COLLEGE of AMERICAN PATHOLOGISTS

Educational Discussion: Lipids

2018 Accuracy-Based Lipids (ABL)

Accurate and reliable lipids and lipoprotein measurements are critical for correctly assessing a person's risk for cardiovascular diseases (CVD) and for monitoring therapies to reduce CVD risk. The analytical performance criteria for lipid and lipoprotein tests used in this Survey are based on recommendations from the National Cholesterol Education Program (NCEP)[1]. The criteria recommended by NCEP states a maximum allowable bias, which is the mean bias calculated using multiple samples and replicates, and a maximum allowable imprecision, which is the average standard deviation or coefficient of variation determined using multiple samples and replicates. Results reported by participants of this Survey are based on a single measurement. Therefore, the criteria applied in this Survey takes into account that a single measurement result reported by a participant can deviate from the target value as a result of both bias and imprecision.

The samples used in this Survey were produced following procedures that minimize matrix effects and produce commutable serum pools with characteristics as close as possible to patient samples. Thus, measurement results can be compared not only within peer group but also across peer groups. Furthermore, measurement results reported by participants on the same sample can be considered replicate measurements. Thus, measurement results from participants can be combined to calculate mean bias and imprecision, which then are compared with the NCEP criteria (Table 1A).

Table 1A: NCEP Recommended Analytical Performance Criteria for Blood Lipids

Analyte	Maximum Allowable Bias	Maximum Allowable Imprecision
Total Cholesterol	±3.0 %	≤3.0 %CV
HDL-C	±5.0 %	≤4.0 %CV at ≥42 mg/dL ≤1.7 mg/dL at <42 mg/dL
LDL-C	± 4.0 %	≤4 %CV
Triglycerides	± 5.0%	≤ 5%CV

For total cholesterol (TC), the majority of reported results (449 results from all 3 samples combined) are within the 9% criterion for this Survey. The distribution of biases calculated from each reported result is similar across samples and manufacturers. The mean bias and precision determined for each sample across all participants are well within the NCEP criteria for mean bias (3%) and imprecision (3%) (Table 1B).

Table 1B: Total Cholesterol and Triglycerides - Mean Bias and Standard Deviation by Sample

Analyte	Parameter	ABL-04	ABL-05	ABL-06	All Samples
Total Cholesterol	Target Value (mg/dL)	207.28	209.40	199.78	N/A
	Mean Bias (%)	-1.19	-1.71	-1.69	-1.53
	SD	2.51	4.13	3.04	3.29
	N	151	151	151	453
Triglycerides	Target Value (mg/dL)	101.7	103.03	300.83	N/A
	Mean Bias (%)	0.05	0.36	-1.22	-0.27
	SD	4.29	4.50	4.21	4.38
	N	152	152	152	456

For triglycerides, reported results for the different detection types (GPO, Colorimetric) and blanking procedures do not appear to be highly different in the samples used in this Survey, suggesting that neither the detection type nor the amount of free glycerol in the samples highly affect measurement results. Therefore, data from different detection types and blanking procedures were combined for this discussion.

The measurement bias for all reported values are within the 15% criterion used in this Survey. The mean bias and imprecision for all values as well as individual samples meet the NCEP criteria for bias (±5%) and imprecision (≤5%) (Table 1B). Similarly, analytical performance criteria recommended by NCEP are met by all manufacturers. While the bias distribution of the Roche and Beckman systems are close to normal, the distributions of the Siemens and Abbott systems show a tailing that occurs in all samples (Figure 1). In this Survey only results from the Abbott Architect c systems were reported, suggesting that the tailing might be related to variability among reagent lots. For the Siemens systems, results reported with the Siemens Dimension Vista instruments consistently shows a positive bias while all other instruments from this manufacture show mainly negative biases, suggesting differences in instrument calibration (Figure 2).

Figure 1: Triglycerides Bias Distribution by Manufacturer*

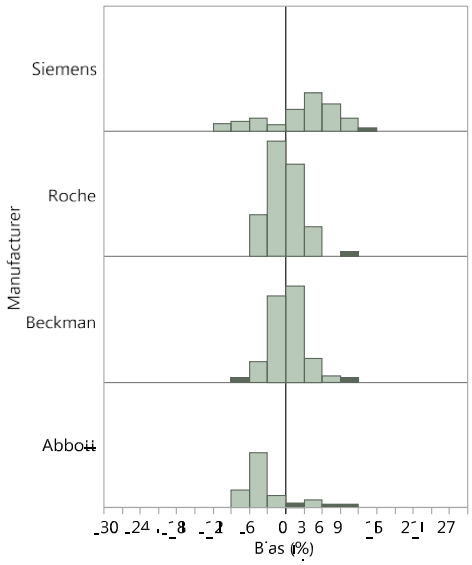
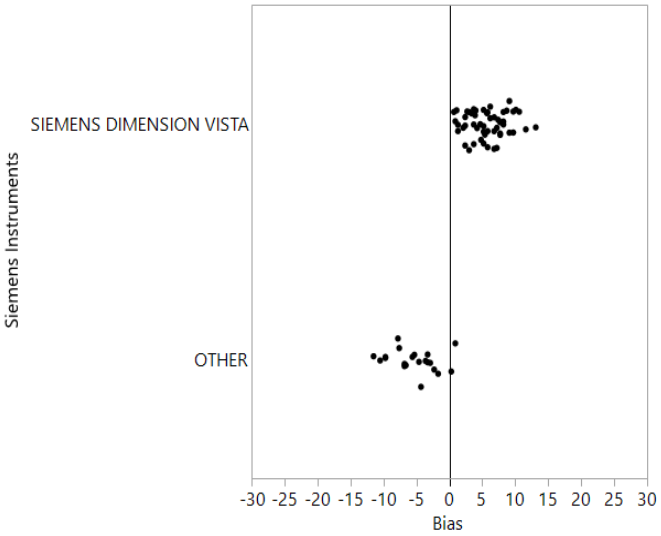


Figure 2: Triglycerides Bias Distribution of Siemens Systems by Instrument



*Only manufacturers with 10 or more values per sample are shown.

For HDL-cholesterol (HDL-C), the majority of measurement results (453 results from all 3 samples combined) are within the criteria used in this Survey. The bias distribution calculated from each reported result appears to shift from a mainly positive bias in sample ABL-04 to a mainly negative bias in sample ABL-06 (Table 2A), with the mean bias of sample ABL-06 being outside the NCEP recommended maximum allowable bias of 5%. This trend is consistent across manufacturers and is most apparent with systems from Roche and Siemens (Table 2B). When assessing the average bias across all three Survey samples for manufactures with more than 10 reported values per sample, all assessed manufacturers meet the NCEP recommended maximum allowable bias. However, when assessing each sample separately, Abbott and Beckman systems exceed the bias in sample ABL-04, and Roche and Siemens systems in sample ABL-06. None of the manufactures met the NCEP recommended imprecision (≤4% CV at concentrations ≥42 mg/dL, and ≤1.7 mg/dL SD at concentrations <42 mg/dL) with highest imprecision observed with sample ABL-06 (Abbott: 7 %CV, Beckman: 8 %CV, Roche:

7 %CV, Siemens: 12 %CV). Sample ABL-06 has the highest triglyceride levels. The high imprecision observed with this sample could be explained in part with inconsistent homogenization of the sample prior to testing.

Table 2A: HDL-Cholesterol - Mean Bias and Standard Deviation by Sample

Parameter	ABL-04	ABL-05	ABL-06	All Samples
Target Value (mg/dL)	51.25	57.22	40.34	N/A
Mean Bias (%)	4.27	-0.67	-5.60	-0.27
SD	5.06	4.29	7.60	7.07
N	151	151	151	453

Table 2B: HDL-Cholesterol - Mean Bias and Standard Deviation by Manufacturer*

Manufacturer	Parameter	ABL-04	ABL-05	ABL-06	All Samples
Abbott	Mean Bias (%)	8.04	1.07	0.81	3.43
	SD	3.22	3.07	2.82	4.64
	N	18	18	18	54
Beckman	Mean Bias (%)	8.52	2.08	0.32	3.64
	SD	3.78	3.33	3.36	4.95
	N	34	34	34	102
Roche	Mean Bias (%)	1.98	-1.70	-9.05	-2.92
	SD	2.47	2.84	2.98	5.36
	N	45	45	45	135
Siemens	Mean Bias (%)	1.31	-4.58	-11.16	-4.80
	SD	3.42	4.33	4.89	6.62
	N	25	25	25	75

*Only manufacturers with 10 or more values per sample are listed.

For LDL-cholesterol (LDL-C), 70 out of 327 reported values (21%) are outside the criterion used in this Survey with the majority of outside results observed with sample ABL-06 having (63 results). The NCEP recommended bias and imprecision are achieved with samples ABL-04 and ABL-05. However, neither bias nor imprecision requirements are achieved with sample ABL-06 (Table 3). A similar pattern is observed when assessing results by manufacturer (Table 4) with none of the assessed manufacturers meeting NCEP recommended criteria for sample ABL-06. In contrast to these findings, only 11 out of 108 calculated LDL-C values (10%) for sample ABL-06 are outside the 12% criterion used in this Survey. Across all samples, only 13 out of 324 calculated LDL-C results (4%) do not meet this criterion. When looking across all calculated LDL-C results reported for each sample, the NCEP criteria are met for all sample.

According to the National Institute of Health[2], LDL-C levels of less than 100 ng/dL are considered healthy. The target value of sample ABL-06 would indicate a healthy patient. Of the 109 results reported for this sample using direct assays, 68 results (58%) would suggest that the patient does not have healthy LDL-C levels, while only 11 calculated results (10%) would indicate non-healthy LDL-C levels.

It appears that calculated LDL-C values reported for ABL-06 are more accurate than those measured with direct assays (Figure 3). Sample ABL-06 has high triglyceride levels and inaccurate results in samples with elevated triglyceride levels have been reported in research studies. The findings in this Survey seem to be in line with observations made in these research studies. Furthermore, the data from this Survey suggest that calculating LDL-C levels in samples with elevated triglyceride levels provides more accurate results than direct assays.

However, the Friedewald equation commonly used in this Survey is considered to be accurate only for samples with triglyceride levels up to 400 mg/dL. The 2016 guideline for the management of dyslipidemia issued by the European Society of Cardiology and European Atherosclerosis Society, suggest using non-HDL-C measurements in patients with high triglyceride levels to overcome limitations of direct HDL-C measurements observed on those patients.

Table 3: LDL-Cholesterol - Mean Bias and Standard Deviation by Sample

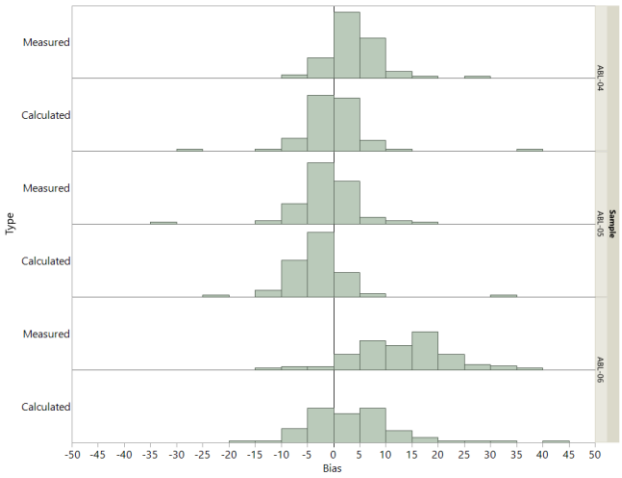
		ABL-04	ABL-05	ABL-06	All Samples
	Target Value (mg/dL)	131.48	133.43	96.12	N/A
Measured	Mean Bias (%)	4.04	-1.05	13.14	5.37
	SD	4.55	5.40	8.81	8.76
	N	109	109	109	327
Calculated	Mean Bias (%)	-0.02	-3.08	2.60	-0.17
	SD	5.83	5.62	12.62	8.94
	N	108	108	108	324

Table 4: LDL-Cholesterol - Mean Bias and Standard Deviation by Manufacturer*

		ABL-04	ABL-05	ABL-06	All Samples
Abbott	Mean Bias (%)	7.46	-2.07	12.10	5.91
	SD	3.77	4.29	9.25	8.36
	N	14	12	12	38
Beckman	Mean Bias (%)	3.70	-1.29	14.87	5.45
	SD	5.64	9.03	8.12	9.67
	N	26	17	17	60
Roche	Mean Bias (%)	3.80	-2.81	10.51	3.83
	SD	3.14	3.69	10.03	7.71
	N	36	22	22	80
Siemens	Mean Bias (%)	2.27	-0.67	12.84	4.69
	SD	5.51	4.70	9.31	8.74
	N	15	13	13	41

*Only manufacturers with 10 or more values per sample are listed.

Figure 3: Bias Distributions of Calculated and Measured LDL-C values



For non-HDL cholesterol, no analytical performance criteria are established. Mean bias and variability are very similar across samples (Table 5). Sample ABL-06 has elevated triglyceride values (300.83 mg/dL) with bias and imprecision similar to the other two samples with normal TG values (101.70 mg/dL and 103.03 mg/dL) suggesting that non-HDL-C is not affected by high TG values.

Table 5: Non-LDL-Cholesterol (Measured) - Mean Bias and Standard Deviation by Sample

	ABL-04	ABL-05	ABL-06	All Samples
Target Value (mg/dL)	150.06	152.18	159.44	N/A
Mean Bias (%)	0.10	-2.58	-0.74	-1.07
SD	3.71	3.74	4.46	4.12
N	58	58	58	174

For apolipoprotein A1 (apoA1) measurement results appear consistent among assay systems with the majority of results being outside the 13% criterion used in this Survey. The measurement bias is the highest in sample ABL-06, which has the highest triglyceride content of all 3 samples, suggesting that measurement results of apoA1 are affected by triglyceride measurements. In contrast, the majority of apolipoprotein B (apoB) results are within the 13% criterion and the bias distribution is consistent across samples. Results from all manufacturer systems show mainly a negative bias, except for results from Roche systems, which show a bias distribution around zero.

Table 6: Apolipoprotein A1 and B - Mean Bias and Standard Deviation by Sample

		ABL-04	ABL-05	ABL-06	All Samples
Apolipoprotein A1	Target Value (mg/dL)	153.8	164.3	131.7	N/A
	Mean Bias (%)	13.1	18.5	29.9	20.5
	SD	5.15	5.69	5.14	8.80
	N	50	50	50	150
Apolipoprotein B	Target Value (mg/dL)	106.9	110.2	106.6	N/A
	Mean Bias (%)	-2.8	-2.8	-6.8	-4.1
	SD	7.49	6.93	8.02	7.68
	N	53	53	53	159

The analytical performance of total cholesterol and triglyceride measurements mostly meet performance requirements used in this Survey and recommended by NCEP. High triglyceride levels appear to affect HDL-C and direct LDL-C measurements, leading to measurement results outside analytical performance requirements. Calculated LDL-C provides notably more accurate results than direct LDL-C measurement. Non-HDL-C and apoB measurements have been suggested to overcome some of the limitations of LDL-C measurements or calculations. Indeed, in this Survey non-HDL-C and apoB appear less affected by triglyceride content and show consistent analytical performance across samples. The strengths of this Survey are in the use of commutable materials with target values assigned by a reference method, and the large number of laboratories and assay systems participating. However, the Survey includes only 3 samples, which may not allow to appropriately assess the analytical performance over all concentration ranges typically observed in patient care.

*Statistics quoted in this discussion were derived prior to outlier review and differ slightly from those in Participant Summary tables.

References:

1. Warnick GR, Kimberly MM, Waymack PP, Leary ET, Myers GM. Standardization of measurements for cholesterol, triglycerides and major lipoproteins. *Lab Med*. 2008;39:481-490.
2. National Heart, Lung, and Blood Institute. High Blood Cholesterol. Available at: https://www.nhlbi.nih.gov/health-topics/high-blood-cholesterol. Accessed December 26, 2018.

Hubert W. Vesper, PhD
Accuracy Based Testing Committee