An Improved Method for LDL-C beta-Quantification for Clinical Drug Trials
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Abstract
Objective
Adapt the standard LDL-C beta quantification method to improve the turnaround time and reduce the amount of specimen required for the assay, and provide a commutable method globally for pharmaceutical clients.

Background
Low density lipoproteins (LDL) are the major cholesterol-carrying lipoproteins in plasma. LDL is primarily cleared from the bloodstream by the liver, but to a lesser extent, by other peripheral tissues. The CDC reference method for the measurement of LDL cholesterol (LDL-C) is beta quantification using ultracentrifugation. The original reference method for LDL-C by beta quantification requires 5 mL of serum; later adaptations apply 1 mL serum. This report describes an adaptation utilizing 0.2 mL of serum with a reduced centrifugation time, which satisfies IRB requirements for pharmaceutical companies investigating PCSK9 inhibitors and other drug development studies designed to improve LDL-C clearance.

Methods
Specimens were centrifuged for 3 hours 54 minutes, as opposed to 20 hours 15 minutes (existing method) in a Model Optima XPN-80 ultracentrifuge with titanium rotor Type 42.2 Ti (Beckman Coulter®, Brea, CA). A Beckman CentriTube™ Slicer and Beckman Coulter Cellulose propionate centrifuge tubes were used. Total cholesterol was measured on Modular Analytics or cobas® 8000 instrumentation (Roche Diagnostics, Indianapolis, IN) using Roche cholesterol reagent (Catalog # 05168538190). LDL-C by direct assay was performed using Roche reagents (Catalog #05171369190). An LDL-C determination by Friedewald calculation was calculated from total cholesterol, high density lipoprotein-cholesterol (HDL-C), and triglycerides (glycerol-blanked) measurements. HDL-C was measured by dextran-magnesium sulfate precipitation and subsequent total cholesterol measurement. Triglycerides (glycerol-blanked) were measured using Roche reagents (Catalog #05976006190). Data reduction utilized EP Evaluator® (Data Innovations, South Burlington, VT). The new method was validated in the US and subsequently in Shanghai, PRC and Geneva, Switzerland. Global correlation studies were conducted according to Covance CLS established methods.

Results
The new process demonstrated an inter-assay imprecision of 2.6%, which satisfied imprecision requirements using an allowable total error of 10.5%. Accuracy, as determined by method comparisons to the Friedewald calculation and LDL-C by direct assay demonstrated concordance within 20% of the LDL-C ultracentrifugation results. The correlation between the 1 mL and 0.2 mL methods, as well as inter-laboratory Global correlations, met acceptance criteria (95% CI for the slope includes 1.00, the 95% CI for the y-intercept includes 0.00, and correlation coefficient, R > 0.95). Global correlations showed met acceptance criteria and proved commutability of method.

Conclusions
The method enhancements for LDL-C by ultracentrifugation demonstrated excellent analytical performance, and provided significant value to pharmaceutical clients and laboratory operations both in turnaround time and fewer test cancellations. Test cancellations due to insufficient specimen volume were reduced from 4.3% to 0.2%. The time required for specimen processing was reduced by 48% leading to significant savings in time and labor expense.

Introduction
Challenge: LDL-C by ultracentrifugation test cancellations because the amount of specimen was insufficient; clinical trials and IRB place restrictions on the amount of specimen.
Challenge: LDL-C by ultracentrifugation takes 24 hours to complete because of centrifugation step; improvement in turnaround time needed.
Challenge: Implement a method using 0.2 mL of serum and reduce the centrifugation time to meet the requirements of IRB and clinical trials investigation in pharmaceutical sponsored studies devoted to the clinical evaluation of PCSK9 inhibitors designed to improve LDL-C clearance.

Background
- Low-density lipoproteins (LDL) – The major cholesterol carrying lipoprotein
- CDC reference method for LDL-cholesterol – Ultracentrifugation uses 5 mL of serum; later adaptations use 1.0 mL
- Ultracentrifugation – Typically the time required for separation of the lipoprotein fractions is 20 hours or more
- Other methods – Friedewald calculation uses triglycerides, HDL-C and total cholesterol
- Clinical trials and IRB restrictions on specimen amount – Led to development of alternatives
- Use of 0.2 mL of serum and a shorter time for the ultracentrifugation step: 3 hours 54 minutes
- Global correlation – Correlation studies with other laboratories included Shanghai PRC and Geneva, Switzerland; acceptance criteria for commutability of data needed in clinical trials

Materials and Methods
- Reagents
  - Roche Diagnostics – Total Cholesterol and Direct LDL-C
  - Roche Diagnostics – Modular Analytics and cobas c702
  - Beckman Coulter – Optima XPN-80 Ultracentrifuge
  - Beckman Coulter – Titanium Type 42.2 Rotor
  - Beckman Coulter – CentriTube Slicer; Cellulose propionate centrifuge tubes
- Methods
  - LDL-C. Roche Diagnostics method Direct LDL-C
  - LDL-C. Existing ultracentrifugation method (20 hrs)
  - LDL-C. Improved ultracentrifugation method (4 hrs)
  - LDL-C. Calculated by Friedewald method
  - EP Evaluator. Used for data reduction and correlations

Description
- Serum (200 μL): Pipette specimen into cellulose propionate centrifuge tube; centrifuge in the ultracentrifuge for 3 hours and 54 minutes
- Slice the centrifuge tubes; remove the top part (chylomicron)
- Transfer the bottom fraction to a testing cup with a Pasteur pipette.
- Add 80 μL of 0.9% saline; rinse within the same cut cellulose propionate tube, to QS
- To ensure complete transfer, rinse centrifuge tube several times, and transfer saline to micropip. Homogenize the sample using the glass transfer pipette; add saline to bring the volume back to 200 μL
- Confirm: (T. Cholesterol – LDL Cholesterol) / Triglycerides ≤ 0.687
- Correlation: Compare results from other labs and use EP Evaluator for data reduction
- Total cholesterol – Measured on Roche Modular Analytics or cobas c702
- LDL-Cholesterol – Direct method measured on the Roche Modular or cobas c702
- LDL-Cholesterol – By Friedewald calculation using Triglyceride (Glycerol Blank) (TG) method and HDL-Cholesterol (dextran sulfate precipitation):
  - LDL-C (mg/dL) = Total Cholesterol – TG5 – HDL-C
- Triglycerides (GB) – Measured on the Roche Modular Analytics or cobas c702
- HDL-C – Total cholesterol measured on the Roche Modular Analytics or cobas c702 after precipitation with dextran (Dextralip 50) sulfate – Magnesium chloride (MgCl2)

Results
- Allowable Total Error (ATE)
  - ATE = 10.5%
- Precision – Two levels of control material
  - Intra-assay imprecision (CV): Less than 2.6% (25% of ATE)
  - Inter-assay imprecision (CV): Less than 3.5% (33% of ATE)
- Accuracy – Three measures:
  - Current method and new method (N=35)
    - Slope = 1.031; Y intercept = 0.089 (includes 0.00)
    - SEE = 0.206; Correlation coefficient (R) = 0.99
  - Friedewald calculation and new method (N=10); acceptable recovery
    - Direct LDL-C method and new method (N=110); step acceptable recovery
  - Global Correlation
    - Split samples: Geneva and Shanghai
    - Correlation met commutability criteria

Conclusion
- Method enhancements for the measurement of LDL-cholesterol by ultracentrifugation demonstrated excellent analytical performance.
- Use of the new method provided significant value to the pharmaceutical clients and laboratory operations; the number of QNS test cancellations was reduced from 4.3% to 0.2%.
- Turnaround time for results was improved; the time required for processing was reduced by 48% leading to a significant savings in time and labor expense.

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