

## Comparison of LDL - cholesterol estimated by direct method and by calculation

**Kinjal Mukeshbhai Badrakiya**, Aashna Darshanbhai Shah, Mayur Goradhanbhai Makadia\*,  
Vishwal Indravadan Patel, Kinjal Prahaladbhai Patel, Kaushik Salubhai Chaudhari and  
Haridas Neelakandan Nilayangode

*Department of Biochemistry, Pramukhswami Medical College, Karamsad, Anand, Gujarat 388325, India*

### \*Correspondence Info:

Dr. Mayur Goradhanbhai Makadia,  
Resident,  
Department of Biochemistry,  
Pramukhswami Medical College,  
Karamsad, Anand, Gujarat 388325, India  
E-mail: [mayurmakadia@gmail.com](mailto:mayurmakadia@gmail.com)

### Abstract

**Introduction:** Total cholesterol (TC) and Low-density lipoprotein cholesterol (LDL-C) are well-established risk factors for the coronary heart disease (CHD). There are many homogenous assays currently available for the estimation of serum LDL-C. Most clinical laboratories determine LDL-C (mg/dL) by Friedewald's formula (FF),  $LDL-C = (TC) - (HDL-C) - (TAG/5)$ . This formula shows the level of LDL-C is dependent on triglyceride (TAG) level.

**Aim and Objectives:** The aim of this study was to find out the relative advantages of direct measurement of cholesterol over the conventional derivation of LDL-C by calculation.

**Material and Method:** The study contained 80 participants above 18 years. LDL-C estimation was done by direct method manually on the spectrophotometer and also calculated using the Friedewald's Formula. An independent t-test was applied to find out the statistically significant difference.

**Results:** It was observed that, the mean LDL-C levels by calculated method and the direct method in the control group ( $TAG \leq 150$  mg/dl) (114.83 and 116.88 mg/dl respectively,  $P=0.81$ ), case group-1 ( $TAG=150-300$  mg/dl) (113.11 and 116.01 mg/dl respectively,  $P=0.82$ ) and case group-2 ( $TAG=300-400$  mg/dl) (112.75 and 116.30 mg/dl respectively,  $P=0.73$ ) show no significant difference, but in the case group-3 ( $TAG \geq 400$  mg/dl) (112.12 and 182.0 mg/dl respectively,  $P<0.001$ ) shows significant difference.

**Conclusion:** Our data suggest that; the estimated LDL-C can be substantially underestimated due to the high triglyceride levels of 400 mg/dl or more. These results in the misclassification of the risk, where the patient's calculated LDL-C may be lower than their true LDL-C, resulting in the missed opportunities for the treatment.

**Keywords:** Direct LDL-C, TAG, Friedewald's formula

### 1. Introduction

Elevated serum Total cholesterol (TC) and Low-Density Lipoprotein Cholesterol (LDL-C) concentration are the well-known atherogenic risk factor for the coronary heart disease (CHD) [1]. The National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) recommends a goal of maintaining serum LDL-C concentration  $< 100$  mg/dl as optimal. The level of LDL-C between 100-129 mg/dl is near/above optimal, the level between 130-159 mg/dl is borderline high, the level between 160-189 mg/dl is high and the level  $\geq 190$  mg/dl is very high for the risk of CHD. Serum LDL-C concentration is also the basis for initiating appropriate treatment and patient's risk stratification [2]. Therefore, it is important for the analysis of serum LDL-C levels to be precise and accurate. The gold standard for measuring LDL-C level is beta ( $\beta$ ) quantification

(the separation of lipoproteins by combining ultracentrifugation and precipitation with polyanions) [3]. Beta quantitation is not suited for routine use, as it is expensive, laborious, requires ultracentrifugation, requires large volumes of samples, is time-consuming and requires expensive instruments [4]. Therefore, its use is confined to research and specialized laboratories [5].

Homogenous assays for direct LDL cholesterol (D-LDL-C) estimation were developed in 1998 [3]. The Cholesterol Reference Method Laboratory Network of the Centres for Disease Control and Prevention has approved the use of five commercially available homogenous assays for LDL-C estimation [3]. The high cost of these assays, however, limits their use in most of the Indian laboratories. In routine practice, most clinical laboratories estimate LDL-C concentrations in serum by Friedewald's formula from the

concentrations of Total Cholesterol (TC), Triglyceride (TAG), and High-Density Lipoprotein Cholesterol (HDL-C). The calculation of LDL-C by the traditional Friedewald's formula (F-LDL-C) is  $F\text{-LDL-C (mg/dl)} = TC - HDL-C - TAG/5$  [6, 7]. The Friedewald's formula cannot be used for LDL-C calculation when the subject is not fasting or in patients with type III or type I hyperlipoproteinaemia [8]. A fasting sample is mandatory for F-LDL-C because the Friedewald's formula assumes that the triglyceride to cholesterol ratio in Very Low-Density Lipoprotein (VLDL) is constant. This ratio is altered in non-fasting samples (containing chylomicrons and chylomicron remnants). Consequently, if a non-fasting sample is used for F-LDL-C, there would be an overestimation of VLDL-C and underestimation of LDL-C [6]. The use of this formula is also not recommended for Type II diabetes mellitus, nephrotic syndrome, and chronic alcoholic patients, because, in these conditions too, the triglyceride to cholesterol ratio in VLDL is altered [9-11].

Therefore, this study was undertaken to determine if, and to what extent, LDL-C level is underestimated/overestimated when it is calculated using the Friedewald's formula compared with direct measurement of LDL-C.

## 2. Material and Method

This study was a cross-sectional study, conducted in the Biochemistry Department at University's Medical College. All the participants of both gender and age >18 years attending the hospital and prescribed for the lipid profile were selected for the study, from whom individuals have selected in the case and the control groups after applying the inclusion and exclusion criteria.

### 2.1 Sampling procedures, inclusion & exclusion criteria

Written informed consents were obtained from all the participants, and then full history was taken. Venous blood sample was drawn in plain vacutainer with strict aseptic precaution after 10-12 hours of fasting; serum was separated by centrifugation at 3000 rpm for 10-15 mins. The tests for Lipid Profile were analysed on fully automated COBAS INTEGRA-400 plus analyser. Serum Total Cholesterol was analysed by Colorimetric assay with CHOD-POD [12], serum HDL was analysed by homogenous enzymatic colorimetric [13], triglyceride was analysed by Colorimetric endpoint GPO-PAP [14], while serum LDL-C and VLDL were calculated by using Friedewald's Formula [7]. LDL-C was also measured manually by the Direct method on Spectrophotometer [15], present in Medical College. The study group consisted of 80 participants above 18 years who were prescribed to do lipid profile. The case group-1 included 20 participants with TAG level between 150-300 mg/dl. The case group-2 included 20 participants with TAG level between 300-400 mg/dl. The case group-3 included 20 participants with TAG level more than 400

mg/dl. The control group included 20 participants with TAG level less than 150 mg/dl. Individuals on treatment with lipid lowering drugs (decided by drug and history) were excluded from both the case and the control groups. Ethical clearance was obtained from Institutional Ethics Committee.

### 2.2 Statistical Analysis

All the data required for this study were collected and analyzed statistically to determine the significance of different parameters by using the commercially available statistical software MedCalc version 14.8.1 and Microsoft Office 2016. All the values were given as mean  $\pm$  SD. Comparison between the case and the control groups were made using Student's t-test and the P value of less than 0.05 was considered statistically significant.

## 3. Result

### 3.1 Demographic data of age and sex

In this study total, 80 participants were included (48 men and 32 women) out of which 20 participants with TAG level  $\leq 150$  mg/dl were included in the control group, and another 60 participants were divided into the case group on the basis of their TAG levels as the case group-1 (TAG = 150 to 300 mg/dl), the case group-2 (TAG = 300 to 400 mg/dl) and the case group-3 (TAG  $\geq 400$  mg/dl). All the study groups were included 60 % of men (12 out of 20) and 40 % of women (8 out of 20) with the age between 18 to 70 years. Table 1 shows the details of demographic data of the study.

### 3.2 Summary of the measurement of various parameters of lipid profile

The mean age of participants in the control group, case group-1, case group-2 and case group-3 was  $50.8 \pm 13.49$ ,  $49.40 \pm 12.58$ ,  $51.95 \pm 12.17$  and  $47.55 \pm 10.88$  years respectively. The mean levels of various parameters of lipid profile like Total Cholesterol, HDL-C, Triacylglycerol, LDL-C by Friedewald's formula and by the Direct method, VLDL, TC/HDL, LDL/HDL for all the study groups are shown in Table 2.

### 3.3 Comparison of LDL-C levels by Student's t-test

The mean LDL-C level by Friedewald's formula and by Direct method in the control group was  $114.83 \pm 26.62$  and  $116.88 \pm 26.81$  mg/dl respectively with the  $P = 0.81$ , in the case group-1 was  $113.11 \pm 40.17$  and  $116.01 \pm 41.10$  mg/dl respectively with the  $P = 0.82$  and in the case group-2 was  $112.75 \pm 31.83$  and  $116.30 \pm 33.04$  mg/dl respectively with the  $P = 0.73$  which show no statistically significant differences. But, the mean level of LDL-C in the case group-3 was  $112.12 \pm 51.84$  and  $182.0 \pm 49.51$  mg/dl by Friedewald's formula and by Direct method respectively, which show statistically significant difference with the P value of  $<0.001$ . There was underestimation of LDL-C by calculation at all the levels of TAG. This underestimation was maximum at the TAG levels of  $\geq 400$  mg/dl with the Mean Difference of - 69.88 mg/dl [Table 3].

Table 1: Demographic data of the Study

| Age (Years) | Control Group | Case Group-1 | Case Group-2 | Case Group-3 | Total |
|-------------|---------------|--------------|--------------|--------------|-------|
| 21-30       | 1             | 1            | 1            | 1            | 4     |
| 31-40       | 5             | 4            | 3            | 4            | 16    |
| 41-50       | 3             | 3            | 4            | 8            | 18    |
| 51-60       | 5             | 9            | 8            | 4            | 26    |
| 61-70       | 6             | 3            | 4            | 3            | 16    |
| TOTAL       | 20            | 20           | 20           | 20           | 80    |

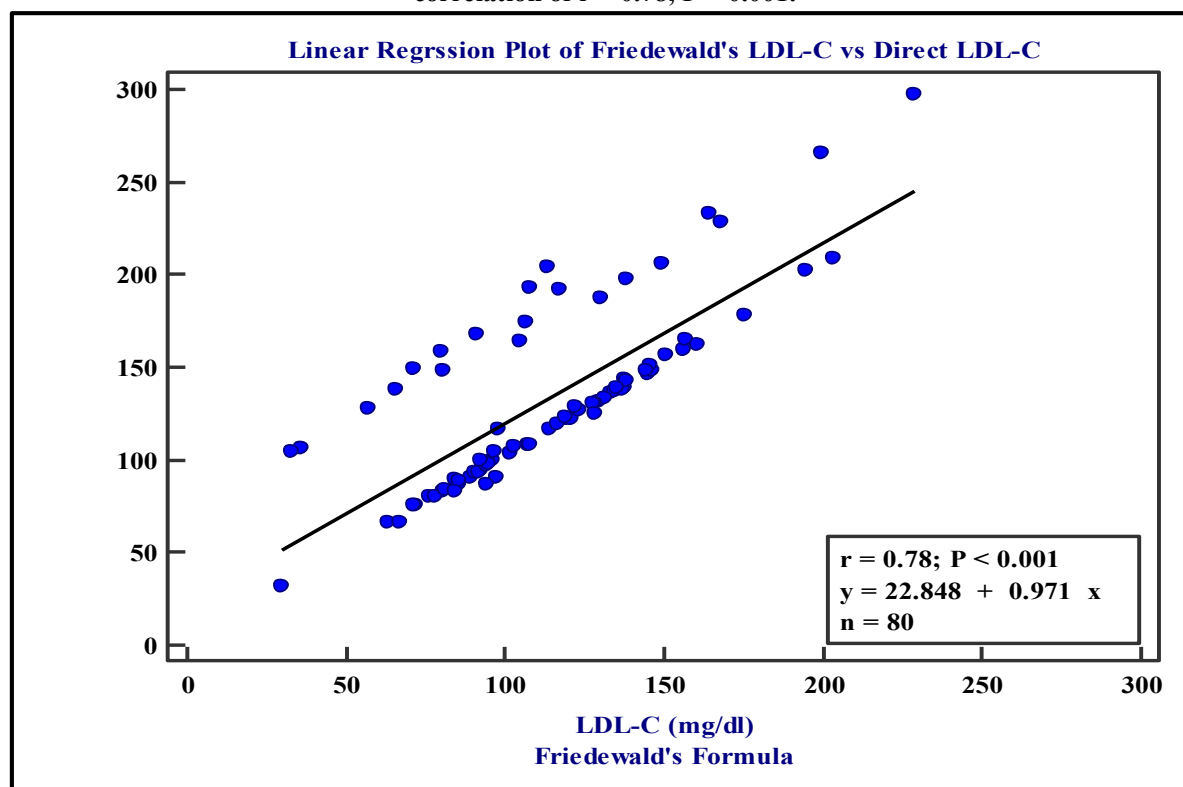
Table 2: Summary of the measurement of various parameters of lipid profile

| Parameters                           | Control Group<br>(Mean $\pm$ SD) | Case Group-1<br>(Mean $\pm$ SD) | Case Group-2<br>(Mean $\pm$ SD) | Case Group-3<br>(Mean $\pm$ SD) |
|--------------------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|
| AGE (years)                          | 50.8 $\pm$ 13.49                 | 49.40 $\pm$ 12.58               | 51.95 $\pm$ 12.17               | 47.55 $\pm$ 10.88               |
| Total cholesterol (mg/dl)            | 179 $\pm$ 30.49                  | 204.25 $\pm$ 45.8               | 216.60 $\pm$ 33.16              | 243.80 $\pm$ 54.35              |
| HDL-C (mg/dl)                        | 45.55 $\pm$ 10.55                | 42.5 $\pm$ 11.65                | 34.55 $\pm$ 4.35                | 35.80 $\pm$ 6.49                |
| Triacylglycerol (mg/dl)              | 93.10 $\pm$ 22.11                | 243.20 $\pm$ 29.7               | 346.50 $\pm$ 23.22              | 479.40 $\pm$ 75.43              |
| LDL-C (Friedewald's Formula) (mg/dl) | 114.83 $\pm$ 26.62               | 113.11 $\pm$ 40.17              | 112.75 $\pm$ 31.83              | 112.12 $\pm$ 51.84              |
| LDL-C (Direct Method) (mg/dl)        | 116.88 $\pm$ 26.81               | 116.01 $\pm$ 41.10              | 116.30 $\pm$ 33.04              | 182.0 $\pm$ 49.51               |
| VLDL (mg/dl)                         | 18.61 $\pm$ 4.41                 | 48.64 $\pm$ 5.94                | 69.25 $\pm$ 4.61                | 95.78 $\pm$ 15.20               |
| TC/HDL                               | 4.07 $\pm$ 0.89                  | 5.31 $\pm$ 1.06                 | 6.34 $\pm$ 1.12                 | 6.10 $\pm$ 1.44                 |
| LDL/HDL                              | 2.63 $\pm$ 0.79                  | 3.09 $\pm$ 0.87                 | 3.29 $\pm$ 1.03                 | 2.85 $\pm$ 1.05                 |

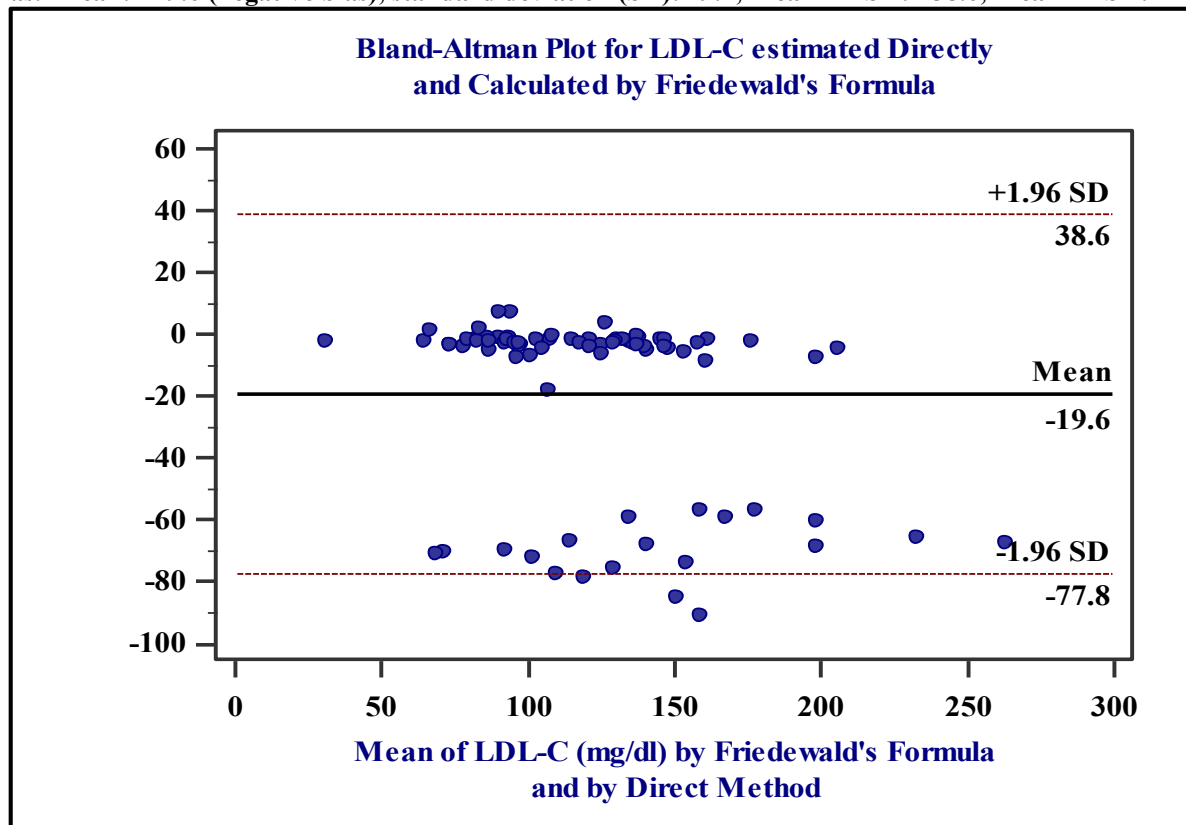
Table 2: Comparison of LDL-C levels by Student's t-test.

| Group         | LDL-C<br>(Friedewald's<br>Formula) (mg/dl)<br>(Mean $\pm$ SD) | LDL-C<br>(Direct Method)<br>(mg/dl)<br>(Mean $\pm$ SD) | Mean<br>Difference<br>(mg/dl) | Pearson's<br>Correlation<br>Coefficient [r] | 95%<br>Confidence<br>interval for r | P<br>value* |
|---------------|---|--|-------------------------------|---|-------------------------------------|-------------|
| Control Group | 114.83 $\pm$ 26.62  | 116.88 $\pm$ 26.81                                     | -2.05                         | 0.999                                       | 0.9975 to 0.9996                    | 0.81        |
| Case Group-1  | 113.11 $\pm$ 40.17  | 116.01 $\pm$ 41.10                                     | -2.9                          | 0.9978                                      | 0.9943 to 0.9991                    | 0.82        |
| Case Group-2  | 112.75 $\pm$ 31.83  | 116.30 $\pm$ 33.04                                     | -3.55                         | 0.988                                       | 0.9694 to 0.9954                    | 0.73        |
| Case Group-3  | 112.12 $\pm$ 51.84  | 182.0 $\pm$ 49.51                                      | -69.88                        | 0.9851                                      | 0.9618 to 0.9942                    | <0.001      |

\*P &lt; 0.05 = Statistically Significant

Figure 1: Linear Regression Plot of Friedewald's LDL-C against Direct LDL-C of the study participants. There was a correlation of  $r = 0.78$ ,  $P < 0.001$ .

**Figure 2: The Bland–Altman Plot for Direct LDL-C and LDL-C Calculated by Friedewald’s formula showing negative bias. Mean: –19.6 (negative bias), standard deviation (SD): 29.1, Mean + 2 SD: +38.6, Mean – 2 SD: –77.8**



### 3.4 Correlation

A strong correlation was observed between the calculated LDL-C by Friedewald’s formula and directly measured LDL-C levels. The correlation was 0.78 between LDL-C by Friedewald’s formula and LDL-C by the Direct method as shown in the linear regression plot [Figure 1]. The correlation [r] between calculated LDL-C and Direct LDL-C was 0.999 in the control group, 0.9978 in the case group-1, 0.988 in the case group-2 and 0.9851 in the case group-3 by using Pearson’s correlation coefficient [Table-3].

### 3.5 Method Comparison

The Bland–Altman plot was used to compare and to find the agreement between the direct and calculated LDL-C methods. This plot provides a graphical comparison of the level of agreement between the two methods of assessment by plotting the difference between the two methods versus the mean between them. The graph in Figure 2 shows the limits of agreement were 38.6, -77.8 and a mean difference of -19.6 mg/dl. The negative bias and wide limits of agreement in them indicate that although the two methods correlate to one another they are not analytically replaceable.

## 4. Discussion

The availability of an accurate and precise method to evaluate LDL-C is the key importance in the clinical assessment of patients at risk for CHD. This is because serum LDL-C concentration is a well-known atherogenic risk factor. It is also the basis for risk stratification of CHD as well as the deciding factor for treatment strategies. This cross-sectional

study was undertaken to assess the performance of a homogeneous method for direct LDL-C measurement, as compared with the LDL-C estimate by using the Friedewald’s formula. Despite the technological innovations, the Friedewald’s formula continues to be used in many clinical laboratories currently.

The most important finding of my study is that the Friedewald’s equation tends to underestimate LDL-C most when accuracy is most crucial. Martin SS *et al.* also found underestimation of LDL-C by Friedewald’s formula [16].

The mean LDL-C level by Friedewald’s formula and by Direct method in the control group, the case group-1 and the case group-2 show no statistically significant differences ( $P = 0.81, 0.82, 0.73$  respectively) but, in the case group-3 was  $112.12 \pm 51.84$  and  $182.0 \pm 49.51$  mg/dl, which show statistically significant difference with the  $P$  value of  $<0.001$ . Reignier A *et al.* also found a significant difference in calculated and direct LDL-C level [17].

A good correlation was observed between the calculated LDL-C and directly measured LDL-C levels. The correlation was 0.78 between Friedewald’s LDL-C and directly measured LDL-C. This finding is similar to that found in other studies where the correlation ranged between 0.78 to 0.93 [18,19].

The Bland–Altman plot showed a negative bias in spite of the good correlation mentioned above. This could be due to a difference in the results obtained by the direct and calculated methods. At different levels of TAG, the study found that calculated LDL-C was always lower than the

directly measured LDL-C. This difference increases at higher levels of TAG. The mean difference between the two methods was highest (69.88 mg/dl) at TAG levels  $\geq 400$  mg/dl. While the mean differences were 2.05, 2.9 and 3.55 mg/dl in the participants with TAG levels of  $\leq 150$ , 150-300 and 300-400 mg/dl respectively. Direct LDL-C is higher than calculated LDL-C at TAG levels  $\geq 400$  mg/dl. Compared with direct measurement, the Friedewald's calculation underestimates the risk for ischemic heart disease. Bansal E *et al* also found the similar result [20].

The direct method used for measuring LDL-C has very good performance, with good reproducibility and a coefficient of variation, which is hardly obtained with the Friedewald's formula. One of the objectives was to discuss that the two methods do not have identical results, it is still a consensus that the Friedewald's formula may be used in patients with triglyceride levels up to 400 mg/dL, who have neither chylomicrons, nor IDLs (type III of the Fredrickson classification) [8]. In fact, many laboratories still continue to estimate LDL-C levels by using that formula, because of the costs of the reagents for the existing direct LDL-C measuring methods. With a decrease in those costs and a better assessment of the performance of the reagents, the direct methods tend to be more widely used in laboratories, providing a better classification of the patients, with more reliable LDL-C level results, according to the NCEP criteria. Certain populations would benefit extremely from the use of the direct methods, such as diabetic patients, who are naturally prone to developing coronary artery disease, and whose LDL-C levels are not correctly estimated with the Friedewald's formula [9-11].

Limitation of the present study was that we did not take into consideration the co-morbidities of each study participants and these comorbidities could also have a role in influencing calculated LDL-C levels. Another major drawback was small sample size.

## 5. Conclusion

Most of the laboratories are measuring LDL-C by the calculated method. Estimated LDL-C can be substantially underestimated due to the high triglyceride levels of 400 mg/dl or more. These results in the misclassification of the risk, where the patient's calculated LDL-C may be lower than their true LDL-C, resulting in the potential missed opportunities for the treatment.

Calculated LDL-C has been the standard method for many years because of the Framingham Heart Study and major clinical trials were done in the era when the more modern direct method of measuring LDL-C was neither available nor validated. Many persons, particularly those with obesity or multiple risk factors, like metabolic syndrome, have elevated triglyceride levels which can compromise the accuracy of a calculated LDL-C measurement. In addition, as the accuracy of calculated LDL-C measures is reduced, and

the potential for misclassification is increased even in those with borderline triglyceride levels such as between 200-400 mg/dl, there may be the clear advantage to considering a direct LDL-C measurement in these cases.

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