

The Fats of Life

Inside This Issue...

Editor's Notes by G. Russell Warnick 2

The Use of Serum Amyloid A–Low-density Lipoprotein (SAA-LDL) as a Cardiometabolic Disease Marker

Kazuhiko Kotani, MD, PhD 6

Is it or is it not Isolated Low HDL-C?

Thomas Dayspring, MD 9

Establishment of Practical Procedure for Measurement of Total Cholesterol by Isotope Dilution /Gas Chromatography/Mass Spectrometry

I. Koyama, H. Iso, M. Kiyama, et al 14

Is Directly Measured Low-density Lipoprotein Clinically Equivalent to Calculated Low-density Lipoprotein?

Reprinted from the *Journal of Clinical Lipidology* 17

Editor's Notes: Homogeneous (Direct) Assays for HDL-C and LDL-C Show Potential but Have Not Been Fully Validated

Reprinted from *The Fats of Life*, Winter 2002 25

Letters: Lack of Agreement of Homogeneous Assays with the Reference Method for LDL-Cholesterol May Not Indicate Unreliable Prediction of Risk for Cardiovascular Disease

Reprinted from *Clinical Chemistry* 2002 29

Editor's Notes: G. Russell Warnick

Reprinted from *The Fats of Life*, Fall 2003 33

The policy of the AACC is that only the President, President-Elect, Secretary, Treasurer, Executive Vice-President and the Association's Legal Counsel may make official statements on behalf of the Association. Therefore, all views expressed herein are solely those of the Contributors and Members of the Editorial Board and not necessarily those of the Association or the LVDD.

To Join the Division, go to
<http://www.aacc.org/AACC/members/divisions/lipids/> and click the
 Join Division link (you'll need to log in to AACC's website).

Have Questions about lipoproteins and vascular disease-related topics? If so, join the Division Listserv:

<https://my.binhost.com/lists/listinfo/aacc-lipo-vasc-div>



Division Officers

Daniel M. Hoefner, PhD, Chair &

Information Officer

Health Diagnostic Laboratory, Inc.

737 N. 5th Street, Suite 103

Richmond, VA 23219

(804) 343-2718

Sridevi Devaraj, PhD, Chair-Elect

UC Davis Medical Center

Laboratory for Atherosclerosis and Metabolic Research

4635 Second Avenue

Sacramento, California 95817

(916) 734-6594

Gyorgy Csako, MD, Past Chair

National Institutes of Health

Clinical Center

Department of Laboratory Medicine

Bethesda, MD 20892-1882

(301) 496-1924

Faith Clendenen, MPH, MPA, Secretary

Dartmouth Hitchcock Medical Center

Laboratory

1 Medical Center Drive

Lebanon, NH 03756-0001

(603) 650-4697

Tonya Mallory, Treasurer

Health Diagnostic Laboratory, Inc.

737 N. 5th Street, Suite 103

Richmond, VA 23219

(804) 986-3660

Editorial Staff

G. Russell Warnick, MS, MBA, Editor

grwarnick@hotmail.com

Joseph McConnell, PhD, Associate Editor

jmcconnell@hldlabinc.com

Katsuyuki Nakajima, PhD, Associate Editor

nakajimak05@ybb.ne.jp

Alan Remaley, MD, PhD, Associate Editor

aremaley1@cc.nih.gov

Daniel Hoefner, PhD, Associate Editor

& Producer

dhoefer@hldlabinc.com

Editorial Advisory Board

J. Contois, PhD; Windham, ME

J. Maciejko, PhD; Detroit, MI

M. Nauck, MD; Greifswald, Germany

N. Rifai, PhD; Boston, MA

D. A. Wiebe PhD; Madison, WI

Establishment of Practical Procedure for Measurement of Total Cholesterol by Isotope Dilution / Gas Chromatography / Mass Spectrometry

I. Koyama, H. Iso, M. Kiyama, T. Shimamoto, Y. Ishikawa and M. Nakamura, Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan, Osaka Medical Center for Health Science and Promotion, Osaka, Japan

Traceability of total cholesterol (TC) measurements can be verified by comparing manufacturers' and clinical laboratories' methods with the Abell-Kendall (AK) reference measurement procedure (RMP) in the CDC/CRMLN certification program. CDC previously presented a RMP for cholesterol using Isotope Dilution / Gas Chromatography / Mass Spectrometry (ID/GC/MS)¹. We have also completed a practical measurement system for cholesterol using ID/GC/MS for the purpose of more accurate TC standardization in Japan. Sample preparation is the same as for the AK method except a preliminary dilution with tris-HCl buffer (50mM, pH7.4) and addition of ¹³C₂-cholesterol as an internal standard. Diluted samples including the internal standard ¹³C₂-cholesterol are hydrolyzed with ethanolic potassium hydroxide (EtOH/KOH) at 50°C for 1 hr followed by hexane extraction and evaporation under vacuum. The cholesterol is derivatized with N, O-Bis (trimethylsilyl) - acetamide before GC/MS analysis. A magnetic sector-type mass spectrometer (JMS GC mateII) is operated in electron impact ionization mode and mass ion fragments m/z 368.4 and m/z 370.4 corresponding to the native and labeled cholesterol fragment ions, respectively, are used for selective ion monitoring. The total run time from injection to MS detection is less than 5 minutes. Three quality control pools (MQ10, Q27, Q28) from CDC and SRM1951b from the National Institute of Science and Technology (NIST) were analyzed in quadruplicate in 20 analytical runs. The data were compared with the assigned values of CDC and NIST. As a result, r² for the standard calibration range (0-400mg/dL) was 0.9998. The within-run (n=4) CV and the among-run (20 assays) CV ranged from 0.08% - 1.57% and 0.20% - 0.51%, respectively. The average bias from CDC and NIST ID/GC/MS methods was 0.39% and 0.03%, respectively. These results demonstrate sufficient

precision and accuracy that is required for the method to be considered as a potential RMP.

Introduction

CDC previously presented a RMP for cholesterol using Isotope Dilution / Gas Chromatography / Mass Spectrometry (ID/GC/MS) using a multi-level linear standard calibration approach.¹ We have also developed a practical measurement system for cholesterol using our own instruments for purpose of more accurate TC standardization in Japan.

Methods

1. 250ul samples (serum, cholesterol standard, control serum) were preliminarily diluted with 5mL of 50mM Tris-HCl buffer, pH7.4, containing 0.5% Emulgen 108.
2. 500ul of diluted samples was mixed with the ethanolic potassium hydroxide (EtOH/KOH) solution for hydrolysis.
3. 2mL of internal standard ¹³C₂-cholesterol (5mg/dL) was added in each tube.
4. Sample mixtures including the internal standard ¹³C₂-cholesterol are hydrolyzed with ethanolic potassium hydroxide at 50°C for 1 hr.
5. After hydrolysis, 7mL of H₂O was added, and tubes were incubated at 25°C for 15min in water bath.
6. 6mL of hexane was added, set on the shaker for extraction, and allowed to stand for more than 15min to entirely divide the hexane layer.
7. Aliquots of hexane layers were transferred into empty vials for GC/MS, and evaporated under vacuum at 55°C.
8. Before GC/MS analysis, vials were reconstituted by hexane (100ul), and derivatized with N, O - Bis (trimethylsilyl) - acetamide (100ul) at 70°C for 1hr.
9. A magnetic sector-type mass spectrometer (JMS GC mateII) was operated in electron impact ionization mode and mass ion fragments m/z 368.4



and m/z 370.4 corresponding to the native and labeled cholesterol fragment ions, respectively, were used for selective ion monitoring.

Results

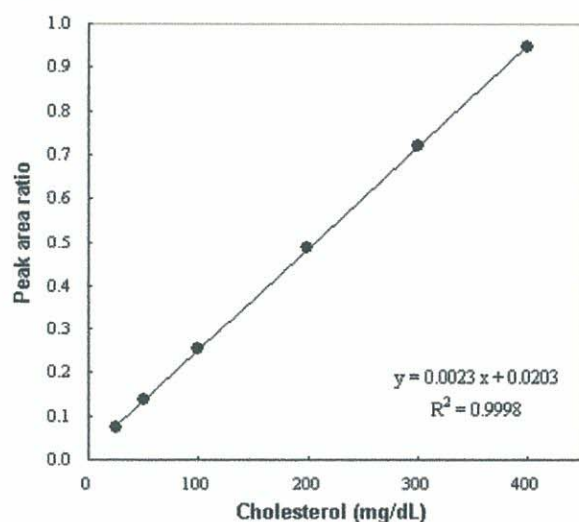


Figure1. Calibration plot for cholesterol ID/GC/MS analysis. $^{13}\text{C}_2$ -Cholesterol was used as the internal standard. The linear regression equation and the correlation coefficient are shown on the figure.

Table 1. Three quality control pools (MQ10, Q27, Q28) from CDC and SRM1951b from the National Institute of Science and Technology (NIST) were analyzed in quadruplicate in 20 analytical runs.

Serum	Target value (mg/dL)	ID/GC/MS (mg/dL)	Bias (mg/dL)	%Bias	Within-run CV%	Among-run CV%
CDC Control						
MQ10	51.01	51.45±0.26	0.44	0.86	0.51	0.83
Q27	275.45	275.25±0.62	-0.20	-0.07	0.23	0.40
Q28	-	170.47±0.53	-	-	0.31	0.47
NIST SRM 1951b						
Level 1	185.76±0.55	186.01±0.38	0.25	0.13	0.20	0.45
Level 2	266.58±0.84	266.40±0.56	-0.18	-0.07	0.21	0.27
4 aliquot per sample 20 runs for N = 80 samples						

Table 2. Ten frozen pooled serum and CDC controls (MQ10, Q27) were measured by the AK method ($n=2$) and ID/GC/MS method ($n=3$)

Serum pool	Abell-Kendall (mg/dL)	CV (%)	ID/GC/MS (mg/dL)	CV (%)	%Bias
No.1	245.1 ± 0.14	0.06	239.1 ± 0.32	0.13	-2.51
No.2	199.8 ± 0.14	0.07	195.7 ± 0.87	0.45	-2.10
No.3	184.2 ± 0.00	0.00	182.9 ± 0.66	0.36	-0.71
No.4	173.3 ± 0.28	0.16	173.1 ± 1.64	0.95	-0.12
No.5	220.8 ± 0.14	0.06	219.0 ± 0.37	0.17	-0.82
No.6	133.1 ± 0.14	0.11	131.8 ± 0.95	0.72	-0.99
No.7	227.3 ± 0.14	0.06	224.2 ± 0.27	0.12	-1.38
No.8	261.8 ± 0.35	0.14	255.8 ± 0.76	0.30	-2.35
No.9	163.3 ± 0.07	0.04	162.3 ± 0.53	0.32	-0.62
No.10	211.6 ± 0.00	0.00	210.3 ± 0.24	0.11	-0.62
MQ10	52.5 ± 0.30	0.02	51.3 ± 0.30	0.88	-2.36
Q27	279.3 ± 0.83	0.01	275.3 ± 1.22	0.44	-1.44
Q28	171.0 ± 0.34	0.01	169.7 ± 1.85	1.10	-0.77
Average	194.1	0.06	191.6	0.47	-1.29

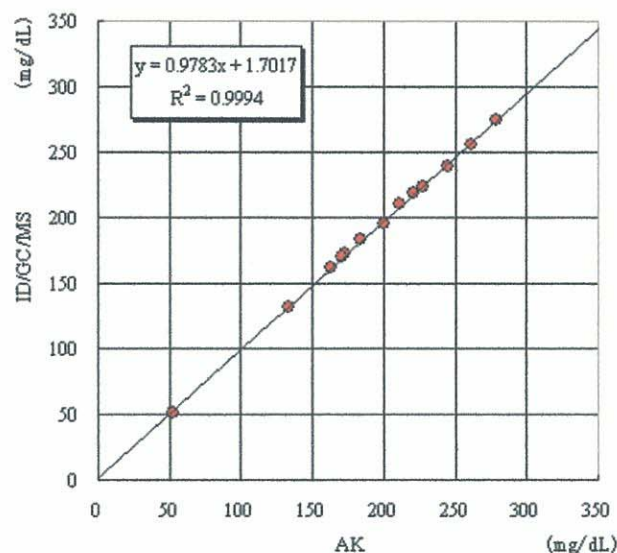


Figure2. The correlation between the AK method and the ID/GC/MS.

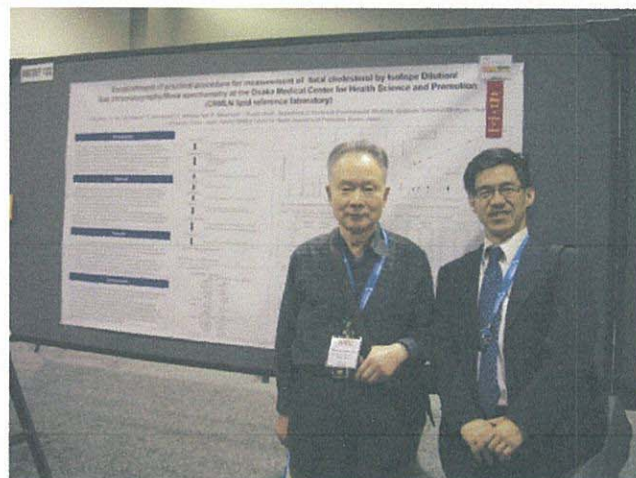
Conclusions

We developed a practical system for measurement of cholesterol using gas chromatography (Agilent 6890A) and the magnetic sector-type mass spectrometer (JMS GC mate II). The within-run and among-run CV values were less than 1%. Lower deviations from the CDC control and the NIST SRM 1951b were observed. Differences between measurements obtained by the ID/GC/MS method and by the AK method were reported by NIST (Ref.2, average %bias = -1.60%) and presented by CDC (Ref.1, average %bias = -1.39%). Average %bias calculated from our results

was -1.29%. These findings demonstrate sufficient levels of precision and accuracy required for a practical method to be considered as a potential RMP.

References

1. S.H. Edwards, S.D. Pyatt, S.L. Stribling, R. Washburn, M.M. Kimberly, G.L. Myers. Clin Chem 2007; 53(6S), A43 [Abstract]
2. Ellerbe P., Myers GL., Cooper GR., Hertz HS., et al., Clin Chem., 36(2) 1990, 370 – 375.
3. Cohen A., Hertz HS., Mandel J., Paule R., et al., Clin Chem., 26(7) 1980, 854 – 860.
4. Pelletier O., Wright L., Breckenridge C., Clin Chem., 33(8), 1987, 1403 – 1411.
5. Abell, LL., Levy BB., Brodie BB, Kendall, FE., J. Biol. Chem., 195, 1952, 357 – 366.



Gas chromatography (Agilent 6890A) and the magnetic sector-type mass spectrometer (JMS GC mate II)