Pitfalls in Lipid Profile Testing
血脂分析的检测陷阱

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彭永祥 博士

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NCEP ATP-III

Accuracy and comprehensiveness in lipid profile testing are increasingly critical because it is used both to stratify a patient's risk for cardiovascular disease and to guide the initiation and monitoring of lipid-modifying therapy to reduce that risk

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Lipid Profile Testing

- Total cholesterol
- HDL-C, and
- Triglycerides
- LDL-C (calculated or direct measurement)
- Apolipoproteins (apo A1, apo B)
- Lipoprotein Pattern (electrophoresis)
- Lp(a)

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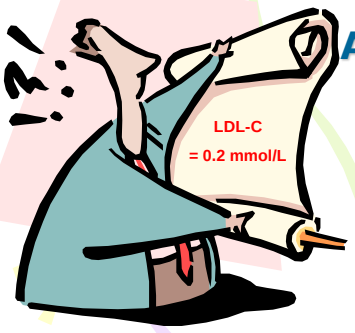
What are those Challenges?



Analytical performance
&
Interpretation of results

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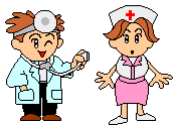
Variations

- Pre-analytical
  - Patient preparation
  - Specimen collection
- Analytical
  - Standardization
  - Interferences
- Post-analytical
  - Reference ranges
  - Interpretation (clinical correlation)



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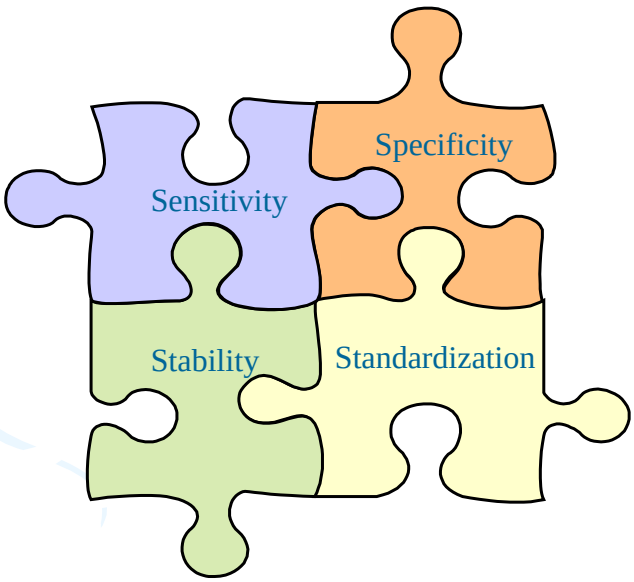

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Lab No: 05C8014245

Diabetes Monitoring & Lipid Profile

HKID No: [redacted]
Name: [redacted]
Hosp No: [redacted]
DOB: 24/03/1953
Sex/Age: M/52Y
Ref: DR 3262

Hospital: Queen Mary Hospital
Unit/Ward/Bed: QMH/FMSY/%FMSY
Request Loc: QMH/FMSY/%FMSY
Requesting Dr: [redacted]

			Ref. Range	Units
Collect Date :	02/09/04	**01/08/05**		
Collect Time :	13:45	**15:00**		
Request No. :	C9024173	**C8014245**		
Remark :	F/HT	DM/LLD CVD		
Cholesterol	5.1	4.8	< 5.2	mmol/L
Triglycerides	2.7 H	--	< 1.7	mmol/L
HDL-C	0.70 L	0.73 L	> 1.0	mmol/L
LDL-C (calc)	3.1	--	< 3.4	mmol/L
Non-HDLC	4.4 H	4.1	< 4.2	mmol/L
Appearance	Clear	Clear		
Drug	Lopid	Lopid		
dosage	600 MG	600 MG		
frequency	BD	BD		

?

2.2

非空腹标本?

3.1

贝特类降脂（甘油三酯）药物 Gemfibrozil 吉非贝齐

Comment:
05C8014245 Non-fasting sample unsuitable for full lipid profile measurement.

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Humans do not possess the pathways necessary to synthesize the essential precursor fatty acid, a-linolenic acid (18:3), essential for production of the longer bioactive omega-3 fatty acids. These long-chain polyunsaturated omega-3 fatty acids must be obtained from plant sources or direct intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) primarily from marine or dietary source.

Omega 3 脂肪酸

Ecosapentaenoic acid (EPA) 和 Docasahexaenoic acid (DHA)

Collect Date :	22/12/04	26/12/04	07/02/05	29/03/05	**01/08/05**		
Collect Time :	10:00	10:45	10:30	10:30	**10:00**		
Request No. :	CC224085	CC264010	C2074105	C3294075	**C8014131**	Ref. Range	Units
Remark :	F/HIGH TG DRUG	LOW K GOUT/LLD	F/HYPOKAL HIGH LIPID/DRUG	F/HYPERTR IGLY DIET	Hyperlipidemia.Hyperlipidemia.Lipid-		
Cholesterol	10.4 H	8.1 H	9.2 H	6.6 H	8.2 H	< 5.2	mmol/L
Triglycerides	16.1 H	5.2 H	4.5 H	3.3 H	7.4 H	< 1.7	mmol/L
HDL-C	1.11	1.14	1.15	1.02	1.06	> 1.0	mmol/L
LDL-C (calc)	--	--	6.0 H	4.1 H	--	< 3.4	mmol/L
LDL-C	4.4 H	5.6 H	6.4 H	--	4.9 H	< 3.4	mmol/L
Non-HDLC	9.3 H	7.0 H	8.1 H	5.6 H	7.1 H	< 4.2	mmol/L
Appearance	Turbid	Clear	Sl.Turbid	Clear	Sl.Turbid		
Apo A1	1.28	--	1.39	--	--	1.20 - 2.00	g/L
Apo B	2.11 H	--	2.12 H	--	--	0.41 - 1.07	g/L
Drug	Lipanthyl	Lopid	Maxepa	--	--		
dosage	200 G	200 MG	5 GM	--	--		
frequency	QD	QD	QD	--	--		
since	07/09/04	--	--	--	--		
Drug	Maxepa	--	Lipanthyl	--	--		
dosage	5 G	--	200 MG	--	--		
frequency	QD	--	QD	--	--		
since	07/09/04	--	--	--	--		

Comment:
05C8014131 Triglycerides > 4.5 Calculated-LDL not available
05C2074105 Lipo Pattern LDL fraction was increased. VLDL fraction was also mildly elevated. Type IIb hyperlipidaemia?
04CC264010 Triglycerides > 4.5 Calculated-LDL not available
04CC224085 Cholesterol Verified.
Triglycerides Verified. > 4.5 Calculated-LDL not available
HDL-C Verified.
Lipo Pattern An electronegative subfraction of pre-beta lipoproteins was noted. Chylomicrons also present. Fish oil supplements may interfere with the electrophoretic mobility of all lipoproteins.

Biochemist: Dr. Richard Pang

This laboratory is accredited by CAP. Accreditation Number 71755-25

Report Date/Time: 03/08/05 16:32 Printed on 03/08/05 16:34
Report Destination: QMH/MED/MECM - Endocrine Clinic L2, QMH

HA2372/QM (Rev.11/03) Page: 1/1

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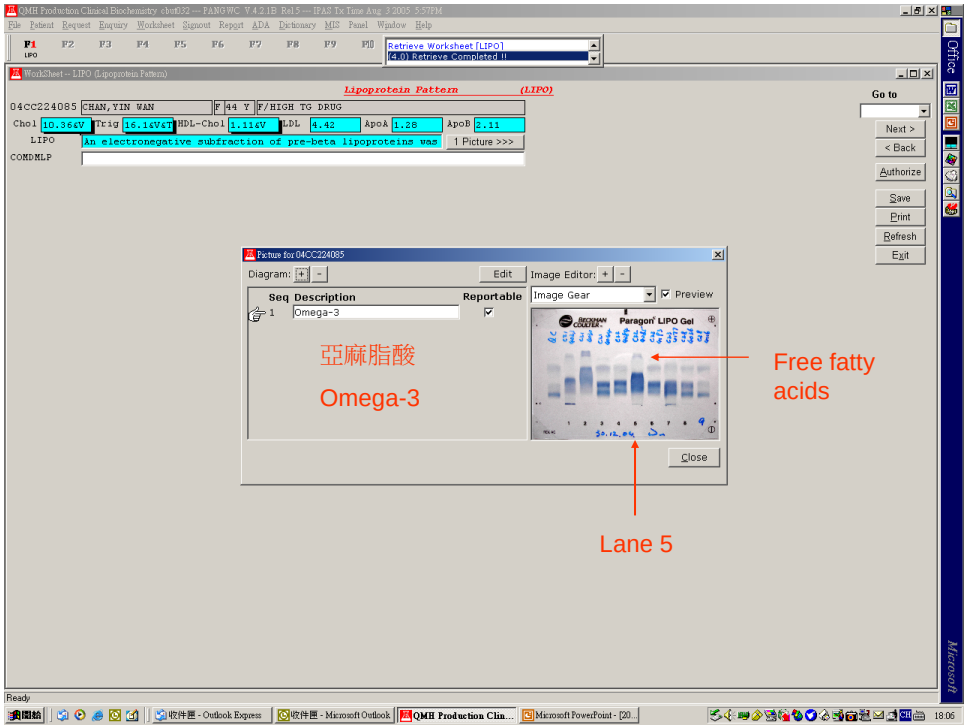

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Friedewald Formula

LDL-C = Total Cholesterol – [(TG/2.2) + HDL-C]

影响血脂准确测定的因素很多，如标本的来源、测定方法、仪器和试剂等，其中分析前即临床实验室进行测定之前的因素对实验结果的影响往往被忽视，应特别引起关注。

? TG/2.2 = VLDL-C

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Limitations

- Estimating VLDL concentration from plasma triglycerides is not valid when the triglycerides exceed 400 mg/dL (4.5 mmol/L).
- The Friedewald equation, LDL-C = TC – HDL-C – triglyceride/5, is used to estimate LDL cholesterol.
- The term “triglyceride/5” is used to estimate the VLDL-C concentration. In estimating VLDL from triglycerides, one assumes there are no chylomicrons in the sample and that there are no unusual lipoproteins in the sample that would alter the typical ratio of triglycerides to VLDL particles.
- It is well documented in the literature that the Friedewald equation begins to give less reliable results when the plasma triglycerides exceed 200–300 mg/dL (2.3–3.4 mmol/L), and
- It is not recommended when the triglycerides are greater than 400 mg/dL (4.5 mmol/L) because the term “triglyceride/5” no longer provides a reliable estimate of the VLDL-C.

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Surrogate Markers

	Chylo-micron	VLDL	IDL	LDL	HDL
Density (g/mL)	<0.95	0.950–1.006	1.006–1.019	1.019–1.063	1.063–1.210
Components (% dry weight)					
Protein	2	8	15	22	40–55
Triacylglycerol	86	55	31	6	4
Free cholesterol	2	7	7	8	4
Cholesterol esters	3	12	23	42	12–20
Phospholipids	7	18	22	22	25–30
Apoprotein composition	A-I, A-II, B-48, C-I, C-II, C-III	B-100, C-I, C-II, C-III, E	B-100, C-I, C-II, C-III, E	B-100	A-I, A-II, C-I, C-II, C-III, D, E

Sources: Data from D. E. Vance and J. E. Vance (eds.), *Biochemistry of Lipids and Membranes* (Redwood City, Calif.: Benjamin/Cummings, 1985); and J. F. Mead, R. B. Alfin-Slater, D. R. Howton, and G. Popják, *Lipids* (New York: Plenum, 1986).

The typical ratio of triglycerides to VLDL particles = 5

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TG/2.2 = VLDL-C

- Fasting
- Triglyceride < 4.5 mmol/L

虽然有人认为TC测定可不用禁食，但应注意饱餐后TC会有所下降；对于TG和其他脂蛋白检测则需至少禁食12小时采血。具体做法：如在采血前一天晚8点钟开始禁食（包括零食），可少量饮水。于次日早上8至10点采取静脉血，也就是应空腹12h~14h晨间取血。

Requirements

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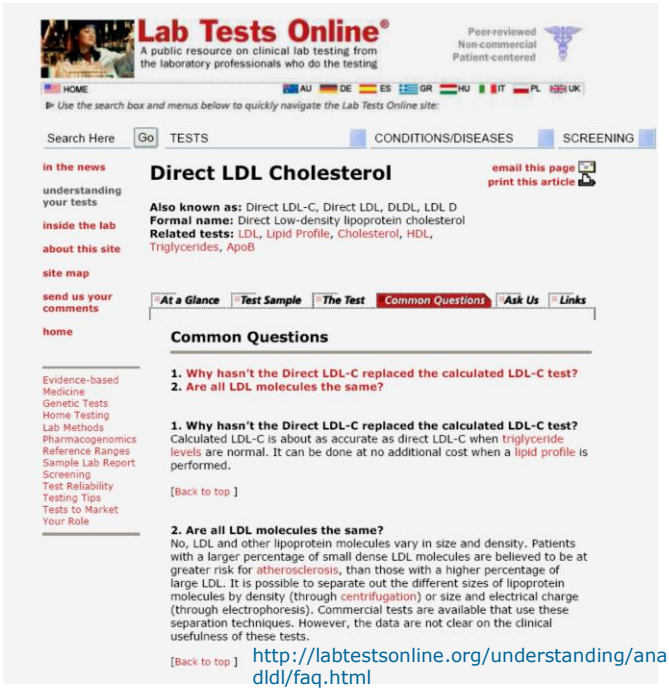

http://labtestsonline.org/understanding/analytes/dldl/faq.html

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LDL-C

- An important clinical consideration is when two LDL-C values (calculated vs direct measurement) are available but different
- Particularly when the value is borderline, requiring a decision to treat or not to treat, such a difference would be of concern

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Limitations

- Discrepancies between the results of calculated LDL-C and the results of the direct homogeneous LDL-C assays are primarily caused by elevated triglycerides and, to a lesser extent, by associated insulin resistance, liver or kidney diseases, and genetic defects in lipid and lipoprotein metabolism.

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Limitations

- Dyslipidemia is a hallmark of the metabolic syndrome and insulin resistance. The underlying mechanism of the dyslipidemia of the metabolic syndrome is the altered metabolism of triglyceride-rich lipoproteins, such as VLDL and IDL remnants.
- Patients with type III hyperlipoproteinemia, characterized by cholesterol enrichment of the VLDL because of impaired clearance of remnant lipoproteins, were also reported to give erroneous values with the homogeneous assays for HDL-C and LDL-C.

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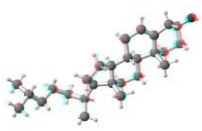
Total Cholesterol

- HDL
  - HDL_2
  - HDL_3
- LDL
  - LDL-native
  - Lp(a)
  - IDL
  - LDL-size
- VLDL
  - $VLDL_{1,2}$
  - $VLDL_3$



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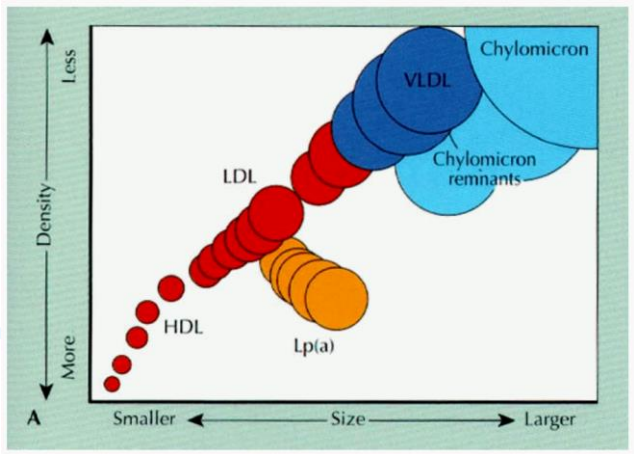
Density and Size



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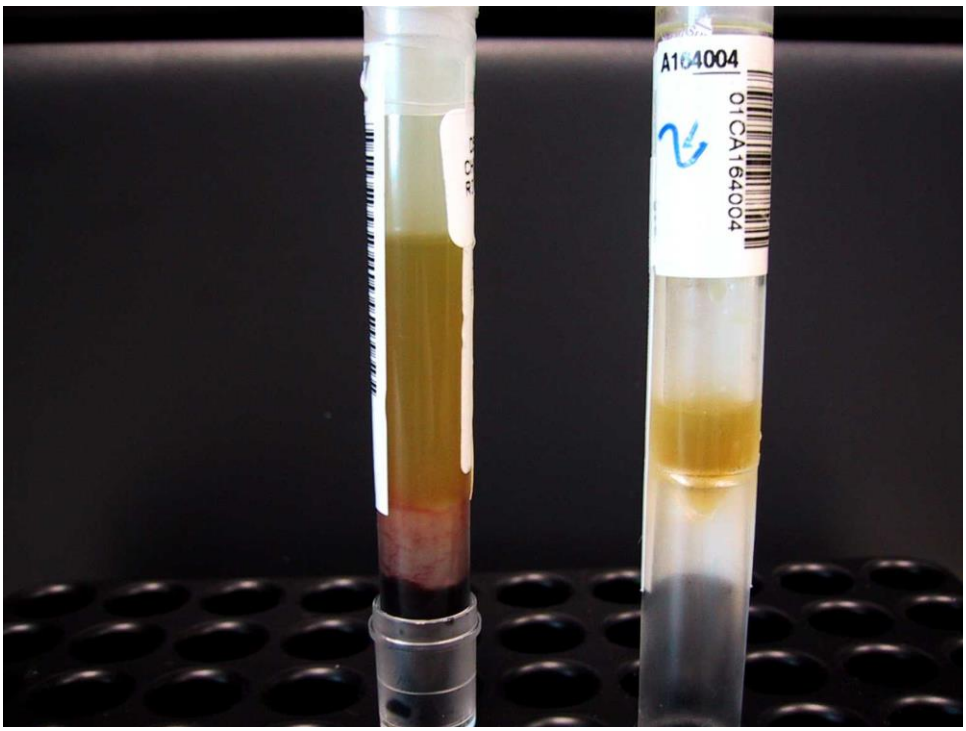

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01CA164004

Cholestasis & Jaundice

Delta Bilirubin = 132 umol/L

TC = 31.5

TG = 4.3

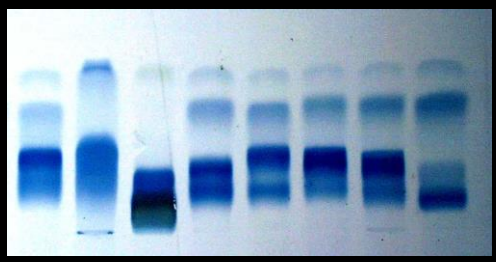
HDL-C = 0.52

LDL-C = 29.0 (by calculation)

LDL-C = 5.0 (by direct method)

Apo A1 = <0.25

Apo B = 2.77



← **HDL**

← **VLDL**
← **LDL**
← **Chylomicrons**

↑ Lp-X?

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Lipids in Health and Disease

BioMed Central

Research

Open Access

LDL cholesterol estimation in patients with the metabolic syndrome

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Published: 06 April 2006

Lipids in Health and Disease 2006, 5:8 doi:10.1186/1476-511X-5-8

Received: 22 February 2006
Accepted: 06 April 2006

This article is available from: http://www.lipidworld.com/content/5/1/8

http://www.lipidworld.com/content/5/1/8

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Friedewald (LDL-F) [4]

LDL-F = TC - HDL-C - TG/5 (in mg/dL), excluding patients with TG concentrations ≥ 400 mg/dL.

Planella (LDL-P), which focuses on the inclusion of apoB levels in the estimation of LDL-C levels [21]

LDL-P = 0.41*TC - 0.14*TG + 0.66*apoB - 10.43 (in mg/dL).

Hattori (LDL-H), an equation very similar to that proposed by Friedewald [22]

LDL-H = 0.94*TC - 0.94*HDL-C -0.19*TG (in mg/dL).

AMORIS study (LDL-A), including apoA-I levels in the equation for LDL-C estimation [24]

LDL-A = 18.53 + 0.99*TC - 0.1*TG - 0.61*apoA-I (in mg/dL).

Wagner (LDL-W), also including apoB levels in the formula for the estimation of LDL-C concentration [20]

LDL-W = 0.358*TC + 0.776*apoB - 0.149*TG (in mg/dL).

Lipoprint System (LDL-L) [25, 26]

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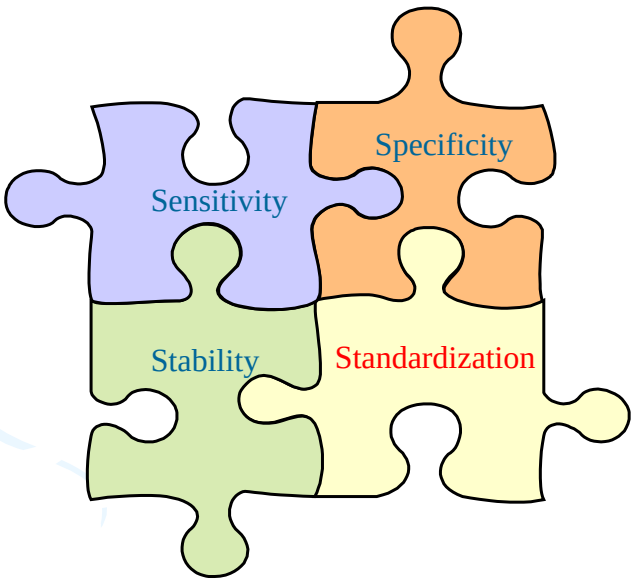

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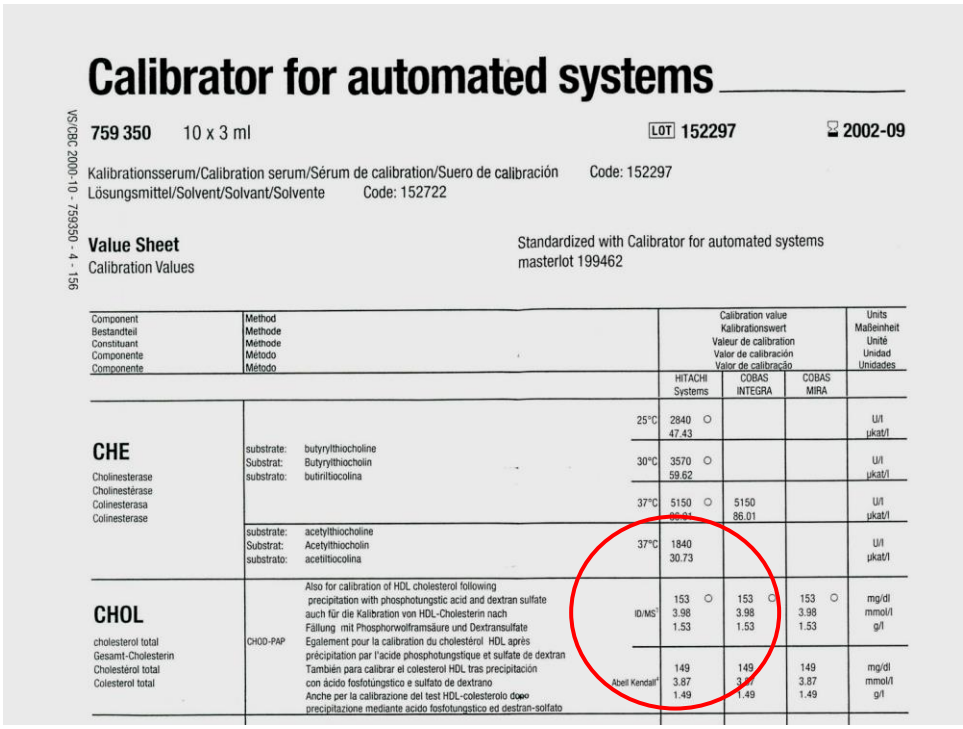

Calibrator for automated systems

VS/CBC 2000-10 - 759350 - 4 - 156

759 350 10 x 3 ml **LOT 152297** 2002-09

Kalibrationsserum/Calibration serum/Sérum de calibration/Suero de calibración Code: 152297
Lösungsmittel/Solvent/Solvant/Solvente Code: 152722

Value Sheet
Calibration Values

Standardized with Calibrator for automated systems
masterlot 199462

Component / Bestandteil / Constituant / Componente / Componente	Method / Methode / Méthode / Método / Método		Calibration value / Kalibrationswert / Valeur de calibration / Valor de calibración / Valor de calibração HITACHI Systems	COBAS INTEGRA	COBAS MIRA	Units / Maßeinheit / Unité / Unidad / Unidades
CHE Cholinesterase, Cholinestérase, Colinesterasa, Colinesterase	substrate: butyrylthiocholine; Substrat: Butyrylthiocholin; substrato: butiriltiocolina	25°C	2840 ○ 47.43			U/l µkat/l
		30°C	3570 ○ 59.62			U/l µkat/l
		37°C	5150 ○ 86.01	5150 86.01		U/l µkat/l
	substrate: acetylthiocholine; Substrat: Acetylthiocholin; substrato: acetiltiocolina	37°C	1840 30.73			U/l µkat/l
CHOL cholesterol total, Gesamt-Cholesterin, Cholestérol total, Colesterol total	CHOD-PAP	ID/MS[3]	153 ○ 3.98 1.53	153 ○ 3.98 1.53	153 ○ 3.98 1.53	mg/dl mmol/l g/l
		Abell Kendall[4]	149 3.87 1.49	149 3.87 1.49	149 3.87 1.49	mg/dl mmol/l g/l

Also for calibration of HDL cholesterol following precipitation with phosphotungstic acid and dextran sulfate
auch für die Kalibration von HDL-Cholesterin nach Fällung mit Phosphorwolframsäure und Dextransulfate
Également pour la calibration du cholestérol HDL après précipitation par l'acide phosphotungstique et sulfate de dextran
También para calibrar el colesterol HDL tras precipitación con ácido fosfotúngstico e sulfato de dextrano
Anche per la calibrazione del test HDL-colesterolo dopo precipitazione mediante acido fosfotungstico ed destran-solfato

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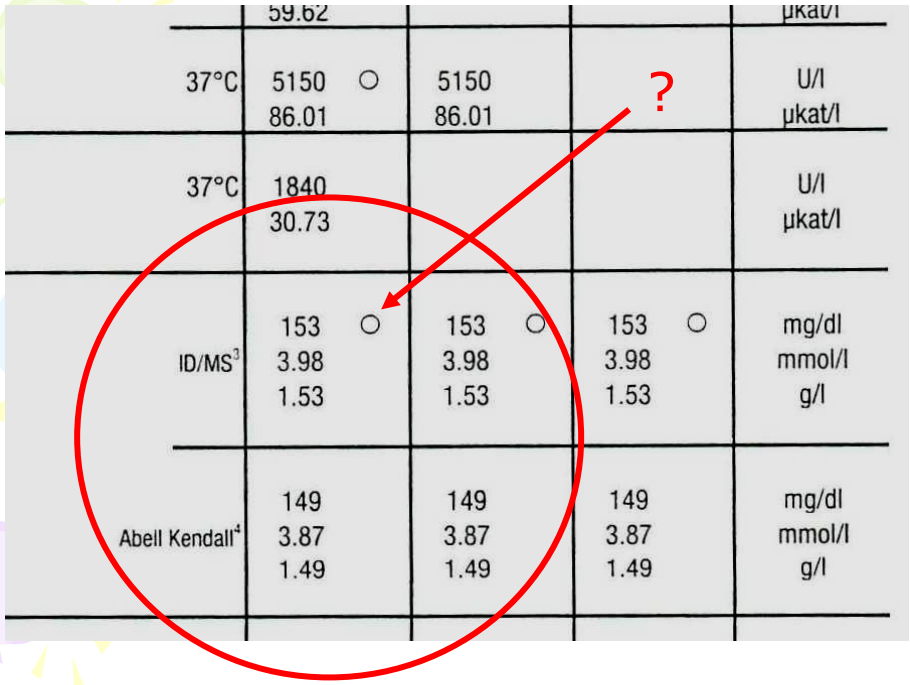

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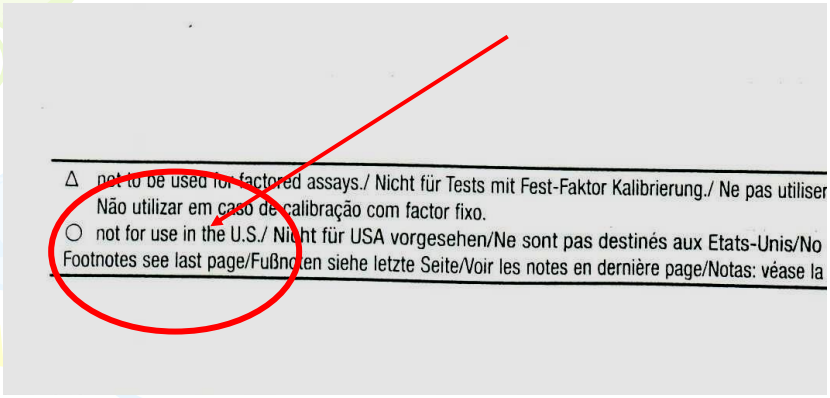

?Merging of Roche with Boehringer Mannheim/Hitachi

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CDC Lipid Reference Laboratory

"Most US NIH funded epidemiological and interventional studies performed over the last 30 years have traced their total cholesterol values to the reference methods performed at the CDC Lipid Reference Laboratory"

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CDC Lipid Reference Laboratory

"The National Cholesterol Education Program (NCEP) recommends that total cholesterol measurements be traceable to the CDC reference (i.e., Abell-Kendall) method"

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CDC Lipid Reference Laboratory

"Thus, NIST and the CAP anticipate that most manufacturers of total cholesterol reagents and calibrator materials, at least for distribution in the United States, will wish to use the CDC reference values, and NOT the NIST definitive method (ID/MS) values at this time"

Mass Spectrometer

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LDL-C (Calculated vs Direct)

- There are numerous pitfalls and inaccuracies in the routine lipid profile testing.
- When two LDL-C values (calculated vs direct measurement) are available but different; particularly when the value is borderline, requiring a decision to treat or not to treat, such a difference would be of

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Myths or Reality

- It is really the number of LDL particles and their composition that are critical, not just concentration of LDL-C.
- The other important issue of note is that most laboratories are measuring the cholesterol content of the high-density lipoprotein, that is, HDL-C, and that does not always correlate with the content of protein moiety in the HDL particle.

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