

Evaluation of a New Point of Care Device for Lipid Panel Analysis: the Pixotest

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Abstract

Introduction: The PixoTest (iXensor) has been introduced for point-of-care testing (POCT), utilizing immunocolorimetric assays to assess total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL), and calculated LDL cholesterol (cLDL) within a 3-minute turn-around time. We evaluated the PixoTest's analytical performance for the above assays against our automated laboratory Roche Cobas (Roche Diagnostics, Singapore) auto-analyser.

Methods: The performance evaluation included assay linearity, analytical precision, regression and Bland-Altman analysis against the Cobas 8000 lipid assays. Concordance analysis and Bland-Altman analysis was also performed for the evaluation of cLDL. Statistical analyses were performed using MedCalc software v18.11.3 (MedCalc, Ostend, Belgium). Our laboratory is a College of American Pathologists (CAP) accredited laboratory. As this work was part of our routine evaluation of new diagnostic assays, it was exempt from institutional review board approval.

Results: The PixoTest assays were linear from 2.69-9.10 mmol/L, 0.60-6.03 mmol/L and 0.75-2.28 mmol/L for TC, TG and HDL respectively. Inter-assay Precision was good with CV% of 4.7/5.8%, 5.1/4.6% and 8.0/6.9% for TC, TG and HDL respectively. Passing-Bablok regression analysis showed good agreement and was $r = 0.913$, 0.933 and 0.756 for TC, TG and HDL respectively. However, there were significant differences between the lipid assays, with mean differences of -0.43 mmol/L, -0.09 mmol/L and -0.07 mmol/L for TC, TG and HDL respectively. cLDL evaluation showed good correlation ($r = 0.904$), although a significant difference between assays remained (mean difference = -0.31).

Conclusion: The performance of the PixoTest lipid panel is good, within the manufacturer's claims, comparable to the Roche Cobas 8000 assays and fit for operational use.

Keywords

PixoTest; Point-of-care testing; Evaluation; Cobas; Lipids

Article highlights

- The PixoTest is a new point-of-care testing device for lipid panels with a 3-minute turn-around time.
- We compared the performance of the PixoTest to the Roche Cobas 8000 laboratory auto-analyser.
- The PixoTest has acceptable linearity, precision, and correlation when compared with the Cobas.

Abbreviations

POCT: Point-of-Care Testing
TC: Total Cholesterol
TG: Triglycerides
HDL: HDL Cholesterol
cLDL: Calculated LDL Cholesterol
CV%: Coefficient of variation
CLSI: Clinical and Laboratory Standards Institute
CAP: College of American Pathologists
EAS: European Atherosclerosis Society
EFLM: European Federation of Clinical Chemistry and Laboratory Medicine

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Introduction

The ability to quickly analyse lipid levels during the same clinic session to guide the management of hyperlipidemia is useful. Point of care testing (POCT) for lipid panels have been examined in several studies, and devices such as the Accutrend GCT/Plus (Roche Diagnostics) [1], Cholestech LDX (Alere) and CardioChek PA (Polymer Technology Systems) [2,3] have been shown to be comparable to mainstream auto-analysers. The PixoTest (iXensor) has been introduced as a convenient POCT device with a 3-minute lipid panel assay that can measure total cholesterol (TC), triglycerides (TG) and HDL cholesterol (HDL) with a calculated LDL cholesterol (LDL) (Friedewald). We evaluated the PixoTest’s analytical performance for the above assays against our laboratory Cobas (Roche Diagnostics) auto-analyser.

Methods

The PixoTest is an immunocolorimetric assay with a short turn-around time of around 3 minutes. 35uL of whole blood is applied onto a proprietary single-use lipid testing strip and a purple coloured product is produced that is read by reflectance photometry. Test strips have a shelf-life of 18 months and need to be stored in a clean, dry environment with a recommended storage temperature range of 2-32 degrees Celsius. The recommended operating temperature range for testing is 18-32 degrees Celsius. Prior to daily use, the PixoTest POCT device must be calibrated with a proprietary calibration card. The PixoTest POCT device for HbA1c has been evaluated [4]. However, the PixoTest HbA1c and lipid panel tests employ different test strips (of different shape and size) that fit into two different device ports. The correct port for each assay (HbA1c/lipids) must also be selected using a sliding switch on the device before testing (Image 1).

In the PixoTest TC assay, cholesterol esters react with cholesterol esterase and cholesterol oxidase to generate hydrogen peroxide, which then reacts with peroxidase to produce a coloured quinoneimine dye. TG reacts with lipoprotein lipase, glycerol kinase and glycerophosphate oxidase, generating hydrogen peroxide that reacts with peroxidase to liberate a coloured product. The VLDL and LDL cholesterol in the sample is then depleted using dextran sulphate, following which cholesterol esterase/cholesterol oxidase/peroxidase are used to derive a coloured product from the remaining HDL. The PixoTest device calculates cLDL using the Friedewald equation. The manufacturer’s claimed measuring range of TC is 2.59-11.64 mmol/L, TG 0.51-7.34 mmol/L, HDL 0.65-2.46 mmol/L and cLDL 1.71-5.82 mmol/L. The pooled Coefficient of Variation (CV%) reported by the manufacturer for TC was 2.5%/0.72% at 4.23/8.29 mmol/L, for TG 2.3%/1.44% at 1.14/4.25 mmol/L, and for HDL 4.53%/6.17% at 0.89/1.42 mmol/L respectively (Table 1).

The Roche Cobas 8000 (Roche Diagnostics, Singapore) TC assay is also an enzymatic immunocolorimetric assay, with the TC assay using a similar reaction to the PixoTest. For the Cobas HDL assay, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to Polyethylene-glycol-modified enzymes. Cholesterol esters are then broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. PEG-cholesterol oxidase is then used to release hydrogen peroxide from HDL, which then reacts with 4-amino-antipyrine and



Image 1: Photograph of the PixoTest POCT device

sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline to form a purple-blue dye. The Roche Cobas TG assay is also an enzymatic colorimetric assay similar to the PixoTest TG assay.

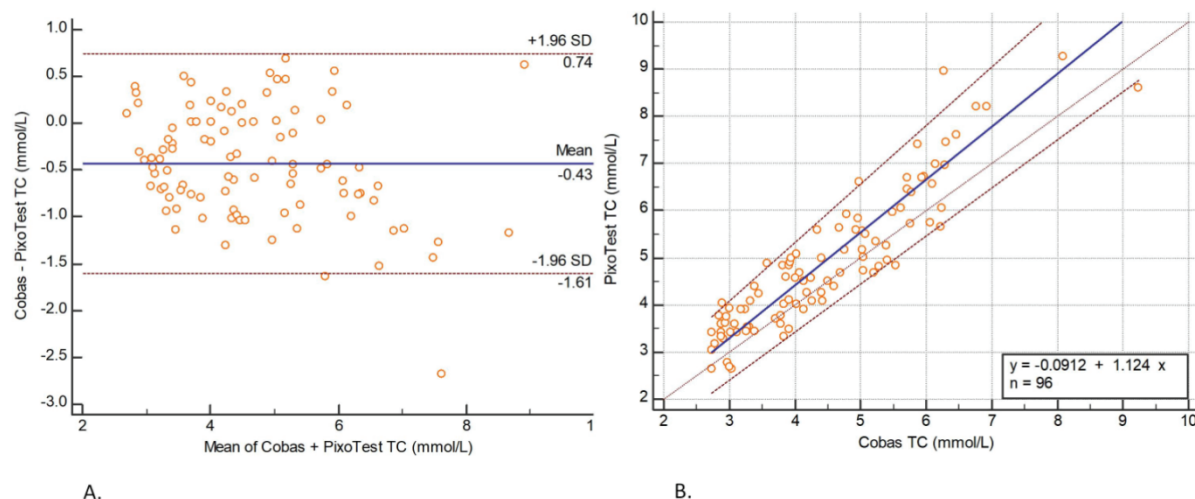
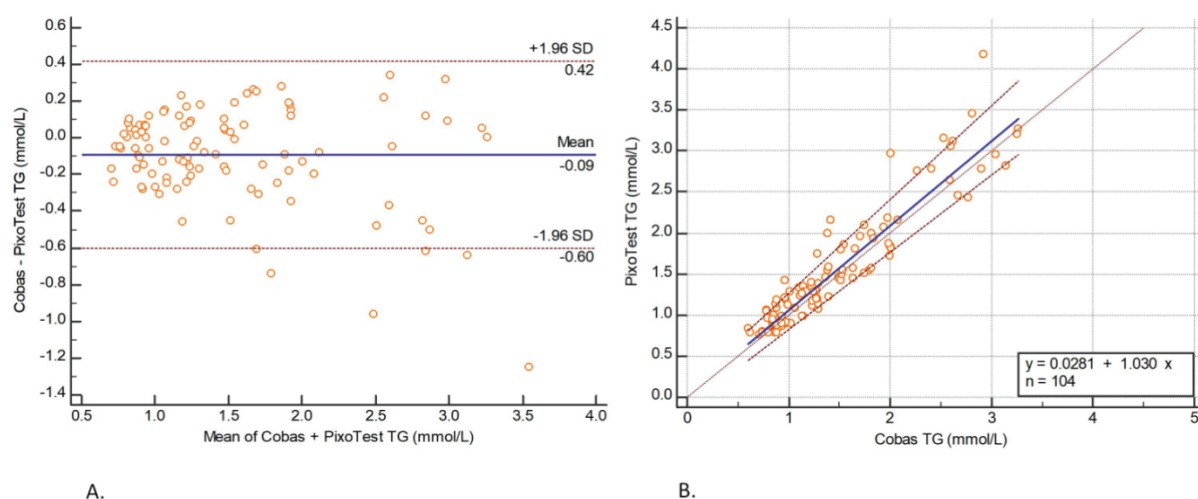
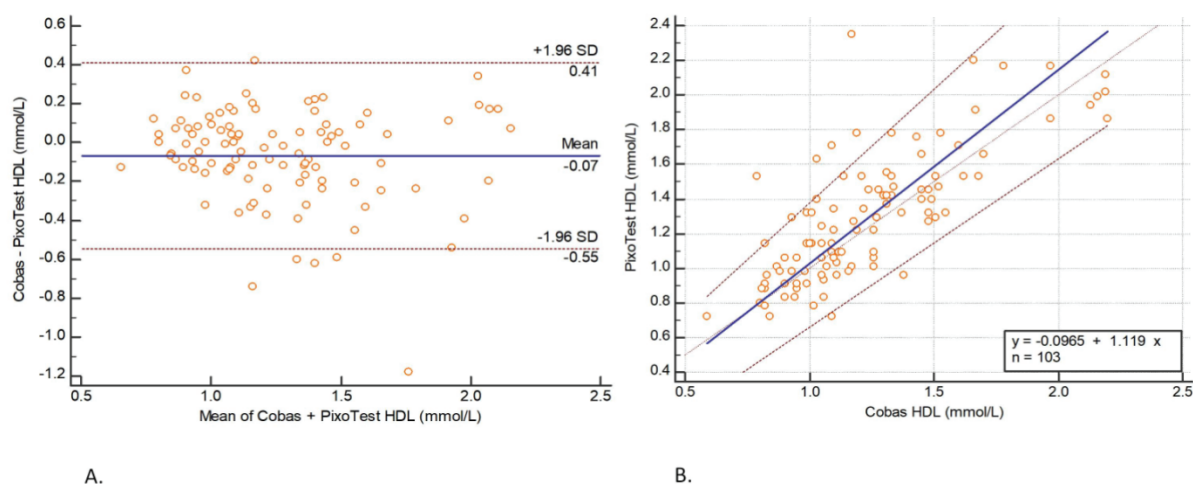
Linearity was studied following the Clinical and Laboratory Standards Institute (CLSI) EP-6 protocol [5]. Using patient serum samples with a low and high lipid values collected in plain gel-separator tubes (Becton-Dickinson, Singapore), a series of mixtures of samples pools covering a clinically relevant range of lipid concentrations were created for linearity testing. Precision was assessed according to the CLSI EP05-A3 protocol [6]. For method comparison, we used the CLSI EP-9 method [7]. Consecutive anonymised leftover serum samples that had been previously tested on the Cobas for lipid panels across a broad clinically relevant range and stored at 4°C were recruited (n =104). These samples were then examined on the PixoTest within 16 hours of receipt by the laboratory. As this work was part of our routine evaluation of new diagnostic assays, it was exempt from an institutional review board approval. Our laboratory is certified by the College of American Pathologists (CAP) and our performance for the Cobas 8000 autoanalyser lipid assays on the CAP external quality assurance program has been satisfactory. Statistical analyses including Bland-Altman analysis and regression were performed using MedCalc software v18.11.3 (MedCalc, Ostend, Belgium).

Results

The PixoTest assays were linear from TC 2.69-9.10 mmol/L, TG 0.60-6.03 mmol/L and HDL 0.75-2.28 mmol/L. Inter-assay precision (CV%) was 4.7/5.8%, 5.1/4.6%, and 8.0/6.9% at TC 2.68/7.48 mmol/L, TG 1.61/4.98 mmol/L and HDL 1.08/1.98 mmol/L respectively. In our method comparison analysis of 104 consecutive serum samples, some results for TC/TG/HDL were below the reported measuring range of the PixoTest (PixoTest reported values as LOW), therefore, for method comparison N = 96/104/103 for TC/TG/HDL respectively. Despite this, all three assays showed good agreement with the Cobas 8000 with Passing-Bablok r =0.913, 0.933 and 0.756 for TC, TG and HDL respectively. Bland-Altman analysis showed significant differences for all three analytes between the two assays, with a mean difference of -0.43 mmol/L (p<0.0001, 95% CI of -0.5529 to -0.3100) for TC, -0.09 mmol/L (p =0.0005, 95% CI -0.1435 to -0.04190) for TG and -0.07 mmol/L (p =0.0065, 95% CI of -0.1147 to -0.01911) for HDL.

Table 1: Analytical Characteristics for the PixoTest

Parameters	Claimed measuring range (mmol/L)	Claimed CV% (concentration mmol/L)
TC	2.59 - 11.64	2.5/0.72 (4.23/8.29)
TG	0.51 - 7.34	2.3/1.44 (1.14/4.25)
HDL	0.65 - 2.46	4.53/6.17 (0.89/1.42)
cLDL	1.71 - 5.82	-

**Figure 1: A. Bland-Altman and B. Passing-Bablok analysis for TC****Figure 2: A. Bland-Altman and B. Passing-Bablok analysis for TG****Figure 3: A. Bland-Altman and B. Passing-Bablok analysis for HDL**

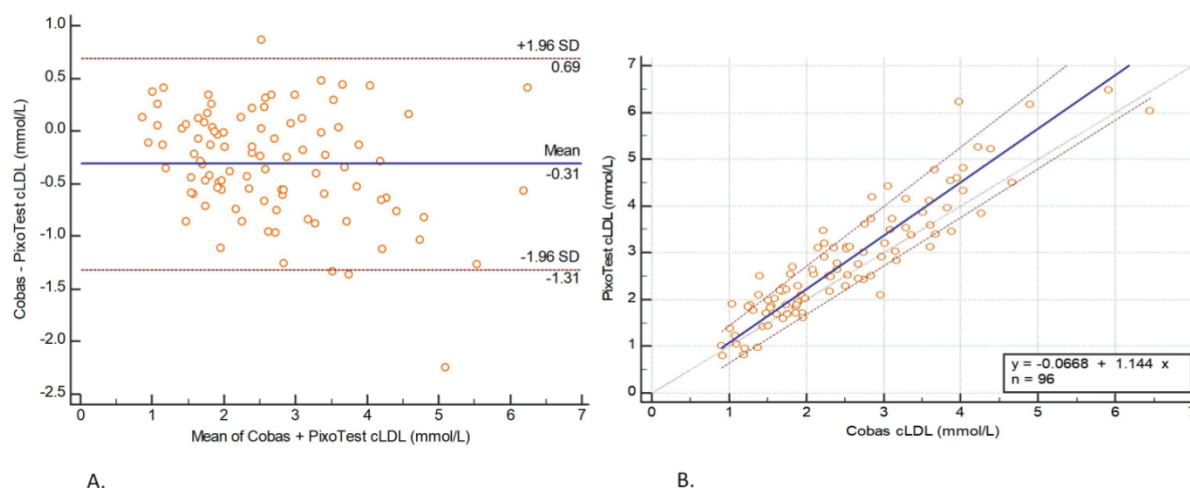


Figure 4: A. Bland-Altman and B. Passing Bablok analysis for cLDL

Out of the 104 consecutive serum samples tested for cLDL, 25 samples were reported as LOW (<1.71 mmol/L) and 4 samples reported as HIGH (>5.82 mmol/L) by the PicoTest because the values exceeded/fell below the specified limits of detection of cLDL. Using the Friedewald equation, we calculated the cLDL values for these samples if they had a measurable TC/TG/HDL. After our own computation, we were able to use N = 96 for cLDL method comparison. Passing-Bablok analysis showed a good correlation for cLDL between the two assays ($r = 0.904$, $p < 0.0001$, 95% CI 0.860-0.935), although a significant difference between assays remained (mean difference = -0.3103, $p < 0.0001$, 95% CI -0.4141 to -0.2065) (Figures 1-4).

Discussion

Our evaluation of the PicoTest lipid panel assay confirms that its performance is good, within the manufacturer's claims and is comparable to the Cobas 8000 analyser. The PicoTest assay precision for TC, TG and HDL were acceptable in the clinically significant range. To simulate real-world conditions, precision testing was performed by 4 operators on 4 different instruments. Several other capillary blood lipid panel POCT analysers are available in the market, including the Cholestech LDX (Alere, UK) and Professional CardioChek PA (Polymer Technology Systems, Indiana, US), which have a turn-around time of 5 minutes and <2 minutes respectively [8]. The PicoTest POCT device, with a turn-around time of around 3 minutes, provides a competitive turn-around time compared with existing POCT lipid assays. While the correlation for the HDL assays between the Cobas and the PicoTest was acceptable ($r = 0.756$), it was lower compared to that of the other lipids assessed. This may be due to differences in the precipitation step for both assays to remove VLDL and LDL cholesterol.

One limitation of the PicoTest is in the reporting of cLDL results. Should TC/TG/HDL be available, a cLDL value should be able to be calculated. However, the PicoTest is only programmed to display cLDL values between 1.71-5.82 mmol/L. As such, out of 104 consecutive serum samples, 29 samples (28%) had unreported cLDL values on the PicoTest. Using the TC/TG/HDL values from the PicoTest to calculate cLDL, we were able to calculate the cLDL for 21 of these samples, resulting in 96 cLDL results we could compare with. The cLDL we calculated using results from LOW samples ranged from 0.80-1.70 mmol/L, and the cLDL based on the Cobas lipid panels for these samples that had LOW cLDL on the PicoTest ranged from 0.90-1.96 mmol/L. The 4 cases that had HIGH PicoTest LDL had a cLDL from 6.04-6.48 mmol/L using the PicoTest values, and their Cobas cLDL was 3.98-6.45 mmol/L. Ideally, cLDL should not have a restricted measuring range as it is a calculated value.

Another limitation of our study is that we were not able to discriminate between samples from fasting/non-fasting patients. However, studies have shown that fasting and non-fasting HDL/TC have similar prognostic value [9-11], and the UK NICE guidelines [12] have endorsed non-fasting lipid testing since 2014. This is also supported by the European Atherosclerosis Society/European Federation of Clinical Chemistry and Laboratory Medicine (EAS/EFLM) guidelines [13]. The advantages of non-fasting lipid testing extend into patient compliance with lipid testing, thanks to their added convenience [14]. Even TG has non-significant increases after habitual food intake [15]. Fasting is thus not essential for the routine assessment of the plasma lipid profile. Another limitation of our study is that we used serum samples rather than whole blood for lipid testing. The PicoTest lipid panel assay is designed to utilize capillary whole blood or fresh whole blood in heparin/EDTA tubes. However, our hospital collects blood for lipid testing in plain tubes/gel separator tubes to isolate patient serum for testing on the Roche Cobas platform. Samples used for this study were drawn from this pool of samples. This could have contributed to the differences found between the two assays. Despite this, correlation between the two assays remained satisfactory. A study by Cloey et al [16], reported a plasma/serum TC difference of 4.7%; however, later studies [17] found that the mean plasma/serum ratio for TC, TG and HDL was significantly different across five analytical methods (including the Abell-Kendall TC reference method). It is thus difficult to use a standard calculation to readily convert serum lipid panel results into plasma or whole blood lipid panel results.

Conclusion

The performance of TC, TG and HDL assays on the PicoTest is good, within the manufacturer's claims, comparable to the Roche assays and fit for operational use. Rapid lipid testing using accurate POCT devices is desirable, and with a rapid assay time of 3 minutes, the PicoTest can aid the clinician in quick decision-making.

References

1. Mendez-Gonzalez J, Bonet-Marques R, Ordonez-Llanos J. Lipid Profile in Ambulatory Subjects Using 3 Point-of-Care Devices and Comparison With Reference Methods. *Point of Care*. 2010 Jun;9(2):102-107.
2. Pluddermann A, Thompson M, Price CP, Wolstenholme J, Heneghan C. Point-of-care testing for the analysis of lipid panels: primary care diagnostic technology update. *Br J Gen Pract*. 2012;62(596):e224-e226.
3. Bastianelli K, Ledin S, Chen J. Comparing the Accuracy of 2 Point-of-Care Lipid Testing Devices. *J Pharm Pract*. 2017;30(5):490-497.

4. Lau CS, Tong CH, Goh R, Aw TC. Evaluation of a New Point-of-Care HbA1c Assay: The Pixotest. *International Journal of Clinical Chemistry and Laboratory Medicine*. 2019;5(4):1-5.
5. CLSI. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI Document EP06-A. Wayne, PA: Clinical and Laboratory Standard Institute; 2003.
6. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standard Institute; 2014.
7. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Third Edition. CLSI document EP09-A3. Wayne, PA: Clinical and Laboratory Standard Institute; 2013.
8. Dale RA, Jensen LH, Krantz MJ. Comparison of two point-of-care lipid analyzers for use in global cardiovascular risk assessments. *Ann Pharmacother*. 2008;42(5):633-639.
9. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation*. 2008;118(20):2047-2056.
10. Langsted A, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58 434 individuals from the Copenhagen General Population Study. *Clin Chem*. 2011;57(3):482-489.
11. Doran B, Guo Y, Xu J, Weintraub H, Mora S, et al. Prognostic value of fasting versus nonfasting low-density lipoprotein cholesterol levels on long-term mortality: insight from the National Health and Nutrition Examination Survey III (NHANES-III). *Circulation*. 2014;130(7):546-553.
12. NICE clinical guideline CG181. Lipid modification: cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease.
13. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points – a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur Heart J*. 2016;37(25):1944-1958.
14. Khera AV, Mora S. Fasting for lipid testing: is it worth the trouble? *Arch Intern Med*. 2012;172(22):1710-1712.
15. Sidhu D, Naugler C. Fasting time and lipid levels in a community-based population: a cross-sectional study. *Arch Intern Med*. 2012;172(22):1707-1710.
16. Cloey T, Bachorik PS, Becker D, Finney C, Lowry D, et al. Reevaluation of serum-plasma differences in total cholesterol concentration. *JAMA*. 1990;263(20):2788-2789.
17. Beheshti I, Wessels LM, Eckfeldt JH. EDTA-plasma vs serum differences in cholesterol, high-density-lipoprotein cholesterol, and triglyceride as measured by several methods. *Clin Chem*. 1994;40(11):2088-2092.