharose, no lipoprotein separation could be achieved in CRMs 4–11, using the original CDC HDL-chol Reference Method. Consequently, the CDC Reference Method was modified. In essence, the saccharose concentration was “diluted” to 80 g/L saccharose by pipetting 2.00 mL of CRM in a bell-top Quick-Seal ultracentrifugation tube (Beckman Instruments) and by overlaying with 3.00 mL of an electrolyte-albumin solution. The composition of the electrolyte-albumin solution was critical because it affected the HDL-chol recovery in the subsequent precipitation step, i.e., HDL-chol recoveries were only complete if the overlaying “diluent” contained physiological concentrations of sodium, potassium, calcium, magnesium, chloride, and protein. Bovine albumin (60 g/L) was used as a protein source. Other than this modification, all other steps and solutions used were similar to those of the CDC Reference Method procedure. Note that after UC, chylomicrons and/or VLDL were eliminated by cutting at a similar, fixed height as in the 5-mL procedure. In addition, subsequent recovery of the infranatant, which contained

HDL and LDL, and the serum proteins was performed by aspiration, washing of the inner side of the tube, and dilution with 0.15 mol/L NaCl solution up to a final volume of 5.00 mL. CRMs 4–11 were assigned values with the modified CDC Reference Method, on the basis of quadruplicate determinations in four independent assays.

The accuracy and the precision of the 2-mL procedure was validated and compared with the analytical performance of the 5-mL procedure, using fresh frozen human sera and a frozen human serum control from the CDC.

Field HDL-chol methods. Four types of commercial precipitation methods were evaluated by the participating clinical laboratories. Five of the participating laboratories investigated, in parallel with their precipitation methods, two recently introduced homogeneous HDL-chol assays.

The HDL-chol chemical precipitation methods were assessed as follows: (a) the PEG 6000 method was assessed at seven laboratories, using precipitation reagents obtained from Merck (cat. no. 807491; three laboratories) or from Instruchemie (cat. no. 2073; three laboratories), or reagents prepared in house (one laboratory) (20, 21); (b) the PTA/MgCl₂ method was assessed at nine laboratories, using precipitation reagents obtained from Boehringer Mannheim (cat. no. 543004; eight laboratories) or from Merck (cat. no. 114210; one laboratory); (c) the DS (M_r 50 000)/MgCl₂ method was assessed at six laboratories, using precipitation reagents obtained from Beckman, Johnson and Johnson, and Sigma; and (d) the PEG/DS/MgCl₂ was assessed at three laboratories, using precipitation reagent obtained from Instruchemie (cat. no. 2258). In the PEG/DS/MgCl₂ precipitation method, the DS was M_r 15 000 (22). In addition, the lyophilized version of the homogeneous HDL-chol method from Boehringer (direct HDL-chol reagent, cat. no. 1661426; calibrator f.a.s. HDL/LDL-c, cat. no. 1778501) and the lyophilized N-geneous™ HDL-chol method from Genzyme (direct HDL-chol reagent, cat. no. 2570; direct HDL-chol calibrator, cat. no. 2574) were evaluated.

In the homogeneous HDL-chol method from Boehringer, sulfated α -cyclodextrin and DS form, at pH 7 and in the presence of MgCl₂, water soluble complexes with LDL, VLDL, and chylomicrons (reagent 1), which are not accessible to PEG-coupled cholesterol esterase and cholesterol oxidase (reagent 2). In the Genzyme homogeneous HDL-chol method, a polyanion and synthetic polymer (reagent 1) together form complexes with chylomicrons, VLDL, and LDL particles and prevent them from reacting with the second reagent, which is a mixture of enzymes (cholesterol esterase, cholesterol oxidase, and peroxidase), 4-aminoantipyrine, detergent, and buffer. The HDL particles are disrupted by the detergent, thereby releasing the cholesterol and cholesteryl esters. The HDL concentration is then determined enzymatically, using a two-point reaction. The homogeneous HDL-chol methods are hereafter denoted as " α -cyclodextrin sulfate/PEG-coupled enzyme" and "polymer/polyanion" assays, respectively.

In the chemical precipitation methods, the precipitation steps for prior isolation of HDL were done manually, whereas the cholesterol in the supernates was measured on automated clinical chemistry analyzers. In the homogeneous HDL-chol methods, sample pretreatment was no longer required, and fully automated applications were used. Application was done according to the instructions of the manufacturers, and all assays were performed with calibrators that were included in the kits. Results were scored after several assays in which reproducible results were obtained.

STATISTICS

In the method comparison study using native specimens, overall analytical imprecision (CV_a) was calculated from measurement repeats, whereas mean laboratory biases were calculated from the linear regression line fitted between the HDL-chol data produced in the field (y -axis) and the data produced with the HDL-chol DCM in the LRL (x -axis). TE was calculated as: $1.96 \times CV_a$ (%) + absolute mean bias (%). For final acceptance or rejection of HDL-chol method performance, a TE criterion of $\leq 13\%$ was used (5).

To investigate the suitability of the CRMs for HDL-chol standardization purposes or accuracy assessment, laboratory means and method group means (\pm SD) were calculated using basic statistics. Laboratory means of the CRMs were compared with the target values assigned by the LRL with either the CDC Reference Method or the modified CDC Reference Method for HDL-chol. A significance level of $\alpha = 0.05$ was used throughout the study.

Results

METHOD COMPARISON OF FIELD HDL-chol METHODS AGAINST THE CDC HDL-chol DCM USING NORMOTRIGLYCERIDEMIC, NATIVE HUMAN SPECIMENS

In the precipitation method group ($n = 25$), the mean (minimum–maximum range) percent TE, bias, and overall imprecision were 11.5% (2.7–25.2%), -0.65% (-14.2% to 11.5%), and 2.8% (0.8–8.0%), respectively (Fig. 1); i.e., 24 of 25 laboratories met the former interim TE goal of $\leq 22\%$, whereas 18 of 25 laboratories satisfied the 1998 TE goal of $\leq 13\%$ (Fig. 1A). The bias criterion $\pm 5\%$ or less was met in 12 of 25 laboratories (Fig. 1B), and the precision criterion of $\leq 4\%$ was reached in 20 of 25 laboratories (Fig. 1C). The laboratories with HDL-chol biases exceeding $\pm 5\%$ were distributed equally across different precipitation methods; therefore, bias differences could not be attributed to a specific manufacturer's reagents or to a specific analyzer (Fig. 1B).

In the homogeneous HDL-chol method group, the mean (minimum–maximum range) percent TE, bias, and overall imprecision were 9.5% (6.0–17.3%), 3.3% (-6.4% to 13.4%), and 1.9% (0.7–2.4%) for the α -cyclodextrin sulfate/PEG-coupled enzyme assay, and 15.7% (3.3–30.7%), 1.66% (-13.1% to 34.2%), and 1.9% (1.2–2.8%) for the polymer/polyanion assay. In the α -cyclodextrin sulfate/PEG-

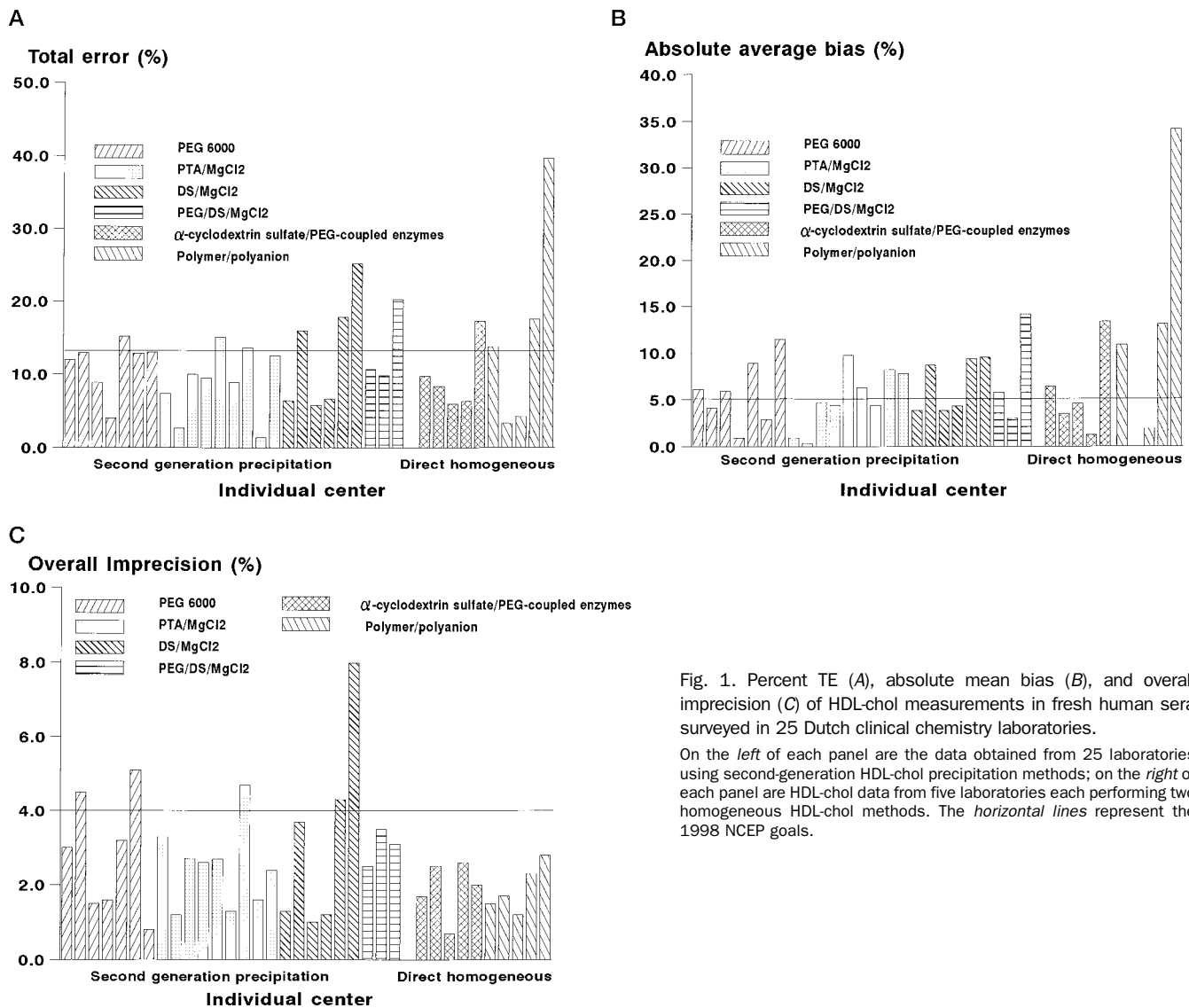


Fig. 1. Percent TE (A), absolute mean bias (B), and overall imprecision (C) of HDL-chol measurements in fresh human sera surveyed in 25 Dutch clinical chemistry laboratories.

On the left of each panel are the data obtained from 25 laboratories using second-generation HDL-chol precipitation methods; on the right of each panel are HDL-chol data from five laboratories each performing two homogeneous HDL-chol methods. The horizontal lines represent the 1998 NCEP goals.

coupled enzyme assay group, one of five laboratories did not meet the 1998 TE criterion, whereas in the polymer/polyanion assay group, three of five laboratories exceeded the 13% TE criterion because of excessive biases (Fig. 1).

The results of the linear regression analyses and the analytical performance, averaged for the participating laboratories per HDL-chol method group, are presented in Table 1. In general, the slopes and intercepts scatter around one and zero, respectively. However, for the homogeneous polymer/polyanion assay, the slopes and intercepts of the regression lines were significantly different from one and zero, respectively ($P < 0.05$), both overall and for individual participating laboratories. Overall imprecision was far below 4% in each individual laboratory performing either of the two homogeneous HDL-chol methods. Conversely, the imprecision criterion was not reached unanimously in individual laboratories performing chemical precipitation methods, with the exception of

the three laboratories performing the PEG/DS/MgCl₂ precipitation method. The range of interlaboratory biases, i.e., minimum–maximum range, was smallest and closest to 0% in the PTA/MgCl₂ method group (−6.3% to 9.8%), and largest in the homogeneous polymer/polyanion method group (−13.1% to 34.2%). TE was smallest for the PEG and PTA/MgCl₂ precipitation method groups, i.e., the maximum TE was ~15% and was largest for the homogeneous polymer/polyanion method group.

CHARACTERIZATION OF THE HDL-chol CRMs

After reconstitution of the CRMs a slight turbidity was visible in CRMs 4–8, whereas a stronger turbidity was observed in CRMs 1–3 and in CRMs 9–10. CRM 11 was found to be lipemic. The CRMs under study, both with 50 and 200 g/L saccharose, exhibited well-defined α - and β -lipoprotein bands, but faint or missing pre- β -bands, especially in CRMs 1–3. Notably, some precipita-

Table 1. Analytical performance of second-generation precipitation methods and third-generation homogeneous methods for HDL-chol vs the HDL-chol DCM from the CDC.

	Mean (minimum–maximum)					
	PEG n = 7 ^a	PTA/MgCl ₂ n = 9	DS/MgCl ₂ n = 6	PEG/DS/MgCl ₂ n = 3	α-Cyclodextrin sulfate/PEG-coupled enzymes n = 5	Polymer/polyanion n = 5
Linear regression analysis						
Field HDL-c method group (<i>y</i> -axis) vs HDL-chol DCM (<i>x</i> -axis) ^b						
Slope	1.017 (0.935–1.153)	1.071 (0.947–1.209)	1.008 (0.907–1.102)	0.953 (0.864–1.007)	1.055 (0.939–1.214)	0.802 (0.719–0.858)
Intercept, mmol/L	–0.026 (–0.139 to 0.133)	–0.056 (–0.145 to 0.042)	–0.032 (–0.004 to 0.027)	–0.044 (–0.182 to 0.091)	–0.019 (–0.204 to 0.126)	0.240 (0.037–0.729)
<i>r</i>	0.998 (0.984–1.000)	0.998 (0.994–1.000)	0.998 (0.997–0.999)	0.993 (0.981–1.000)	0.997 (0.991–1.000)	0.987 (0.955–0.999)
Analytical performance characteristics of field HDL-chol method groups						
Overall CV _a , %	2.8 (0.8–5.1)	2.5 (1.2–4.7)	3.3 (1.0–8.0)	3.0 (2.5–3.5)	1.9 (0.7–2.6)	1.9 (1.2–2.8)
Average bias, %	–0.5 (–8.9 to 11.5)	2.6 (–6.3 to 9.8)	–2.2 (–9.4 to 9.5)	–7.7 (–14.2 to –3.0)	3.3 (–6.4 to 13.4)	1.7 (–13.1 to 34.2)
TE, % ^c	11.3 (4.0–15.2)	10.1 (2.7–15.1)	13.0 (5.8–25.2)	13.6 (9.9–20.3)	9.5 (6.0–17.3)	15.7 (3.3–39.7)

^a n, number of laboratories.
^b Field HDL-chol analyses were performed locally in 25 Dutch clinical chemistry laboratories using fresh, normotriglyceridemic human sera covering the HDL-chol measuring range (n = 6; duplicate analyses during three consecutive working days). HDL-chol DCM analyses were done centrally at the LRL Rotterdam, out of frozen aliquots (duplicate analyses in one analytical run). Linear regression analysis was performed for each participating laboratory. Subsequently, the calculated parameters were averaged per method group.
^c TE (%) = 1.96 × CV_a (%) + absolute average bias (%).

tion appeared at the application site as well as a smear of indiscriminate staining between the application and the β-region, being again much more pronounced in CRMs 1–3 than in CRMs 4–10. As an example, lipoprotein patterns of CRMs 1–3 and CRMs 4–6 on agarose gel electrophoresis are shown in Fig. 2, A and B, respectively. For comparison, a normotriglyceridemic control was run in lanes 7 and 8 of Fig. 2A.

In agreement with previous results (17), density-gradient UC (data not shown) revealed sharp and well-separated HDL₂ and HDL₃ bands in all CRMs. In addition, tiny flakes were observed at the interphase with the atmosphere after UC. Flakes were also observed in the CDC Reference Method: after UC at serum density minuscule flakes were observed in the infranatants in all CRMs. More flakes were noted in CRMs 1–3 compared with CRMs 4–11.

VALIDATION OF THE MODIFIED CDC HDL-chol REFERENCE METHOD PROCEDURE

The mean HDL-chol ± SD (CV) for AQ15, as measured in duplicate in 20 separate assays, was 1.288 ± 0.029 mmol/L (2.3%) when the 2-mL procedure was used. In comparison, the mean HDL-chol ± SD (CV) for AQ15, as measured in duplicate in 20 assays, was 1.292 ± 0.027 mmol/L (2.1%) when the regular 5-mL procedure was used and 1.287 ± 0.009 (0.7%) when the CDC DCM was used.

Analogously, in fresh-frozen single donor sera measured using duplicate spins in three separate assays, the

overall CVs obtained with the 2-mL procedure was 0.8% at 2.24 mmol/L and 1.9% at 1.25 mmol/L.

Moreover, the accuracy of the modified CDC UC 2-mL procedure for HDL-chol in the presence of 80 g/L saccharose, i.e., the final saccharose concentration in the modified 2-mL procedure, was evaluated. Previously, the HDL-chol concentration in a saccharose-supplemented serum pool was compared with the HDL-chol target value, as determined with the original 5-mL procedure in an undiluted serum pool and with the modified 2-mL procedure in the same, non-saccharose-supplemented, diluted serum pool. Because weighing 80 g/L of saccharose into serum pools diluted the pool 1.135-fold, the non-saccharose-supplemented serum pool was, in case of the 2-mL procedure and before UC, diluted to a similar extent. The diluent was saline solution (9 g NaCl/L). In each condition, HDL-chol value assignments were done in quadruplicate in two different UC runs. When the 5-mL UC procedure was used, the HDL-chol target value was 1.219 ± 0.009 mmol/L (0.7%); in the 2-mL procedure, the mean HDL-chol concentration was 1.214 ± 0.035 mmol/L (2.9%) in the non-saccharose-supplemented serum pool and 1.203 ± 0.021 (1.8%) mmol/L in the 80 g/L saccharose-containing serum pool (not significant; *P* ≥ 0.05).

VALUE ASSIGNMENT TO THE HDL-chol CRMs

The HDL-chol values assigned by the LRL Rotterdam to the SKZL CRMs are presented in Table 2. Each target value is the mean of quadruplicate analyses measured in

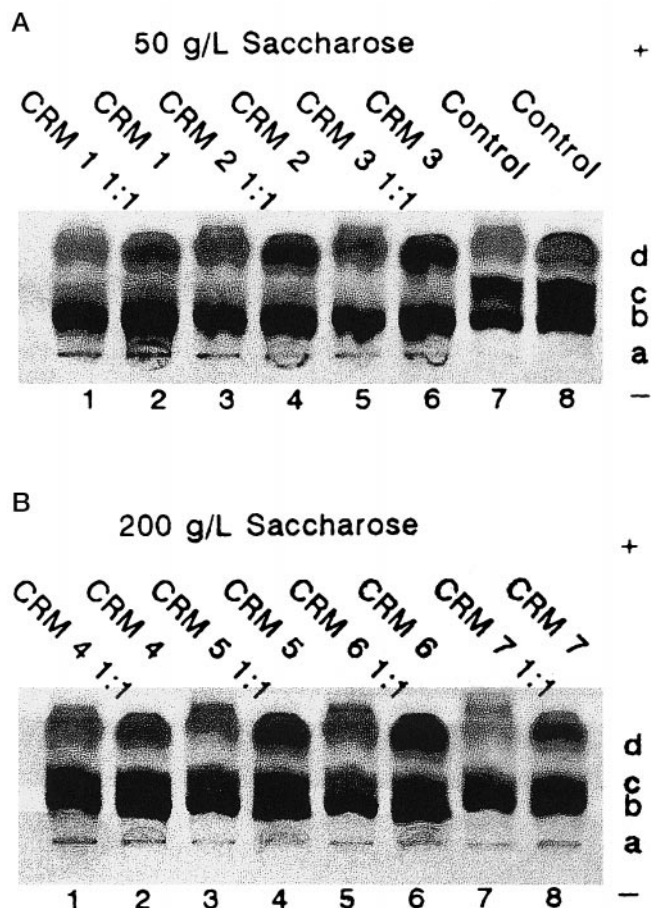


Fig. 2. Agarose gel electrophoresis of seven lyophilized HDL-chol CRMs (CRMs 1–7), containing either 50 g/L saccharose (A) or 200 g/L saccharose (B).

β -, pre- β -, and α -lipoproteins migrated at distances *b*, *c*, and *d*, respectively. Some nonmigrating material was present at the application zone (*a*). CRMs 1–3 contained 50 g/L saccharose, whereas CRMs 4–7 contained 200 g/L saccharose. All CRMs were applied twice, i.e., either diluted 1:1 with physiological saline solution or undiluted. (A), lanes 7 and 8, fresh control serum.

four different assays, being produced with the CDC Reference Method in the case of CRMs 1–3 and with the modified CDC Reference Method in the case of CRMs 4–11. CRMs were reconstituted freshly and pooled before each UC run. Notably, SDs ranged between 0.043 and 0.166 mmol/L and CVs between 2.7% and 10% (Table 2); i.e., SDs and CVs in processed CRMs were three- to fivefold higher than those obtained in frozen sera (see above).

PERFORMANCE OF HDL-chol CRMs 1–11 FOR ACCURACY ASSESSMENT OF HDL-chol PRECIPITATION METHODS

The dispersion of the mean HDL-chol concentrations obtained in CRMs 1–11 by each participating laboratory—the mean being based on duplicate analyses repeated during 3 consecutive days—around the target values of CRMs 1–11 is presented in Fig. 3. The outer horizontal lines display the uncertainty on the values assigned by the (modified) CDC Reference Method and represent the

mean \pm 2 SD. From Fig. 3 it is obvious that there is a large scatter of the mean HDL-chol values around the target values. Nevertheless, most data fit within the mean plus or minus the measuring error (expressed as SD) of the reference range established with the (modified) CDC Reference Method. Within each method group, interlaboratory variability was similar for CRMs 1–10; however, in the case of the chylomicron-rich CRM 11 the interlaboratory dispersion was more pronounced. Across method groups, the PEG 6000 and the PTA/MgCl₂ precipitation methods performed better than the DS/MgCl₂ method both in terms of accuracy and in terms of interlaboratory differences (Table 2). Conversely, the DS/MgCl₂ had the worst performance in combination with the lyophilized, saccharose-containing CRMs, especially in the CRM containing 200 g/L saccharose.

PERFORMANCE OF HDL-chol CRMs 1–11 FOR ACCURACY ASSESSMENT OF HDL-chol HOMOGENEOUS METHODS

In the α -cyclodextrin sulfate/PEG-coupled enzyme assay, the dispersion of the mean HDL-chol concentrations measured in CRMs 1–11 by each participating laboratory (four laboratories; one laboratory failed to analyze the CRMs in combination with the homogeneous HDL-chol methods) around the target values of CRMs 1–11 was very moderate, the mean from the participating laboratories being well within the mean \pm 2 SD confidence limits of the CDC Reference Method (Fig. 3). From Table 2 it is obvious that for CRMs 2–6 the mean HDL-chol values were within \pm 1% of the assigned values. In the diluted CRM 7, the mean HDL-chol values displayed a positive bias for all four laboratories (overall mean bias, 5.4%), whereas in the “concentrated” CRM 9 mean HDL-chol values were negatively biased (overall mean bias, –7.2%). Even in hypertriglyceridemic CRMs, i.e., CRMs 10 and 11, which contained 4.85 and 15.8 mmol/L triglycerides, respectively, biases were less than \pm 5%.

In the polymer/polyanion assay, a large spread in mean HDL-chol values around the target values was observed (Fig. 3 and Table 2), irrespective of the CRM analyzed. These observations are in line with those observed in native human sera.

Discussion

STATE-OF-THE-ART ANALYTICAL PERFORMANCE OF HDL-chol MEASUREMENTS IN THE NETHERLANDS

In HDL-chol assays, accuracy and precision are critical because clinical decisions are made using nationally defined cutoff points that do not allow for methodological error (3, 4). Whereas the precision of HDL-chol analyses is monitored periodically in the SKZL, the Dutch proficiency testing program provided by the Dutch National External Quality Assurance Society, accuracy can only be checked relative to the scores of colleagues (grand mean). Absolute accuracy control is not possible at present because of a lack of suitable and stable (lyophilized) refer-

Table 2. HDL-chol analysis on CRMs 1–11 by 25 Dutch clinical chemistry laboratories using the indicated four, most commonly used second-generation HDL-chol precipitation methods, and by 4 laboratories using two types of third-generation HDL-chol methods.^a

CRM	[Saccharose] _{final} g/L	Assigned value (LRL Rotterdam)	Mean ± SD, mmol/L (CV, %)							
			PEG 6000 (7 labs)	PTA/MgCl ₂ (9 labs)	DS/MgCl ₂ (6 labs)	PEG/DS/MgCl ₂ (3 labs)	α-Cyclodextrin sulfate/PEG-coupled enzymes (4 labs) ^b	Polymer/polyanion (4 labs) ^b		
1	50	0.833 ± 0.051 (6.2%)	0.793 ± 0.057 (7.3%)	0.790 ± 0.056 (7.1%)	0.766 ± 0.051 (6.7%)	0.686 ± 0.059 (8.7%)	0.779 ± 0.031 (4.0%)	0.890 ± 0.140 (16%)		
2	50	1.152 ± 0.043 (3.7%)	1.177 ± 0.067 (5.7%)	1.164 ± 0.052 (4.5%)	1.123 ± 0.075 (6.7%)	1.061 ± 0.062 (5.8%)	1.150 ± 0.016 (1.4%)	1.192 ± 0.108 (9.1%)		
3	50	1.611 ± 0.044 (2.7%)	1.691 ± 0.113 (6.7%)	1.600 ± 0.094 (5.9%)	1.581 ± 0.106 (6.7%)	1.541 ± 0.058 (3.8%)	1.615 ± 0.015 (0.9%)	1.598 ± 0.079 (5.0%)		
4	200	0.911 ± 0.052 (5.7%)	0.913 ± 0.073 (8.0%)	0.866 ± 0.085 (9.9%)	0.818 ± 0.121 (15%)	0.783 ± 0.059 (7.5%)	0.903 ± 0.025 (2.8%)	0.982 ± 0.111 (11%)		
5	200	1.314 ± 0.075 (5.7%)	1.312 ± 0.096 (7.3%)	1.271 ± 0.071 (5.6%)	1.223 ± 0.280 (23%)	1.168 ± 0.084 (7.2%)	1.316 ± 0.018 (1.4%)	1.315 ± 0.066 (5.0%)		
6	200	1.858 ± 0.062 (3.4%)	1.850 ± 0.113 (6.1%)	1.818 ± 0.083 (4.5%)	1.631 ± 0.369 (23%)	1.726 ± 0.079 (4.6%)	1.869 ± 0.022 (1.2%)	1.794 ± 0.062 (3.5%)		
7	200	0.867 ± 0.063 (7.2%)	0.943 ± 0.128 (14%)	0.865 ± 0.057 (6.6%)	0.771 ± 0.074 (9.6%)	0.811 ± 0.059 (7.3%)	0.914 ± 0.015 (1.7%)	0.931 ± 0.065 (7.0%)		
8	200	1.241 ± 0.068 (5.5%)	1.290 ± 0.103 (8.0%)	1.218 ± 0.086 (7.1%)	1.131 ± 0.255 (23%)	1.137 ± 0.058 (5.1%)	1.271 ± 0.014 (1.1%)	1.290 ± 0.051 (4.0%)		
9	200	1.987 ± 0.106 (8.4%)	1.826 ± 0.153 (8.4%)	1.921 ± 0.072 (3.7%)	1.779 ± 0.307 (17%)	1.683 ± 0.086 (5.1%)	1.843 ± 0.022 (1.2%)	1.881 ± 0.074 (3.9%)		
10	200	1.068 ± 0.111 (10%)	1.044 ± 0.088 (8.4%)	1.004 ± 0.065 (6.4%)	0.870 ± 0.219 (25%)	0.920 ± 0.059 (6.4%)	1.020 ± 0.033 (3.2%)	1.131 ± 0.125 (11%)		
11	200	0.742 ± 0.073 (9.8%)	0.822 ± 0.115 (14%)	0.863 ± 0.226 (26%)	0.679 ± 0.232 (34%)	ND ^c	0.733 ± 0.039 (5.3%)	1.002 ± 0.293 (29%)		

^a Each participating laboratory assayed the freshly reconstituted CRMs in duplicate on 3 different days. Results are given as means ± SD (CV) and are compared with those obtained with the (modified) CDC reference method.

^b One laboratory failed to analyze the CRMs together with the native sera in the same series.

^c ND, not determined.

ence materials that have a long shelf half-life. To meet this need, a pilot HDL-chol survey was conducted in 25 Dutch clinical chemistry laboratories that aimed at documenting the state-of-the-art analytical performance of HDL-chol measurements in The Netherlands. Emphasis was put on accuracy assessment using native, normotriglyceridemic human sera. Because no performance recommendations for HDL-cholesterol measurements have been developed by the Dutch Clinical Chemistry Society thus far, the calculated bias, imprecision, and TE were interpreted in the light of the performance guidelines issued by the American NCEP Working Group on Lipoprotein Measurement (5).

Excessive bias, exceeding ±5%, was found to be present in ~50% of the clinical chemistry laboratories performing chemical precipitation methods (Fig. 1B). In addition, because mean biases ranged between -14.2% and 11.5%, interlaboratory differences between HDL-chol measurements were as large as 25%, the latter observation underscoring the findings of Crook (9) in 32 lipid clinics in the United Kingdom. Excess bias was also observed in three of five laboratories that implemented the homogeneous HDL-chol methods. In the direct α-cyclodextrin sulfate/PEG-coupled enzyme method, the overall mean bias of 3.3% was in line with previous findings of our group (14). In the direct polymer/polyanion assay, apparent improper reagent formulation and/or application rather than improper value assignment of the calibrator explained the observed findings (data not shown). Although the survey was based on a small group, it seems that the NCEP precision criterion of ≤4% was amply met for the homogeneous HDL-chol methods compared with the precipitation methods (Fig. 1C), probably because the direct HDL-chol assays make the cumbersome manual pretreatment step for isolation of HDL redundant.

The major advantages of this bias survey are related to the fact that the targeting of the native sera was done in a CDC Network Laboratory, using the recommended HDL-chol accuracy bases (15, 16). In addition, firm conclusions could be drawn from this survey because biases were calculated from HDL-chol measurements performed in fresh and native human sera, and the analysis of six human sera on 3 consecutive days per laboratory led to an acceptable uncertainty for the bias and TE estimates (Mulder and Cobbaert, submitted for publication).

Assuming that the observed analytical performance of the participating clinical laboratories for HDL-chol is representative for The Netherlands, we concluded that especially the accuracy, and to a lesser extent the precision, of conventional HDL-chol precipitation methods were insufficient to satisfy the 1998 NCEP TE goal in one-third of the clinical chemistry laboratories (Fig. 1A). Whereas precision of the direct HDL-chol methods was excellent (Fig. 1C), inaccurate results were obtained in about one-half of the participating laboratories (Fig. 1B). Consequently, the results from this pilot HDL-chol survey underscore the urgent need for stable and commutable calibration materials that emulate patient sera.

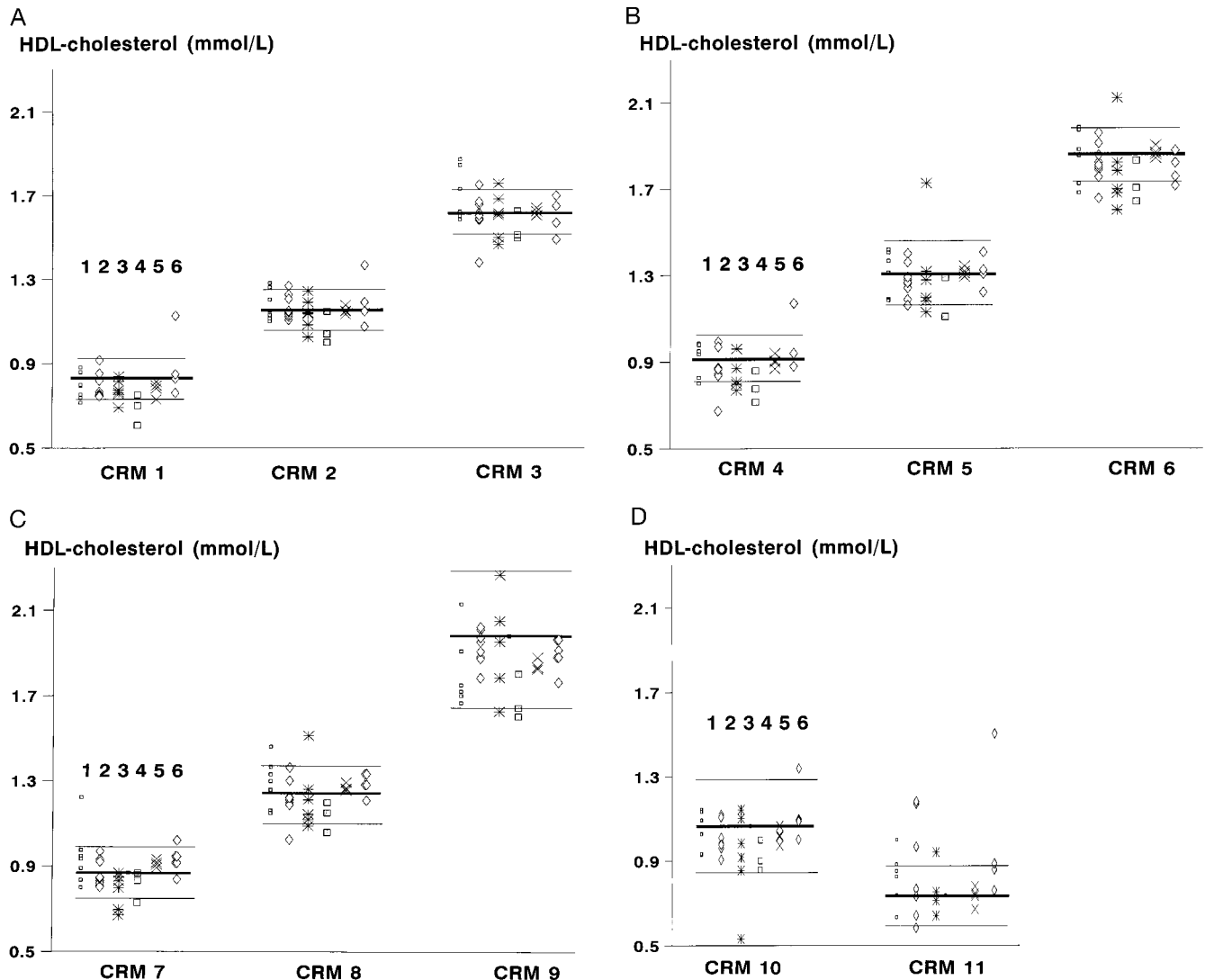


Fig. 3. HDL-chol recovery in CRMs 1–11 as measured by four precipitation methods and two homogeneous HDL-chol methods .

1, PEG 6000; 2, PTA/MgCl₂; 3, DS/MgCl₂; 4, PEG/DS/MgCl₂; 5, α -cyclodextrin sulfate/PEG-coupled enzymes; 6, polymer/polyanion. The *thick horizontal lines* represent the assigned values as measured in 16-fold with either the CDC Reference Method or the modified CDC Reference Method for HDL-chol. The *thin horizontal lines* represent the uncertainty on the assigned values, calculated as mean \pm 2 SD. For each CRM and method group, the individual HDL-chol values of each participating laboratory are given. *Symbols* indicate participating laboratories.

EVALUATION OF HDL-chol CRMs

Because improved nationwide accuracy awaits the availability of reference materials that are free of matrix effects, are sufficiently stable to be shipped by overnight postage without freezing, and have long-term stability (preferably at 4 °C), an attempt was made to develop such CRMs for HDL-chol. To this end, either 50 or 200 g/L saccharose was weighed into serum pools to create different degrees of cryo- and lyoprotection upon freezing and subsequent lyophilization (17). A final saccharose concentration of 200 g/L was found to be a better protectant because macroscopic turbidity after reconstitution of the lyophilized CRMs was significantly less in CRMs 4–9 compared with CRMs 1–3, the latter being cryoprotected with only 50 g/L of saccharose. However, reconstituted CRMs were slightly turbid even in the presence of 200 g/L saccharose,

pointing to some degree of lipoprotein disintegration and/or apolipoprotein denaturation. The observed turbidity in the CRMs could be established on the basis of the absorbance of the reconstituted materials at 620 nm (data not shown), the presence of nonmigrating material at the application site after agarose gel electrophoresis (Fig. 2), and the presence of tiny flakes in the infranatants after UC at serum density or at the interphase with the atmosphere after density gradient centrifugation (data not shown). The minuscule flakes probably were denatured apoB that could no longer be dissolved, which was supported by the faint or absent pre- β bands on agarose gel electrophoresis.

Subsequent value assignment of the CRMs with the CDC HDL-chol Reference Method was hampered by the high saccharose concentration, as reflected by the three- to fivefold increases in CVs for targeting CRMs (Table 2)

compared with those of frozen single-donor sera and frozen quality-control material (see *Results*). To target CRMs 4–11, which contained 200 g/L saccharose, the CDC HDL-chol Reference Method had to be modified. In essence, the saccharose concentration in CRMs 4–11 was diluted to 80 g/L before UC to enable lipoprotein separation, which automatically adds a dilution factor of 2.5, contributing greatly to the higher CVs. Because the accuracy of the modified CDC HDL-chol Reference Method was within 0.5% of the target value obtained with the original 5-mL procedure (mean \pm SD of 1.288 ± 0.029 mmol/L for AQ15, using the 2-mL procedure, compared with 1.292 ± 0.027 mmol/L, using the official 5-mL procedure) and because value assignment of each CRM was based on 16 spins using either the Reference Method or the Modified Reference Method, i.e., performing quadruplicate spins in four different UC runs, "target" values for HDL-chol can be considered reliable and accurate.

In the present pilot HDL-chol survey, clinical chemistry laboratories analyzed freshly reconstituted CRMs in duplicate in three separate assays. Per participant and per CRM measured HDL-chol concentrations were averaged. Fig. 3 shows that these HDL-chol concentrations were highly variable both within and between precipitation method groups. Because the between-run CVs of the CRMs produced by individual laboratories did not differ significantly from the between-run CVs produced with internal, non-saccharose-based, quality-control material ($P \geq 0.05$; data not shown), it is unlikely that pipetting errors caused by the high viscosity of the CRMs explain the interlaboratory and intermethod differences. Probably, the high amount of saccharose in the (manual) pretreatment step interfered with HDL isolation. From the scattered HDL-chol results around the assigned values (Fig. 3), we concluded that saccharose-based reference materials cannot be used for standardizing precipitation methods.

HDL-chol concentrations in the CRMs as measured by all four laboratories that performed the α -cyclodextrin sulfate/PEG-coupled enzyme assay were similar to those obtained for fresh material, ranging between 93% and 105%. Likely, the 101-fold dilution of the saccharose concentration in the final reaction mixture (4 μ L of CRM was incubated with 300 μ L of R1 and 100 μ L of R2) did not interfere with assay specificity and HDL-chol recovery. More specifically, HDL-chol recoveries were almost ideal in CRMs 4–6, being $100\% \pm 1\%$. Because the aforementioned CRMs 4–6 were pools of the highest quality, these data support the validity of the targeting procedure in the CRMs with the modified CDC Reference Method. However, for CRM 7, the mean HDL-chol recovery was 105.4%, whereas for CRM 9, the mean HDL-chol recovery was 92.8%. The positive bias obtained in CRM 7 and the negative bias in CRM 9 can be linked to the dilution or concentration of these pools, respectively, and point to the sensitivity of the homogeneous method for large variations in protein concentration. In CRMs 10 and 11, characterized by moderately increased concentrations of

triglycerides, HDL-chol recoveries were 96% and 99%, respectively, illustrating the relative insensitivity of the Boehringer direct HDL-chol method to hypertriglyceridemia.

In the polymer/polyanion method, the HDL-chol concentrations measured in the CRMs could not be interpreted because an unacceptable, method-related bias was already disclosed in native human sera during the pilot HDL-chol survey. The bias issue of the direct polymer/polyanion method from Genzyme currently is solved in the liquid formulation of the reagent because the reagent formulation of the liquid HDL-chol assay differs distinctly from that of the lyophilized version in that there is no polymer in the first reagent, the magnesium concentration has been reduced, an HDL-selective detergent has been introduced in the second reagent, and the chromogens 4-aminoantipyrine and DSBmT have been segregated into the first and second reagent, respectively. The different modifications have been aimed successively at preventing precipitation with alkaline wash solutions on analyzers with reusable cuvettes; at improving assay specificity, especially among hypertriglyceridemic specimens; at reducing the background absorbance during the R1 phase; and at improving the stability of the reagent blank. In addition, the assay has incorporated a true endpoint reading that is independent of analyzer cycling time, and the wavelength selection has been changed (600 nm/700 nm instead of 546 nm/660 nm for the main and subsidiary wavelengths, respectively) to reduce hemoglobin interference. Consequently, the liquid version of the Genzyme assay displays an overall mean bias of $\ll \pm 5\%$ against the CDC HDL-chol DCM in normotriglyceridemic sera. Moreover, preliminary data from our group reveal that the CRMs tested perform equally well with the latest liquid version of the Genzyme direct HDL-chol method and with the Boehringer direct HDL-chol method.

In conclusion, more effort must be made in The Netherlands to standardize HDL-chol methods to the CDC Reference Method and/or to the CDC DCM and to reduce method bias to less than $\pm 5\%$. To this end, the lyophilized, saccharose-containing, human sera investigated seem to have ample potential for use in standardizing homogeneous HDL-chol methods. More specifically, the selectively collected CRMs 4–6, prepared from lipoprotein-stabilized serum pools and having a normal protein matrix with the exception of the presence of 200 g/L saccharose, seem to be the first choice candidates for future standardization of homogeneous HDL-chol assays because the mean HDL-chol concentrations obtained in CRMs 4–6 by the α -cyclodextrin sulfate/PEG-coupled enzyme method were within 1% of the assigned values obtained by the modified CDC Reference Method. Nevertheless, more work must be done to investigate whether the data can be extrapolated to all homogeneous HDL-chol assays. Conversely, the viscous CRMs 1–11 cannot be used for standardization of the HDL-chol precipitation methods. However, it can be expected that virtually all

clinical laboratories will switch to direct HDL-chol methods in the near future, abolishing the need for standardization of precipitation methods.

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Appendix

PARTICIPATING DUTCH CLINICAL LABORATORIES

Academisch Ziekenhuis Leiden, Leiden; Academisch Ziekenhuis Nijmegen, Nijmegen; Academisch Ziekenhuis Rotterdam, Rotterdam; Academisch Ziekenhuis Vrije Universiteit Amsterdam, Amsterdam; Baronie Ziekenhuis, Breda; BCO, Breda; Catharina Ziekenhuis, Eindhoven; Eemland Ziekenhuis Lichtenberg, Amersfoort; Groot Ziekengasthuis, 's Hertogenbosch; Hofpoort Ziekenhuis, Woerden; Holy Ziekenhuis, Vlaardingen; Leyenburg Ziekenhuis, Den Haag; Maasland Ziekenhuis, Sittard; Medisch Spectrum, Enschede; Sint-Anna Ziekenhuis, Geldrop; Sint-Lucas Ziekenhuis, Winschoten; Sint-Maartens Gasthuis, Venlo; Spaarne Ziekenhuis, Heemstede; Stichting KCL, Leeuwarden; Streekziekenhuis Koningin Beatrix, Winterswijk; Streekziekenhuis Walcheren, Vlissingen; Westeinde Ziekenhuis, Den Haag; Ziekenhuis Amstelveen, Amstelveen; Ziekenhuis de Weezelanden, Zwolle; and Zuiderziekenhuis, Rotterdam.

References

- Lewis B, Assmann G, Tikkanen M, Mancini M, Pometta D. Prevention of coronary heart disease: scientific background and new clinical guidelines. Recommendations of the European Atherosclerosis Society, prepared by the International Task Force for Prevention of Coronary Heart Disease. *Nutr Metab Cardiovasc Dis* 1992;2:113–56.
- NIH Consensus Development Panel on Triglyceride, High Density Lipoprotein, and Coronary Heart Disease. *JAMA* 1993;269:505–10.
- Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Adult Treatment Panel II. *JAMA* 1993;269:3015–23.
- Behandeling en preventie van coronaire hartziekten door verlaging van de plasmacholesterolconcentratie. Consensus Cholesterol, tweede herziening, CBO/MWR, 1998:96 pp.
- Warnick GR, Wood PD for the National Cholesterol Education Program Working Group on Lipoprotein Measurement. National Cholesterol Education Program recommendations for measurement of high-density lipoprotein cholesterol: executive summary. *Clin Chem* 1995;41:1427–33.
- Wiebe DA, Warnick GR. Measurement of high-density lipoprotein cholesterol concentration. In: Rifai N, Warnick GR, eds. *Laboratory measurement of lipids, lipoproteins and apolipoproteins*. Washington, DC: AACC Press, 1994:91–105.
- Demacker PNM, Vos-Jansen HE, Hijmans AGM, van't Laar A, Janssen AP. Measurement of HDL-cholesterol in serum: comparison of six isolation methods with enzymatic cholesterol analysis. *Clin Chem* 1980;26:1780–6.
- Warnick GR, Cheung MC, Albers JJ. Comparison of current methods for high-density lipoprotein cholesterol quantification. *Clin Chem* 1979;25:596–604.
- Crook D. A survey of biases in the measurement of plasma lipid and lipoprotein concentrations in 32 lipid clinics in the UK. *Ann Clin Biochem* 1996;33:82–3.
- Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, Miyauchi K. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated α -cyclodextrin. *Clin Chem* 1995;41:717–23.
- Harris N, Galpichian V, Rifai N. Three routine methods for measuring high-density lipoprotein cholesterol compared with the reference method. *Clin Chem* 1996;42:738–43.
- Nauck M, März W, Haas B, Wieland H. Homogeneous assay for direct determination of high-density lipoprotein cholesterol evaluated. *Clin Chem* 1996;42:424–9.
- Nauck M, März W, Jarausch J, Cobbaert C, Sägers A, Bernard D, et al. Multicenter evaluation of a homogeneous assay for HDL-cholesterol without sample pretreatment. *Clin Chem* 1997;43:1622–9.
- Cobbaert C, Zwang L, Ceriotti F, Modenese A, Cremer P, Herrmann W, et al. Reference standardization and triglyceride interference of a new homogeneous HDL-cholesterol assay compared with a former chemical precipitation assay. *Clin Chem* 1998;44:779–89.
- Myers GL, Cooper GR, Henderson LO, Hassemer DJ, Kimberly M. Standardization of lipid and lipoprotein measurements. In: Rifai N, Warnick GR, eds. *Laboratory measurement of lipids, lipoproteins and apolipoproteins*. Washington, DC: AACC Press, 1994:177–205.
- Myers GL, Cooper GR, Henderson LO, Hassemer DJ, Kimberly M. Standardization of lipid and lipoprotein measurements. In: Rifai N, Warnick GR, Dominiczak MH, eds. *Handbook of lipoprotein testing*. Washington, DC: AACC Press, 1997:223–50.
- Baadenhuijsen H, Demacker PNM, Hessels M, Boerma GJM, Penders TJ, Weykamp C, Willems HL. Testing the accuracy of total cholesterol assays in an external quality-control program. Effect of adding sucrose to lyophilized control sera compared with use of fresh or frozen sera. *Clin Chem* 1995;41:724–30.
- McNamara JR, Leary ET, Ceriotti F, Cobbaert C, Cole TG, Hassemer DJ, et al. Status of lipid and lipoprotein standardization. *Clin Chem* 1997;43:1306–10.
- Wiebe DA, Warnick GR. Measurement of high-density lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. *Handbook of lipoprotein testing*. Washington, DC: AACC Press, 1997:127–44.
- Demacker PNM, Hijmans AGM, Vos-Janssen HE, van't Laar A, Jansen AP. A study of the use of polyethylene glycol in estimating cholesterol in high density lipoprotein. *Clin Chem* 1980;26:1775–9.
- Demacker PNM, Hessels M, Toenhake-Dijkstra H, Baadenhuijsen H. Precipitation methods for high-density lipoprotein cholesterol measurement compared, and final evaluation under routine operating conditions of a method with a low sample-to-reagent ratio. *Clin Chem* 1997;43:663–8.
- Lippi U, Graziani MS, Manzato F, Schinella M. Assay of cholesterol in high-density lipoprotein in hypertriglyceridemic sera [Letter]. *Clin Chem* 1986;32:2118–9.