Analytical Performance of the Cholesterol Profile Measurement by Vertical Auto Profile [VAP]: Analysis of NCEP-III Guidelines Lipid Analytes

Atherotech Diagnostic Laboratory

Accuracy of the Vertical Auto Profile [VAP] cholesterol test is verified every six months by performing a split sample comparison with the Core Laboratory for Clinical Studies at Washington University School of Medicine in St. Louis, Mo.; one of the few reference laboratories for lipoprotein analysis. This comparison uses beta quantification, a standard procedure for lipoprotein analysis based on ultracentrifugation.

Method

The VAP® Test is an inverted rate zonal, single vertical spin, density gradient ultracentrifugation technique that simultaneously measures cholesterol concentrations of all five lipoprotein classes HDL, LDL, VLDL, IDL, and Lp(a) and their subclasses[1,2]. Unlike more laborious preparative ultracentrifugation methods, the VAP method separates all lipoproteins in less than one hour. The VAP procedure comprises three steps: a two-layer density gradient is prepared with the bottom layer containing a 1:40 serum dilution; this is centrifuged at 65,000 rpm [~1 hour]; and then analyzed for cholesterol using a continuous flow VAP cholesterol analyzer.

Results

VAP was compared to the Washington University Beta quantitation [gold standard] method as well as a regional reference laboratory, another medical school laboratory, and the Abbott ARCHITECH® Lipid assays.

Low Density Lipoproteins:

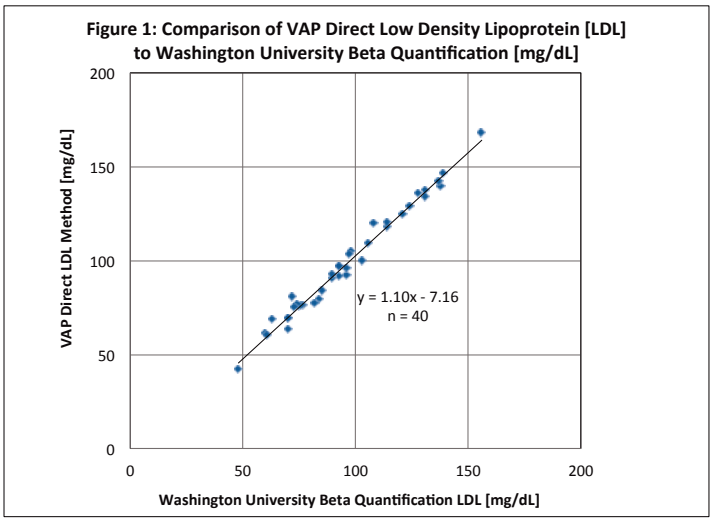

Table 1: Agreement of Atherotech Low Density Lipoprotein [LDL] Measurement to Other Reference LDL Methods

External Comparison	Mean Low Density Lipids [LDL] mg/dL	Correlation [r]	Slope	Y-intercept
Atherotech - Direct LDL	99			
Washington University Beta Quantitation	96	0.9918	1.10	-7.16
S.E. Medical School Laboratory - Direct LDL	98	0.9609	0.91	8.25
Regional Reference Laboratory - Direct LDL	103	0.9850	0.97	7.01
Abbott ARCHITECT Direct LDL	103	0.9908	0.91	3.67
Friedewald Calcalated LDL	102	0.9755	0.91	12.61

Bias of VAP Direct LDL compared to Beta Quantitation = 2.3%

Precision of VAP Direct LDL [%CV] = 2.9%

LDL

- The VAP Test directly measures LDL in serum in contrast to a Standard Lipid Panel [SLP] which uses the Friedewald formula to estimate LDL concentration. The Friedewald technique is known to be inaccurate in many cases[3].
- VAP showed excellent correlation [r = 0.9918] to the Wash U Beta Quantitation ultracentrifugation method for direct LDL. Agreement to other laboratories was also very good.
- Bias of the VAP LDL measurement compared to Beta Quantitation was 2.3%.
- Precision of the VAP LDL measurement was determined using 32 patient samples replicated 17 times with different autodiluters, centrifuges and VAP cholesterol analyzers. VAP LDL precision [CV] was found to be 2.9%.

High Density Lipoproteins

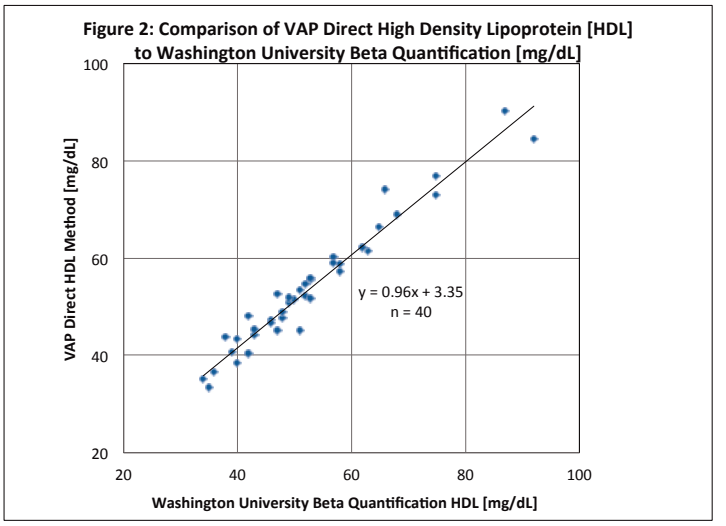

Table 2: Agreement of Atherotech High Density Lipoprotein [HDL] Measurement to Other Reference HDL Methods

External Comparison	Mean High Density Lipids [HDL] mg/dL	Correlation [r]	Slope	Y-intercept
Atherotech - Direct HDL	54			
Washington University Beta Quantitation	53	0.9758	0.96	3.35
S.E. Medical School Laboratory - Direct HDL	54	0.9847	1.03	-1.14
Regional Reference Laboratory - Direct HDL	52	0.9791	0.89	3.63
Abbott ARCHITECT Direct HDL	53	0.9805	0.87	5.83

Bias of VAP Direct HDL compared to Beta Quantitation = 1.9%

Precision of VAP Direct HDL [%CV] = 2.9%

HDL

- High Density Lipoprotein is an increasingly important target for therapy and appears to exert a protective effect through multiple mechanisms[4].
- The VAP HDL assay showed very good correlation [r = 0.9758] to the Wash U Beta Quantitation ultracentrifugation method for direct HDL. Agreement to other laboratories was also very good.
- Bias of the VAP HDL measurement compared to Beta Quantitation was 1.9%.
- Precision of the VAP HDL measurement was determined using 32 patient samples replicated 17 times with different auto diluters, centrifuges and VAP cholesterol analyzers. Precision [%CV] of the VAP HDL assay was found to be 2.9%.

Total Cholesterol

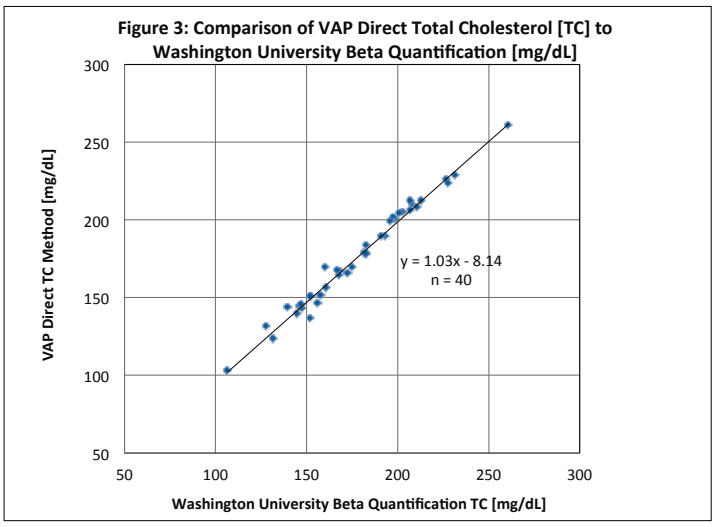

Table 3: Agreement of Atherotech Total Cholesterol [TC] Measurement to Other Reference TC Methods

External Comparison	Mean Total Cholesterol [TC] mg/dL	Correlation [r]	Slope	Y-intercept
Atherotech - Direct TC	177			
Washington University Beta Quantitation	179	0.9917	1.03	-8.14
S.E. Medical School Laboratory - Direct TC	180	0.9840	0.86	27.24
Regional Reference Laboratory - Direct TC	172	0.9911	0.90	12.84
Abbott ARCHITECT Direct TC	179	0.9904	0.91	19.46

Bias of VAP Direct TC compared to Beta Quantitation = 1.9%

Precision of VAP Direct TC [%CV] = 2.6%

TC

- The VAP TC assay showed excellent correlation [r = 0.9917] to the Wash U Beta Quantitation ultracentrifugation method for direct TC.
- Agreement to other laboratories was also very good.
- Bias of the VAP TC measurement compared to Beta Quantitation was 1.9%.
- Precision of the VAP TC measurement was determined using 32 patient samples replicated 17 times with different auto diluters, centrifuges and VAP cholesterol analyzers. Precision [%CV] of the VAP TC assay was found to be 2.6%.

Non-High Density Lipids

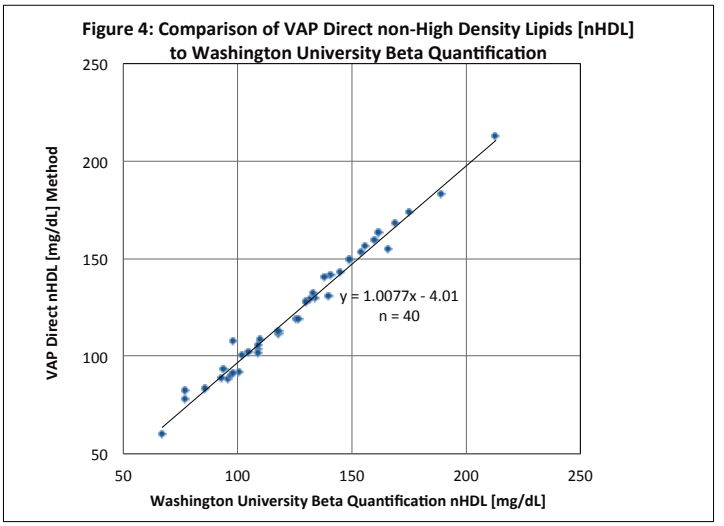

Table 4: Agreement of Atherotech non High Density Lipoprotein [nHDL] to Other Reference nHDL Methods				
External Comparison	Mean nHDL [mg/dL]	Correlation [r]	Slope	Y-intercept
Atherotech - Direct nHDL	123			
Washington University Beta Quantitation	126	0.9920	1.01	-4.01
S.E. Medical School Laboratory - Direct nHDL	125	0.9838	0.89	15.97
Regional Reference Laboratory - Direct nHDL	120	0.9896	0.90	9.15
Abbott ARCHITECT Direct nHDL	127	0.9883	0.90	16.43
Bias of VAP Direct nHDL compared to Beta Quantitation = -2.4%				
Precision of VAP Direct nHDL [%CV] = 2.8%				

nonHDL

- Recent randomized trials assessing the role of statins in patients with established coronary heart disease showed that on-treatment levels of **non**-**HDL**-C were more closely associated with cardiovascular outcomes than levels of low-density lipoprotein cholesterol[5].
- The VAP nonHDL assay showed excellent correlation [r = 0.9920] to the Wash U Beta Quantitation ultracentrifugation method for direct nonHDL. Agreement to other laboratories was also very good.
- Bias of the VAP nonHDL compared to Beta Quantitation was -2.4%.
- Precision of the VAP nonHDL measurement was determined using 32 patient samples replicated 17 times with different auto diluters, centrifuges and VAP cholesterol analyzers. Precision [%CV] of the VAP nonHDL assay was found to be 2.8%.

Triglycerides

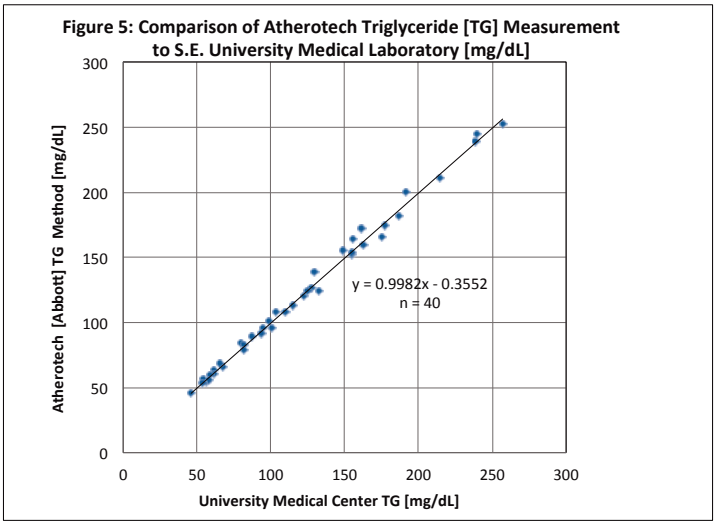

Table 5: Agreement of Atherotech Triglyceride [TG] mg/dL to Other Reference TC Methods				
External Comparison	Mean nHDL [mg/dL]	Correlation [r]	Slope	Y-intercept
Atherotech - Direct nHDL	122			
S.E. Medical School Laboratory - TG	123	0.9968	1.00	1.13
Regional Reference Laboratory - TG	126	0.9994	1.03	1.45
Bias of VAP Triglyceride Method compared to a S.E. Medical School = -0.5%				
Precision of Atherotech Triglyceride Assay = 0.93%				

TG

- Triglycerides are a NCEP secondary therapeutic target, but are increasingly viewed as a primary risk factor leading to small atherogenic lipoprotein particles[6].
- Triglycerides are determined by enzymatic reaction using the Abbott ARCHITECH® CA-8000.
- The Atherotech TG showed excellent agreement to both a S.E. Medical School Hospital Laboratory and a Regional Reference Laboratory.
- Bias of the VAP compared a Medical School Laboratory was -.05%. Precision [%CV] of the Atherotech Abbott ARCHITECH TG assay was 0.93%.

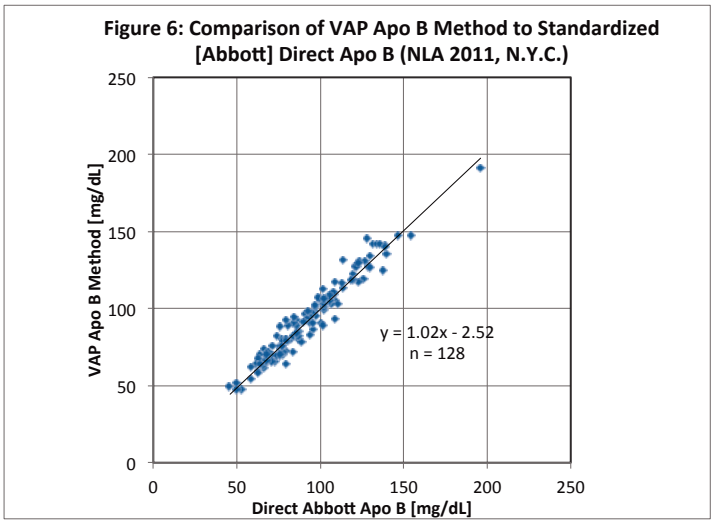

Table 6: Agreement of Atherotech ApoB Method to Abbott Architect Standardized ApoB Method				
Internal Comparison	ApoB [mean] mg/dL	Correlation [r]	Slope	Y-intercept
Abbott ARCHITECT Direct ApoB [standardized]	94			
Atherotech - VAP ApoB	93	0.9732	1.02	-2.52
n=127				
Bias of VAP ApoB compared to Abbott Architect = -0.6%				
Precision of VAP ApoB [%CV] = 3.0%				

VAP ApoB

- The utility of apoB as a measure of atherogenic lipoprotein particles and a primary risk indicator is validated by recent clinical trials and meta analysis[7].
- VAP apoB values are obtained concurrently with the normal VAP cholesterol analysis.
- The VAP apoB assay is compared to an Abbott ARCHITECH® apoB assay referenced to an international standard.
- Agreement to the Abbott ARCHITECH®method was r = 0.973. The precision [%CV] of the VAP apoB assay is 3.0%.

References

1. Kulkarni KR. Cholesterol profile measurement by Vertical Auto Profile method. *Clin Lab Med*. 2006 Dec;26(4):787-802. Review.

2. Kulkarni KR, Garber DW, Marcovina SM, *et.al*. Quantification of cholesterol in all lipoprotein classes by the VAP-II method. *J Lipid Res.* 1994 Jan;35(1):159-68.

3. Jun KR, Park HI, Chun S, Park H, et. al. Effects of total cholesterol and triglyceride on the percentage differencebetween the low-density lipoprotein cholesterolconcentrationmeasured directly and calculated using the Friedewald formula. *Clin Chem Lab Med* 2008;46(3):371-5.

4. Viles-Gonzalez JF, Fuster V, Corti R, Badimon JJ. Emerging importance of HDL cholesterol in developing high-risk coronary plaques in acute coronary syndromes. *Curr Opin Cardiol.* 2003 Jul 18(4):286-94.

5. Rana JS, Boekholdt SM. Should we change our lipid management strategies to focus on non-high-density lipoprotein cholesterol? *Curr Opin Cardiol*. 2010 Nov25(6):622-6.

6. Chapman MJ, Ginsberg HN, Amarenco P, et. al. European Atherosclerosis Society Consensus Panel. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 2011 Jun;32(11):1345-61.

7. Sniderman AD, Williams K, Contois JH, et. al. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. Circ Cardiovasc Qual Outcomes. 2011 May 1;4(3):337-45.