Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Lipids/Lipoproteins

B-131

Development of new HDL-Cholesterol Generation 4 assay on Roche Clinical Chemistry Analyzers

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Medical background

Monitoring of HDLcholesterol (HDL-C) in patients is of clinical relevance as the HDL-C concentration is important in the assessment of atherosclerotic risk. Elevated HDLC concentrations protect against coronary heart disease (CHD), whereas reduced HDL-C concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.

Test principle

NonHDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a watersoluble complex. In this complex the enzymatic reaction of cholesterol oxidase (CHER) and cholesterol oxidase (CHOD) towards nonHDL lipoproteins is blocked. The concentration of HDLcholesterol is determined enzymatically by CHER and CHOD.

Development Goals HDL-Cholesterol Gen.4:

- Improved specificity for HDL-C in human samples due to reduced sensitivity towards denatured lipoprotein
- Extended measuring range of 0.083.88 mmol/L (3.09150 mg/dL)
- Less sensitivity to denatured lipoproteins and less matrix effects
- Increased reactivity towards apo E enriched HDL(Important for people with reduced CETP (cholesterol ester transport protein) activity)
- Reduced high recovery in samples from patients with liver disease and lipid disorder
- Improved stability over shelf life

Results

Measuring range and lower limits of measurement

The linear assay range of the HDLC4 assay is from 0.083.88 mmol/L (3.09150 mg/dL). The LoB, LoQ and LoD are 0.08 mmol/L (3.09 mg/dL).

Traceability

HDLC4 assay has been standardized against the ultracentrifugation method according to CDC (center of disease control) manufacture's protocol.

Limitations and interferences

No interference of bilirubin (conjugated and non-conjugated) up to 1026 µmol/L (60 mg/dL), I-Index of 60, no interference of lipaemia (Intralipid) up to a L-Index of 2000, no significant interference from native triglycerides up to 13.7 mmol/L (1200 mg/dL), no interference of hemoglobin up to 745 µmol/L (1200 mg/dL), H-Index of 1200. Statins (Simvastatin) and fibrates (Bezafibrate) tested at therapeutic concentration ranges do not interfere. Ascorbic acid up to 2.84 mmol/L (50 mg/dL) does not interfere.

Precision - CLSI EP5 - 21 days

Repeatability ≤ 1.8% and Intermediate precision ≤ 2.2% (concentration range 0.25 mmol/L (9.67 mg/dL) to 3.66 (141 mg/dL).

Method comparison study:

Human serum and plasma samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche **cobas c** 501 analyzer (x) (n = 59). Passing/Bablok regression: y = 1.006x + 0.032 mmol/L. The sample concentrations were between 0.11 and 3.69 mmol/L (4.25 and 143 mg/dL).

Conclusions

All development goals for the new HDLC4 assay were met. The use of HDLC4 method in laboratory routine will improve quality of test results for HDL-C.

B-135

Serum Bone Morphogenic Protein 4 levels are conversely correlated with high sensitive- C Reactive Protein in Obese Men

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Background: The prevalence of obesity is increasing in many parts of the world, including Indonesia. The reframing of obesity as an inflammatory condition has had a wide impact on the conceptualization of obesity-associated disease. BMP4 is a growth factor of the transforming growth factor-β superfamily. Initially, BMPs were identified as inducer of ectopic bone formation. Recent in vitro study on cultured human adipocytes have highlighted the important roles of BMP4 in the regulation of adipogenesis as an anti-inflammatory. However the correlation between is serum BMP4 and inflammation in adult obese is unknown yet. The objective of this study was to evaluate the correlation between common inflammation marker, serum hs-CRP and BMP4 in obese men.

Methods: A total of 80 obese adult male subjects were included in the present study, out of which 33 were non metabolic syndrome (nonMetS) and the remaining 47 were metabolic syndrome, age range from 31 to 60 years old. Serum BMP4 and TNFα concentrations were quantified by ELISA principle. Serum hsCRP were quantified by immulite2000 (DPC cat L2KCRP-2). All assays were performed according to the manufacture instruction. Statistical analysis was performed with SPSS for windows ver 20. Significance value were define as alpha level< 0.05 based on two-tailed tests.

Results: The mean levels of serum BMP4 in obese MetS is lower (470 pg/ml) as compared to obese nonMetS group (613 pg/ml). However, inverse results were seen for mean serum hsCRP levels which were higher in obese MetS group (2.69 mg/l) compared to obese nonMetS group (1.89 mg/l). Bivariate analysis (n=80) revealed that serum BMP4 was inversely correlated with hsCRP, TNF-α, triglyceride, blood fasting glucose. A significant inverse correlation were found between BMP4 and hsCRP (r=-0.277; p=0.013).

Conclusion: In conclusion, this study provides evidence that in obese men serum BMP4 inversely correlated with hsCRP. This finding demonstrate the importance of BMP4 as an anti inflammatory protective factor of obesity-related diseases. Further study is needed to validate that BMP4 may serve as a therapeutic target for intervention in the control of metabolic disease.

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Evaluation of Free Fatty Acid Assay adapted on the Abbott Architect c8000 analyser

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Background:

Fatty acids provide a source of energy alternative to glucose for some organs such as skeletal muscle. In liver, fatty acids can be converted to ketones, which in turn provide energy for the brain during starvation or when blood glucose levels are low. In blood, most fatty acids are esterified with either cholesterol or glycerol, and transported by lipoprotein particles, whereas non-esterified (free) fatty acids are bound to proteins in plasma.

In this study, we aimed to study the performance of the Randox non-esterified fatty acid, also known as free fatty acid (FFA) on the Abbott Architect c8000 analyser.

Methods:

We evaluated inter-day imprecision, linearity and limit of quantitation (LOQ) of the FFA assay adapted to the Abbott Architect c8000 analyser and its performance in two external quality assessment (EQA) schemes. Potential interference of the assay by hemolysis and bilirubin was also investigated using serum specimens spiked with known concentrations of hemoglobin or unconjugated bilirubin.

Results:

Inter-day imprecision, CV (%), over 10 days was 4.3% at 0.52 mmol/L and 2.5% at 1.59 mmol/L. The FFA assay demonstrated linearity for measurement between 0.10 and 2.97 mmol/L. Limit of quantitation (LOQ) was found to be 0.16 mmol/L with CV = 2.6%.

EQA results were comparable with peers participating in the Randox International Quality Assessment Scheme (RIQAS) and European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM).

Hemoglobin concentration at 300 mg/dL and 600 mg/dL increased FFA results by 11.7% and 23.9%, respectively, at FFA concentration of 0.51 mmol/L (p<0.001).

Bilirubin concentration at 100 umol/L, 200 umol/L and 400 umol/L decreased FFA results by 12.6%, 21.7% and 29.1%, respectively, at FFA concentration of 0.64 mmol/L (p<0.001).

Conclusion:

We have evaluated the performance (imprecision, linearity and LOQ) of the Randox free fatty acid assay on the Abbott Architect c8000 analyzer. Assay results were found to be satisfactory when compared to all users' means in both RIQAS and ERNDIM external quality assessment programs. Hemoglobin at 300 mg/dL and above resulted in statistically higher FFA results compared with controls, whereas bilirubin at 100 umol/L and above resulted in statistically lower FFA results.

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An Improved Method for LDL-Cholesterol Beta-Quantification for Clinical Drug Trials

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Background: Low density lipoproteins (LDL) are the major cholesterolcarrying lipoproteins in plasma. LDL is primarily cleared from the bloodstream via hepatic LDL receptors, and to a lesser extent, by extra-hepatic 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 7x20 mm 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 met acceptance criteria and proved commutability of the method.

Conclusion: 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 test cancellation because of sample quantity not sufficient for testing. 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.

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Comparison between cholesterol or low-density lipoprotein concentrations obtained by the direct method and those estimated through application of the Martin and Friedewald equations, in a Brazilian population sample: Hypertriglyceridemia limits the accuracy of the Martin equation.

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Background:

In 2013, Martin et al proposed a new equation for estimating cholesterol or low-density lipoprotein levels (LDL-C), [LDL-C(M)], which, by having an adjustable factor in its formula, would be more accurate than the more traditional Friedewald equation, [LDL-C(F)]. In December of 2016, the Brazilian Consensus for the Normalization of Laboratory Determination of Lipid Profiles was published, and this document recommended using LDL-C(M), regardless of the triglyceride (TG) levels. The objective of the current study was to evaluate the correlation and concordance between LDL-C(M) or LDL-C(F) and the direct dosage determination of LDL-C [LDL-C(D)], and to determine the triglyceride concentrations that may limit the accuracy of the LDL-C(M) values.

Methods:

LDL-C(D), TG, total cholesterol and HDL-C concentrations were determined for 680 serum samples, using the Advia 2400 analyser; LDL-C(M) and LDL-C(F) values were calculated by applying the respective equations of Martin or Friedewald. The clinical concordance was evaluated by using the total minimum error based on the biological variation components (17.84%). Statistical analyses were performed using the Pearson correlation, the Cohen?s Kappa test, the Lin Concordance Correlation Coefficient, ROC curve analysis and the Bland-Altman graph.

Results:

The results are shown in Table. For TG values <400mg/dL, the concordance between LDL-C(M) and LDL-C(D) was 97.4% (p-0.238), and between LDL-C(F) and LDL-C(D) was 84,7% (p<0.0001). For TG values ≥400mg/dL, the concordance between LDL-C(M) and LDL-C(D) was 68.3% (p<0.0001), and between LDL-C(F) and LDL-C(D) was 43.3% (p<0.0001), considering a total maximum error of 17.84%. Analysis of the ROC curve showed that a TG concentration of 432 mg/dL limited the accuracy of the LDL-C(M) results.

Conclusions:

For the LDL-C calculation, the Martin equation is more accurate than that of Friedewald, despite presenting limitations with regard to samples with TG values greater than or equal to 432 mg/dL.

Concordance and correlation by Friedewald vs Martin Estimates of Low-Density Lipoprotein Cholesterol

	TG < 400 mg/dL (n=620)				TG ≥ 400 mg/dL (n=60)			
	LDL-C(F)		LDL-C(M)		LDL-C(F)		LDL-C(M)	
	Value	95% CI	Value	95% CI	Value	95% CI	Value	95% CI
Percent Agreement based on Total Error	84.7	81.8 to 87.5	97.4	96.2 to 98.7	43.33	30.79 - 55.87	68.3	56.56 - 80.1
Pearson's correlation coefficient	0.95	0.92 to 0.97	0.98	0.97 to 0.98	0.95	0.92 - 0.97	0.93	0.89 - 0.96
Lin's Concordance Correlation Coefficient (Interpretation)	0.94 (Mod-erate)	0.92 to 0.94	0.92 to 0.94	0.97 to 0.979	0.78 (Poor)	0.70 - 0.84	0.81 (Poor)	0.74 - 0.87
Cohen's kappa coefficient (Interpretation)	0.65 (Good)	0.61 to 0.70	0.85 (Very good)	0.82 to 0.88	0.26 (Fair)	0,13 - 0,41	0.53 (Mod-erate)	0.38 - 0.69
Mean difference, mg/dL	-5.87	-6,74 to -5,00	-0.35	-0,94 to 0,23	-19.8	25,3 to -14,3	16.5	11,9 to 21,1

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Multicenter Evaluation of new HDL-Cholesterol Generation 4 assay (HDLC4) on Roche Clinical Chemistry Analyzers

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Background:

Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. A new formulation of the Roche Diagnostics HDL-Cholesterol Gen.4 reagent (HDLC4) was developed to increase specificity for HDL-C and to widen the measuring range up to 3.88 mmol/L (150 mg/dL). The analytical performance of the assay was tested in three laboratories.

Assay principle:

Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a watersoluble complex. In this complex the enzymatic reaction of cholesterol estrase (CHER) and cholesterol oxidase (CHOD) towards nonHDL lipoproteins is blocked. The concentration of HDLcholesterol is determined enzymatically by CHER and CHOD.

Study Design:

The analytical performance of the new HDLC4 assay was evaluated in three independent laboratories using **cobas c** 702, **cobas c** 502 and **cobas c** 501 instruments. Recovery of Roche PreciControl ClinChem Multi 1 and 2, recovery of Ring Trial samples from RfB and Instand e.V. and controls from Bio-Rad, within-run precision of human samples pools, precision according to CLSI EP5-A3, method comparisons HDLC Gen.3 vs. HDLC Gen.4, instrument-to-instrument (**cobas c** 701 vs **cobas c** 502), method comparison to HDL-C assays from competitors.

Methods and Results:

Repeatability and intermediate precision were measured in the concentration range from 0.66 mmol/L (25.5 mg/dL) to 2.55 mmol/L (98.6 mg/dL) according to the CLSI EP5-A3 protocol using two Roche controls and five human serum pools. For the repeatability the coefficients of variation (CVs) were determined to be less than 1.9 % and for intermediate precision yielded CVs ranging between 1.1 and 2.7 % (two runs/day, 21 days). The recovery of two controls (Roche Diagnostics) was determined in three independent runs measuring 3 aliquots. The recovery of HDL-C target values ranged from 97.4 to 105.6 %. Method comparison experiments were designed in compliance with CLSI EP09-A3, using ≥ 117 serum samples. Passing-Bablok regression analysis of Roche HDL-C methods resulted in slopes in a range of 0.97 to 0.86, intercepts of 0.02 mmol/L (0.77 mg/dL) to 0.13 mmol/L (5.02 mg/dL), and Pearson correlation factors from 0.977 to 0.998. The HDL- C concentration of samples ranged from 0.08 mmol/L (3.09 mg/dL) up to 3.05 mmol/L (117,91 mg/dL). The measured biases at the medical decision points were acceptable (≤ 5 %).

Conclusions:

The results of the multicenter evaluation study prove a good analytical performance of the new HDLC4 assay as well as an increased resistance to endogenous interferences and a increased specificity for HDL-C. The assay is well-suitable for routine use.

B-140

Specific Sandwich-type Enzyme-Linked Immunosorbent Assay (ELISA) for Oxidized High-Density Lipoprotein and Its Clinical Application

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Background: High-density lipoprotein (HDL) has anti-atherogenic functions such as cholesterol efflux. It is also well known that plasma HDL-C levels are negatively correlated with cardiovascular events. Consequently HDL has been considered as ‘’good’’ cholesterol. However, recent studies show that HDL from coronary artery disease patients have poor cholesterol efflux, and such HDL is defined as ‘’dysfunctional HDL’’. Oxidative stress is one of the factors which lead HDL to dysfunctional, so we consider oxidized HDL (Ox-HDL) is useful to assess the quality or functionality of HDL. The aim of this study is to develop a specific sandwich-type enzyme-linked immunosorbent assay (ELISA) for Ox-HDL and to analyze the serum Ox-HDL levels of both normolipidemic healthy subjects and dyslipidemic patients.

Methods: We have developed a sandwich ELISA for Ox-HDL with DLH3 antibody that can specifically recognize oxidized phospholipid. We assessed specificity of the assay using native HDL and LDL as well as Cu-oxidized HDL and LDL. Each lipoprotein was separated from human plasma by sodium bromide stepwise density gradient ultracentrifugation. Additionally we assessed the non-specific reaction of unoxidized phosphatidylcholine (PC) by inhibition assay. Next, we defined Ox-HDL obtained by forced-oxidation of 1 mg/L HDL phospholipids (HDL-PL) as 1 U/L Ox-HDL, and analyzed the sera of 118 healthy normolipidemic controls and 177 dyslipidemic outpatients, respectively.

Results: *Specificity analysis*-In this ELISA assay, Cu-oxidized HDL was specifically detected and neither Cu-oxidized LDL nor native LDL was detected. Native HDL was also recognized but the signal was low. Inhibition assay with various types of phospholipids revealed that lysophosphatidylcholine (lyso-PC) showed concentration-dependent inhibition. On the other hand, no inhibition by unoxidized PC was observed even in the condition of excess concentration. These results strongly suggested that oxidized phospholipid in HDL is specifically recognized in this ELISA assay. *Clinical evaluation*-The serum Ox-HDL levels in healthy controls were 28.3 ± 4.9 U/L (mean ± SD) and in dyslipidemic patients treated with drugs were 27.7 ± 9.3 U/L. Ox-HDL levels were moderately correlated with HDL-PL levels (r=0.59), therefore we also evaluated Ox-HDL/HDL-PL ratio, which represents oxidation degree of phospholipids in the HDL fraction . Patients treated with probucol, which is a potently anti-oxidative and anti-hyperlipidemic drug, showed significantly lower Ox-HDL level (18.7 ± 6.6 U/L vs 30.0 ± 8.1 U/L, p<0.001) and Ox-HDL/HDL-PL ratio (0.206 ± 0.045 vs 0.224 ± 0.049, p=0.043) than those without probucol, suggesting that probucol may prevent oxidation of HDL. These probucol effects were also seen in Familial Hypercholesterolemia patients (Ox-HDL: 16.1 ± 5.8 U/L vs 30.2 ± 5.4 U/L, p<0.001; Ox-HDL/HDL-PL: 0.205 ± 0.037 vs 0.238 ± 0.043, p=0.015, respectively).

Conclusion: Our sandwich ELISA is an assay with a good specificity to Ox-HDL. In addition, clinical evaluation using our assay showed that probucol may prevent the formation of these Ox-HDLs.

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ApoE-rich HDL in pre-heparin plasma may be HDL remnants which remain in the circulation

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Background: High-density lipoprotein (HDL) containing apolipoprotein E (apoE-rich HDL) represents only a small portion of plasma HDL. Hepatic triglyceride lipase (HTGL) is well known to play a major role in HDL metabolism, and promotes the selective uptake of cholesteryl esters from HDL via the scavenger receptor B1. We previously reported that HTGL may be associated with apoE-rich HDL in post-heparin plasma, but it has not been clear whether this observation is found in pre-heparin plasma. In this study, we propose the possibility of this lipoprotein fraction as HDL remnants based on the interaction with HTGL in pre-heparin plasma. **Methods:** A total HDL fraction was isolated from healthy serum samples (pre-heparin plasma) using 13% polyethylene glycol (PEG), and applied to a cation-exchange column (HiTrap SP HP, 1 mL, GE Healthcare) to obtain apoE-rich and apoE-poor HDL fractions. For the determination of HTGL distribution, the column effluent was collected in 0.4-mL fractions and analyzed by a newly developed HTGL-ELISA. Furthermore, the apoE-rich HDL fraction was applied into a gel-permeation column (Superose 6HR) to investigate the interaction between apoE-rich HDL and HTGL. **Results:** Approximately 90% of serum HTGL mass was recovered in the total HDL fraction (PEG supernatant), indicating apoB-containing lipoproteins were not associated with HTGL in pre-heparin plasma. Only 5% of total HDL-cholesterol was found in apoE-rich HDL fraction but 40% of HTGL in the total HDL was detected in apoE-rich HDL fraction. Therefore, the ratio of HTGL (ng/mL) to cholesterol (mg/dL) was much higher (approximately 7.0-fold) in apoE-rich HDL than apoE-poor HDL. Furthermore, the gel-permeation HPLC analysis revealed that HTGL and apoE-rich HDL were co-eluted at larger HDL position. These findings indicate that HTGL interacts preferably with apoE-rich HDL. **Conclusion:** This is the first report that

apoE-rich HDL prevalently carries HTGL in pre-heparin plasma. Although lipoprotein lipase was found in VLDL fraction as remnants, HTGL was found in apoE-rich HDL. Both fractions contain apoE-rich lipoproteins and carry the lipases as bound forms in pre-heparin plasma. Therefore, we propose that apoE-rich HDL is "HDL remnants" which were not rapidly cleared from the circulation.

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Comparability of commercial assays & commutability of evaluated materials for apolipoprotein A1 measurement

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Background: Harmonization & comparability of apolipoprotein A1 (apo A1) measurement results are crucial to assess cardiovascular risk. Non-commutable materials used as calibrators & external quality assessment (EQA) materials can result in misdiagnosis or provide misleading information leading to limited EQA schemes. Potential reference materials should therefore be selected & evaluated for commutability.

Methods: In the present study, we determined apo A1 levels in commercial control materials used in the 2013 EQA Program, human serum pools prepared from leftover samples (LHSPs) & fresh donations (FHSPs), & a set of 50 individual samples using nine commercially available assays. Original or logarithm-transformed results were pairwise-analyzed to estimate the slopes & intercepts by Deming regression. The 95 % prediction interval (PI) & the minimum bias from biological variability were used to analyze the commutability of these materials. Matrix-related biases were also estimated.

Results: All commercially available assays in the present study showed acceptable within-run precision, compared with the minimum specification for imprecision derived from biological variability (4.9 %), & almost all combinations correlated well (r>0.975). The slopes varied from 0.65 to 1.35, & the intercepts varied from -0.45 to 0.43. FHSPs were commutable in more combinations than LHSPs or EQA materials, & both 95 % PI & minimum bias (5.6 %) criteria for commutability yielded similar results for FHSPs. The matrix-related biases of EQA materials, LHSPs, & FHSPs varied from -42.28 % to 25.58 %, -7.29 % to 12.53 %, & -8.38 % to 11.22 %, respectively.

Conclusion: Human serum pools prepared from fresh donations are thus a more appropriate source of reference materials. The metrological traceability of calibrators is therefore essential for the harmonization & comparability of apo A1 measurement procedures.

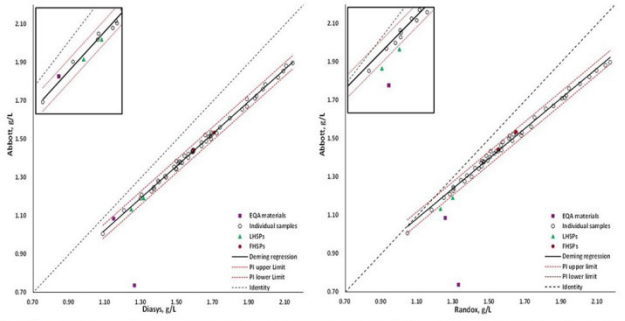

Fig. Examples for commutability assessment of apo A1 measurements in EQA materials and HSPs. The Deming fit with 95 % PI (red dotted lines) were calculated from individual sample results. Evaluated materials within 95% PI were considered commutable, otherwise they were non-commutable. The black dotted lines were identity lines (y = x).

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The laboratory's contribution in the clinical assessment of cardiovascular risk: evaluation of Imprecision and Reference Change Value of lipid profile.

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Background: Variability in measurements of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) has important repercussions on clinical decision-making. To test the impact of analytic variability (Va) of lipid measurements on the cardiovascular risk estimation we evaluated our Va close to decision limits suggested by ACC/AHA 2013 and ESC/EAS 2016 guidelines. Since the state of art suggests the use of Reference Change Value (RCV) to evaluate significant changes in serial lipids results we also define our RCV. **Methods:** Analytical imprecision of lipid tests was evaluated throughout intra-(CVr) and inter-assay (CVd) performed in 10 days with Abbott Architect c8000. Samples: Bio-Rad Quality Control third-part (QC_1, QC_2) and 1 sample with values close to 200, 240, 290 mg/dL TC (S_1-S_2-S_3); 115, 150 mg/dL LDL-C (S_1-S_2); 150, 180 mg/dL TG (S_1-S_2); 45 mg/dL HDL-C (S_1). We further tested 10 patient samples (S_{Var}) close to decision limits (200 mg/dL TC; 115 mg/dL LDL-C; 150 mg/dL TG; 45 mg/dL HDL-C). To define RCV we used biological variation from Ricos's database, we monthly calculated our analytical imprecision (CV_A) performing daily measurements of QC_1 and QC_2 during two years by Unity Real Time software, Bio-Rad Laboratories. Then we compared CV_A with analytical goals (AG) for imprecision derived from biological variation and calculated by Fraser's formulas. **Results:** Table below shows all results expressed as Coefficient of Variation (CV%). RCVs of TC, LDL-C, HDL-C and TG were 17.3, 22.5, 22.1, and 55.5 % respectively (Z = 1.96 for bidirectional changes; p< 0.05). **Conclusion:** The accordance of CVr and CVd versus CV_A obtained from QC highlight good analytical performances at different concentrations close to decision limits. The definition of CVa and RCV is a useful tool used by our cardiologists to assess cardiovascular risk and response to lipid-lowering therapy of patients.

		QC_1 CV%	QC_2 CV%	S_1 CV%	S_2 CV%	S_3 CV%	S_{Var} CV%	Average CV_A%	AG Imprecision CV%
TC	CVr	0.76	0.47	0.51	0.37	0.68	<0.54	1.8	Desirable 3.0
TC	CVd	1.51	1.43	0.86	0.88	1.32	-	1.8	Desirable 3.0
LDL-C	CVr	0.79	0.64	0.50	0.68	-	<0.91	2.3	Desirable 3.9
LDL-C	CVd	2.24	2.34	1.52	1.55	-	-	2.3	Desirable 3.9
HDL-C	CVr	1.76	0.89	0.77	-	-	<0.71	3.2	Desirable 3.6
HDL-C	CVd	1.35	1.69	1.26	-	-	-	3.2	Desirable 3.6
TG	CVr	0.73	0.36	0.37	0.57	-	<0.58	2.4	Optimal 5.0
TG	CVd	1.75	1.88	1.71	1.74	-	-	2.4	Optimal 5.0

B-146

Establishment of an automated assay for cholesterol uptake capacity, a new concept of high-density lipoprotein functionality

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Background: Recent studies have shown that the capacity of high density lipoprotein (HDL) to stimulate cholesterol efflux from lipid-laden macrophages is a better predictor of cardiovascular disease status than overall HDL cholesterol concentration. Recently, we established a cell-free plate assay system to evaluate cholesterol uptake capacity (CUC) of HDL using a fluorescence-labeled cholesterol and an apolipoprotein A1 (apoA1) specific antibody, and demonstrated feasibility of CUC for potential substitution of cholesterol efflux capacity and coronary risk stratification. To apply this concept in the clinical settings, this study aimed to establish a high-throughput method capable of measuring CUC.

Methods: We had developed a fluorescence-based manual plate assay as a pilot method for measuring CUC, which was presented at the AACC 2016 Annual Meeting. In this study, we converted it into automated magnetic bead-based chemi-luminescence detection system. Briefly, HDL was incubated in reaction buffer containing dinitrophenyl (DNP)-labeled cholesterol, followed by its capture to an anti-apoA1 antibody coated onto the beads. Using alkaline phosphatase labeled anti-

DNP antibody and specific substrate, chemi-luminescence signals were detected with our automated immunoassay system HISCL®. To investigate the throughput and analytical performance of our method, we quantified CUC of apoB-depleted serum samples and the recombinant apoA-1 as a standard.

Results: In this system, measurement of CUC was completed about 40 minutes per sample, notably rapid compared with that of conventional cholesterol efflux capacity assay, which requires 2-3 days. Each assay proceed sequentially every one minutes, so more than 10 samples could be analyzed within an hour. The assay system had high reproducibility (CV < 10%) and was linear from 5 to 200 ng/mL. The LoQ was validated at 5 ng/mL. CUC measured by the present automated method correlated well with that of our previous pilot method, which inversely associated with recurrence of coronary artery disease in patients with optimal control of low-density lipoprotein cholesterol.

Conclusion: Our novel automated method can measure CUC quickly and accurately. This method would expand the dysfunctional HDL research from the bench to the clinical arena.

B-147

ELISA system for GPIHBP1 levels in human plasma

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Background: GPIHBP1, a glycosylphosphatidylinositol (GPI)-anchored protein of capillary endothelial cells, is crucial for the lipolytic processing of triglyceride-rich lipoproteins (TRLs). GPIHBP1 binds lipoprotein lipase (LPL) in the interstitial spaces and shuttles the enzyme to its site of action in the capillary lumen. GPIHBP1 is also required for the margination of TRLs along capillaries—so that lipolytic processing can proceed. A deficiency of GPIHBP1 in humans causes severe hypertriglyceridemia (chylomicronemia). Even though GPI-anchored proteins are tethered to the plasma membrane, it has been possible to detect some GPI-anchored proteins (*e.g.*, uPAR) in the plasma. We hypothesized that it might be possible to detect GPIHBP1 in human plasma. To test this hypothesis, we created a sensitive sandwich immunoassay for human GPIHBP1 with two newly created human GPIHBP1-specific monoclonal antibodies.

Methods: We created a solid-phase sandwich ELISA for human GPIHBP1. The wells of 96-well plates were coated with Mab-CH79A4. After first incubating plasma samples at 37°C for 60 min and then washing the plates, the GPIHBP1 captured was detected with horseradish peroxidase-labeled Mab-HE20A6. Recombinant GPIHBP1 was used as a calibration standard.

Results: The ELISA detected GPIHBP1 in human plasma. The median concentration of GPIHBP1 in plasma samples from healthy subjects was range, 570-1523 pg/ml; the detection limit was 10 pg/ml. When plasma samples were "spiked" with recombinant GPIHBP1, the recovery of the spiked GPIHBP1 was excellent (ranging from 85 to 115% of the amount added). The assay was linear over a >4-fold dilution of plasma. The intra- and interassay coefficients of variation were <15%. The plasma levels of GPIHBP1 were not different in pre- and post-heparin plasma samples.

Conclusion: We developed a solid-phase ELISA for GPIHBP1 in plasma and used the assay to measure levels of GPIHBP1 in human plasma. In future studies, we will determine whether plasma levels of GPIHBP1 correlate with plasma lipid levels and whether plasma GPIHBP1 levels are a useful biomarker for metabolic or cardiovascular disease.

B-148

A new enzyme-linked immunosorbent assay system for human serum hepatic triglyceride lipase

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Background: There is no previously established method for measuring human hepatic triglyceride lipase (HTGL) concentration in plasma obtained without heparin injection.

Methods: We developed new monoclonal antibodies (MoAb mouse 9A1, MoAb rat 141A1) that react with both HTGL in serum and in post-heparin plasma (PHP) and established a novel enzyme-linked immunosorbent assay (ELISA) system that was able to measure both serum HTGL concentration and PHP-HTGL concentration.

Results: To confirm the specificity of antibodies, we performed an immunoprecipitation -immunoblotting analysis. Both MoAb mouse 9A1 and MoAb rat 141 A1 were able to immune-precipitate not only recombinant HTGL and PHP-HTGL but also serum HTGL, demonstrating that HTGL exists in plasma obtained without heparin injection. This method yielded a coefficient of variation of less than 6 % in intra- and inter-assays and did not cross-react with lipoprotein lipase (LPL) or endothelial lipase (EL). In clinical analysis on 42 male subjects with coronary artery disease, there were strongly positive correlations of serum HTGL concentration to PHP-HTGL concentration ($r=0.727$, $p<0.01$). Serum HTGL concentrations had positive correlations to serum triglycerides ($r=0.314$, $p<0.05$) and ALT ($r=0.406$, $p<0.01$) and tendency toward positive correlations to LDL-C , small, dense LDL and γGTP.

Conclusion: These results indicate that this new ELISA method for measuring pre-HTGL is applicable in daily clinical practice.

B-149

Homogenous LDL Cholesterol assays - How does the Roche Generation 3 LDL-Cholesterol measure up?

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Background:

Low Density Lipoprotein Cholesterol (LDL-C) is a an important risk assessment biomarker for cardiovascular disease and a primary target of cholesterol lowering therapy, hence accurate measurement is pertinent in routine clinical practice. Homogenous assays for direct measurement of LDL-C are gaining recognition due to its capability of being adapted to automated analysers and its' merits in improved assay precision. However not all homogenous assays perform alike, due to differences in the ability of the assays to reliably detect LDL-C particularly in more complex specimen matrix. Our laboratory currently offers, in addition to the more routinely requested traditional Fridewald calculated LDL-C, testing for direct LDL-C measurements using the Wako LDL-C assay on the Beckman Coulter AU 5800 automated analyser.Lack of appropriate peers and sub-optimal performance in external quality assurance (EQA) programs for our current LDL-C assay have propelled our laboratory to proactively explore alternative assays as part of continuous improvement initiatives.Roche Diagnostics has recently launched a new improved formulated LDL-C Generation 3 (Gen 3) assay, which claims to offer improved specificity for LDL-C and better comparison with the beta-quantification reference method.Our study evaluated the analytical performance of the Roche LDL-C Gen 3 assay on the Roche Cobas c502 and compared it against our current existing LDL-C assay on the Beckman Coulter AU5800.

Methods:

Performance validation parameters of the Roche LDL-C Gen 3 assay included assay imprecision (within and total), lower limit of detection, linearity and carry-over. Imprecision was assessed using manufacturer's quality control materials measured in triplicates over 5 days. Limit of detection, linearity and carry-over studies were performed using appropriate low and high concentration patient samples.Method correlation studies using fresh patient serum samples (n=120) were performed on the AU5800 and Cobas c502 platforms. EQA performance of the Roche LDL-C Gen 3 assay was also assessed using leftover samples from accuracy-based surveys.

Results:

Within run and total imprecision were determined to be ≤1.1% on Roche c502. Roche LDL Gen 3 assay demonstrated linearity across the analytical measurement range of 0.1 - 14.2 mmol/L, with recoveries between 97-100%.The lower limit of detectable concentration was assessed to be in agreement with the manufacturer's claims.Results of carry-over studies were insignificant. Method correlation with Beckman AU revealed Passing Bablok regression slope of 1.16 and intercept of -0.7; mean absolute bias (Altman Bland) of -0.18 mmol/L (95% CI: -0.22 to -0.13) and Spearman's correlation coefficient of 0.98. Indirect assessment of Roche's LDL Gen 3 EQA performance using post-survey samples from accuracy-based surveys showed better agreement with reference method beta-quantification.

Conclusions:

Overall, the Roche LDL-C Gen 3 assay performed within manufacturer's specifications and showed good correlation with beta-quantification.A small but insignificant negative bias is detected in the Roche assay when compared with Beckman AU assay

(Wako). Our study demonstrated that the Roche Gen 3 LDL-C fulfills the accuracy and imprecision assay goals in the National Cholesterol Education Program (NCEP) requirements and represents a good alternative choice of direct DL-C testing for clinical laboratories.

B-150

Small Dense LDL Cholesterol as Strong Predictors for Secondary Cardiovascular Events Compared with LDL Cholesterol in Elder Patients.

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Background: Small dense (sd) LDL particles have been suggested to be highly atherogenic due to their higher penetration into arterial walls, their lower binding affinity for LDL receptors, their prolonged plasma half-life, and their lower resistance to oxidative stress compared to large buoyant (lb) LDL. Though several methods have been developed for the measurement of serum sd LDL levels, the methods are laborious and time-consuming We have developed a simple fully-automated direct homogeneous measurement method of sd LDL-C . With our assay kit, it has been revealed that the rise of sd LDL-C is a risk factor for coronary heart disease (CHD) independent from LDL-C. However, the significance as a risk factor in the secondary prevention of CHD has been unexplained yet. Method: Subjects were 356 male and 73 female patients aged of 65 years and older with stable coronary artery disease (CAD) who did or did not develop major cardiovascular events (MACEs) during 5-year follow-up period. MACEs were defined as all cause death, onset of acute coronary syndrome, need for coronary and peripheral arterial revascularization, hospitalization for heart failure, surgical procedure for any cardiovascular diseases, and/or requiring hospitalization for stroke. Besides sd LDL-C with our assay kit, the following biomarkers were measured or calculated, and then analyzed: LDL-C, lb LDL-C, non-HDL-C, RLP-C, apoA1, apoB, glucose, HbA1c, BNP, and eGFR.Result: First-time MACEs were observed in 141 patients. Male patients with MACEs had significantly higher levels of LDL-C, lb LDL-C, sd LDL-C, non HDL-C, apoB, RLP-C, fasting plasma glucose, HbA1c, BNP, and high-sensitive CRP, and significantly lower levels of HDL-C, apoA1, and eGFR were observed in the patients. Multivariate Cox regression analysis results showed that all of biomarkers except lb LDL-C and eGFR were significantly associated with MACEs. Among lipid biomarkers, only sd LDL-C had a significant association with MACEs in male patients treated with statins (Hazard ratio (HR) 1.022, 95% confidence interval (CI) 1.003-1.042) and diabetic male patients (HR 1.019, 95% CI 1.001-1.036). Kaplan-Meier MACE-free survival curve analysis was conducted for 2 groups dichotomized by sd LDL-C 25 mg/dL, which is the median of whole subject. Significantly decreased MACE-free Survival ratio was observed in high sd LDL-C group.Conclusion: These results confirm that sd LDL-C is the most important residual risk to predict future MACEs in stable CAD patients including patients treated with statins.

B-151

Stability of fatty acids in serum stored at -20ºC for up to 5 years

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In a pilot study, we saw up to 45% degradation of polyunsaturated fatty acids (PUFA) in plasma stored for 6 months at -20°C. It is known that PUFA are highly sensitive to oxidative degradation. This study evaluates the stability of a panel of fatty acids in serum stored with and without butylated hydroxytoluene (BHT) at -20°C ≤ 5 years. The panel includes 11 saturated (SFA), 6 monounsaturated (MUFA), and 13 PUFA fatty acids. A modification of Lagerstedt *et al.* (2001) was used to quantitate unfractionated serum fatty acids. Briefly, total fatty acids were hydrolyzed, hexane-extracted, derivatized to pentafluorobenzyl bromide esters, and detected using electron capture negative-ion gas chromatography-mass spectrometry.

Serum pools were prepared (±)BHT (3.33 g/L methanol). The serum pools (±) BHT were stored at -70°C or -20°C for up to 5 years. All vials were kept frozen and analyzed at designated times, (3 months, 1.5 years, and 5 years). To assess assay performance, serum quality control pools (+)BHT were used. Serum was measured in duplicate at each time point except one storage condition, serum (-)BHT stored at -70°C for 5 years was measured in singlicate due to availability.

For each time period and fatty acid in the panel, concentrations were log transformed and a separate two-way analysis of variance was performed using pool and storage condition as the main effects. The reference condition was storage at -70°C (+)BHT for the specified length of time (3 months, 1.5 years, or 5 years). Relative percent changes for storage at -20°C (±)BHT compared to the reference condition for a given time period were calculated from a two-way ANOVA model with no interaction. However, interactions between the storage conditions and pools were noted if the pool-by-storage-condition interaction was both statistically significant and a > 5% difference existed between any of the pools.

The average percent difference for SFA in serum (±)BHT stored at -20°C ranged from -2% to 0.2% at 3 months and 1.5 years ($p \leq 0.05$); MUFA were ≤ 4% different at 3 months ($p \leq 0.05$). PUFA with ≥ 3 double bonds decreased from 0.4% to 9% (+)BHT and from 16% to 36% (-)BHT in serum stored at -20°C for up to 5 years ($p \leq 0.05$). Eicosapentaenoic acid and docosahexaenoic acid were particularly unstable in the absence of BHT, losing 34% and 35%, respectively, after 5 years at -20°C ($p \leq 0.05$). Serum fatty acids stored for 5 years at -70°C (±)BHT were on average < 1% different across all classes compared to the reference condition.

In conclusion, SFA and MUFA in unfractionated serum are relatively stable when stored at -20°C (±)BHT. However, PUFA degrade when stored without BHT at -20°C for longer than 3 months; generally, greater numbers of double bonds were associated with lower stability. To prevent substantial PUFA degradation, BHT should be added to the serum prior to storage at -20°C or preferably serum should be stored at ≤-70°C.

B-152

Non-HDL cholesterol secundary therapeutic goals based on LDL cholesterol population percentiles: Contribution of Longitudinal Study of Adult Health (ELSA-Brasil)

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Background: Current international guidelines consider non–high-density lipoprotein cholesterol (non-HDL-C) as a goal for the prevention and treatment of atherosclerotic cardiovascular disease. These guidelines established non-HDL-C targets 30 mg/dL higher than the respective low-density lipoprotein cholesterol (LDL-C) cut-off points, considering the very-low-density lipoprotein (VLDL-C), estimated by the Friedewald equation, when the triglyceride concentration is 150 mg/dL. These definitions do not consider the population distribution of the non-HDL-C values, and recent studies in different populations have shown that they were lower than the guidelines cut-off points. The aim of this study is to establish cut-off points for non-HDL cholesterol (non-HDL-C) at the same population percentiles of LDL-C. The frequency of reclassification of therapeutic category using current guideline-based non-HDL-C and population percentiles-based cut-off points were also assessed.

Methods: A total of 14,837 participants from the Longitudinal Study of Adult Health (ELSA-Brasil), aged from 35 to 74 years old and with triglycerides levels < 400 mg/dL were included. Total cholesterol, HDL-C, and triglycerides were measured in the ADVIA Chemistry system after 12 hours of fasting and LDL-C was calculated with the Friedewald equation. Initially, population percentiles were established for the LDL-C values corresponding to the therapeutic goals, and non-HDL-C values equivalent to the respective LDL-C percentiles were assigned. Next, we assessed the reclassification of therapeutic category based on non-HDL-C, defined as the frequency of subjects with LDL-C levels in the recommended treatment category, but non-HDL-C levels higher than the recommended treatment goal.

Results: The LDL-C values of 70, 100, 130 and 160 mg/dL corresponded to the population percentiles 3, 18, 52 and 82, respectively. The non-HDL-C cut-off points for the same percentiles were 92, 122, 156 and 191 mg/dL. When using the latter non–HDL-C cut-off points, 2305 (15.5%) participants were reclassified upwards and 2410 (16.2%) downward. Using current guidelines-based cut-off points, 2068 (14.0%) participants were reclassified upwards and 3957 (26.7%) downward. Among participants with triglycerides between 150 and 199 mg/dL and with concurrently LDL-C level < 70 mg/dL, 26.3% were reclassified upward according to the guideline-based non-HDL-C cut-off point (≥ 100 mg/dL), and 65.8% according to the population percentile-based cut-off point (non-HDL-C ≥ 92 mg/dL). Of participants with LDL-C level between 70 and 99 mg/dL, 14.3% were reclassified upward according to the guideline-based cut-off point (non-HDL-C ≥ 130 mg/dL), and 48.9% according to the population percentile-based cut-off point (non-HDL-C ≥ 122 mg/dL).

Conclusion: In this large cohort of free living Brazilians, ours results indicates that the recommended target value for non-HDL-C should be reduced to match the same population percentile equivalent to LDL-C goals. This change resulted in a significant increase in the frequency of reclassification of patients to a higher therapeutic category, especially in the presence of low LDL-C level and hypertriglyceridemia.

B-153

RNASE L regulates the expression of fatty acid synthase in the mouse liver

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Background:

Interferons (IFNs) are cytokines that participate in the innate immunity against viruses and other microbial pathogens. In addition, they also display anti-tumor, anti-proliferative and immuno-regulatory activities. The function of IFNs is mediated through proteins encoded by IFN-stimulated genes (ISGs). Ribonuclease L (RNase L) is a type of ISGs. Studies have shown that RNase L contributes to anti-viral infection, apoptosis and anti-cell proliferation. RNase L knockout mice are significantly larger than wide type mice in terms of body weight, and have more oil droplets in the organ tissues such as liver and kidney, suggesting that RNase L may be involved in lipid metabolism.

Method and Results:

To determine if RNase L regulates the expression of any genes in the liver, liver tissue extracts from RNase L knockout and wild type mice were subjected to SDS-polyacrylamide electrophoresis and Coomassie blue staining. The differentially expressed protein bands between the two tissue extracts were excised and digested by trypsin, and subsequently analyzed by LC/MS. Interestingly, the results indicated that fatty acid synthase (FAS) was significantly higher expressed in the liver deficient RNase L. The results were further confirmed with Western Blot analysis by using a mouse monoclonal antibody against fatty acid synthase. Further investigation revealed that RNase L regulates its expression at the transcriptional level and is age and gender dependent. This observation is consistent with our previous report that the lipid level is relatively higher in the plasma of RNase L deficient mice.

Conclusion:

Our study reveals that FAS is higher expressed in the liver of RNase l deficient mice, as a result, an increased level of lipid in the plasma of the animals was observed. RNase L transcriptionally regulates the expression of FAS, which could be a potential target of hyperlipidemia and a prognostic marker obesity.

B-154

The Performance of Calculated and Directly-Measured Low Density Lipoprotein Cholesterol in a Pediatric Population

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Introduction: Accurate measurement of low-density lipoprotein cholesterol (LDL-C) is essential for the identification and monitoring of dyslipidemia. Recent studies indicate that the Friedewald equation is biased when LDL-C levels fall below 100 mg/dL and triglycerides are elevated. The objective of this study was to evaluate the use of a novel equation and a direct homogenous assay to correct the bias of the Friedewald equation in a pediatric population. As a secondary goal, the utility of these methods was assessed by determining the proportion of cases which fall into the biased range of the Friedewald equation at Texas Children's Hospital.

Study design: LDL-C concentrations were determined by the Friedewald equation, a direct homogenous assay, the novel equation[1], and the reference method in 127 pediatric samples with triglycerides between 150-399 mg/dL. Bias was measured by regression analysis at selected LDL-C cutoffs and after stratifying samples by triglyceride content. Statistical significance (p=.0001) was determined by the Wilcoxon Signed-Rank test at LDL-C cutoffs of ≤ 70, ≤ 100, and > 100 mg/dL. The concordance of each method, relative to the reference method, was calculated at LDL-C cut-points of <70, 70-99, and 100-129 mg/dL. Retrospective data analysis was carried out to determine the prevalence of low LDL-C (≤100 mg/dL) and elevated TG (150-399 mg/dL) over a 2.5-month period at Texas Children's Hospital in Houston, TX.

Results: The Friedewald equation significantly underestimated pediatric LDL-C concentrations below 100 mg/dL (-21.6%) and the direct-LDL assay was positively biased (10.8%); the performance of both methods worsened with decreasing LDL concentrations and increasing triglyceride content. The novel equation most-effectively reduced the bias (-1.2%) and increased the concordance of sample classification to the reference method. Approximately 19% (157 of 846) of samples submitted for LDL-C and TG measurement between October 2016 and December 2016 fell into the biased range of the Friedewald equation.

Conclusions: The novel equation should be used for accurate measurement of pediatric LDL-C when the concentration is below 100mg/dL and the triglycerides are between 150-399mg/dL. Granted the large percentage of samples having low LDL-C and elevated triglycerides at our institution, values from the novel equation should be included in lipid results.

1. Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310(19):2061-2068.

B-155

Monogenic dyslipidemia in a child followed due disnutrition diagnosis: the importance of clinical suspicion and cascading screening

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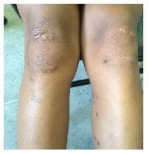
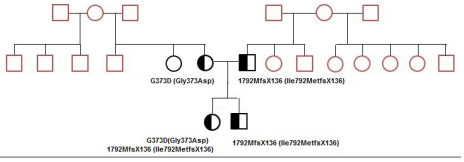
Background: The screening to identify children with lipid disorders, and diagnosis of familial hypercholesterolemia (FH) is uncommon in clinical practice.In general, lipid profile measured in early childhood usually do so as a result of a metabolic condition arising from obesity and the family history of cardiovascular disease in the pediatric age group is not always obtained.

Methods: Case report

Results: We described an 11 year old child attended a pediatric referral center with complains of xanthomas (figure 1) in the knee, elbow and buttocks associated with LDL-c high levels (above 200 mg/dL), despite the rosuvastatin use, since the age 2. Additionally, the child had positive family history with her maternal grandmother surgically revascularized at 47 years old. As the child did not respond to the treatment, the pediatrician decided to refer her for clinical and genetic investigation to a primary dyslipidemia center. At the first visit, the lipid panel showed total cholesterol of 436mg/dL, LDL-c:383mg/dL, HDL-c:38mg/dL and triglyceride:75mg/dL. The calculate Dutch score was 8 with causality classified as "probable" for FH.The genetic sequencing was performed, using the Sanger method and revealed mutation on compound heterozygosis: G373D(Gly373Asp) on exon 8 and 1792MfsX136 (Ile792MetfsX136) on exon 16 of LDL receptor gene. After confirmation of this index-case (IC), a cascade screening was started on her asymptomatic first-degreer elatives and identified the following mutations in heterozygosis at the LDL-c receptor gene: G373D(Gly373Asp) on exon 08 (mother- 27 yrs); 1792MfsX136 (Ile792MetfsX136) on exon 16(father- 45yrs and brother- 5 yrs).

Conclusion: Monogenic conditions due to a single gene defect, such as FH is a rare condition in children.Clinical suspicion in non-obese children is uncommon. The most frequent mutation is that of the LDL gene receptor. First-degree relatives should always be screened.





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Genetic and clinical-demographic profile of patients with familial hypercholesterolemia (FH) followed on an ambulatory of primary dyslipidemia in a university hospital

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Background: FH is a genetic disease caused by mutations in genes related to the metabolism of low-density lipoprotein (LDL-c), resulting in LDL-c high levels and cardiovascular risk (CVR) increase. The course of FH is silent and less than 10% FH patients are diagnosed. **Methods:** This was a cross-sectional study that evaluated

patients from a FH screening program, selected through the LDL-c levels ≥210mg/dl (adults) and ≥170mg/dl (children). The patients were classified according to Dutch criteria and genetic data. The DNA sequencing was performed by Sanger's methodology. After the identification of a causal mutation, a cascade screening (CS) in first-degree relatives was pursued.

Results: For the 63 index cases (IC), the mean age was 51.44 ± 15.36 years and 72.73% (48) were female. On the 31 related cases, from CS, 22 ± 17.3 years and 27.27% (18) were female. From 63 IC,79.36% (50) completed the genetic sequencing with 20.96% heterozygosis mutation confirmed on LDL-c receptor gene. The positivity for those mutation detection on SC patient was 25.8%. The majority of mutations were found on exons 8,9,12,14 and 16.From the population (63 IC and 31 CS), 67.02% (63) patients met DUTCH criteria and 52.38% (11) had a positive family history for cardiovascular disease. Overweight or obesity was not identified in the study population. Less than half of the patients received at least one pharmacological agent for dyslipidemia (45.74%).

Conclusion: The diagnosis of FH is based on clinical criteriaalthough the genetic diagnosis is very relevant. Most of the patients are heterozygote. The CS is crucial on the FH identification, which allows early intervention and cardiovascular mortality decrease.

B-157

Markers of Inflammation in Acne Vulgaris

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Background: Acne vulgaris (AV) is a inflammatory skin disease of unknown etiology. Eicosanoids derived from omega-6 (n-6) polyunsaturated fatty acids (PUFAs) have proinflammatory functions whereas eicosanoids derived from n-3 PUFAs have anti-inflammatory properties. Studies have documented that dietary supplementation with n-3 fatty acids ameliorates AV however serum PUFA levels have not been evaluated in patients with AV. Excess levels of secretory phospholipase A2 (sPLA2) also contributes to inflammatory diseases and studies indicate that lipoprotein lipase (LPL) has differential effects on several inflammatory pathways. This study aimed to determine circulating levels of n-6, n-3 PUFAs, assess serum activity of sPLA2, LPL and evaluate changes in circulating protein levels of angiopoietin-like protein 3 (ANGPTL3), ANGPTL4, cyclooxygenase-2(COX-2) and prostaglandin E2 (PGE2) in patients with AV.

Methods: Serum from 31 AV patients and 21 age and gender matched control subjects were evaluated for levels of sPLA2, COX, PGE2, LPL, ANGPTL3 and ANGPTL4 via commercial assay kits. Serum PUFA levels were measured by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods using autoanalyzers.

Results: No significant differences were found for the lipid profile, routine biochemical and hormone parameters between AV patients and controls. Serum EPA levels were significantly decreased while AA/EPA and DGLA/EPA ratio were significantly increased in AV patients compared to controls. Serum levels of AA, DGLA and DHA showed no significant difference between patients and controls. Serum activity of sPLA2 and LPL were significantly increased in AV compared to controls. No significant differences were found for COX, PGE2, ANGPTL3 and ANGPTL4 levels.

Conclusion: The results of this study reveal the presence of a proinflammatory state in AV as shown by significantly decreased serum EPA levels and increased activity of sPLA2, AA/EPA and DGLA/EPA ratio. Increased LPL activity in the serum of AV patients can be protective through its anti-dyslipidemic actions. This is the first study reporting altered EPA levels and increased sPLA2 activity in AV and supports the use of omega-3 fatty acids as adjuvant treatment for acne patients. **Acknowledgement:** This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK; #115S940).

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Only fresh samples should be allowed for lipid profile evaluation

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Background: This study was aimed to investigate whether the laboratory could assay lipid profile using either fresh or thawed samples.

Methods: Serum from 10 volunteers were immediately assayed for total cholesterol (CHOL), high density lipoprotein (HDL), and triglycerides (TG); then each volunteer's sample was divided in 2 identical aliquots. All aliquots were thawed after two-days freezing at -70°C. Immediately afterwards, the sera of the two paired aliquots were treated using two different techniques: (a) no mixing; (b) reference procedure, entailing 6 gentle inversions by overturning; The significance of the differences against the fresh (no frozen samples) was assessed with Wilcoxon signed rank test. The statistical significance was set at $p<0.05$.

Results: A significant variability was observed for CHOL, HDL, and TG when compared with thawed samples not mixed; whereas thawed samples mixed by inversion showed significant differences for CHOL, and HDL; but not for TG (Table 1).

Table 1. Lipid profile assayed on fresh and thawed samples

Test	Fresh samples	Thawed samples	
		Not mixed	Mixed
TG	1.32 [1.11 – 1.87]	0.82 [0.66 – 1.20]	1.31 [1.10 – 1.86]
		P=0.002	P=0.588
CHOL	5.43 [5.18 – 5.68]	3.22 [2.90 – 3.42]	5.33 [5.10 – 5.65]
		P=0.002	**P=0.008**
HDL	1.66 [1.38 – 1.75]	0.92 [0.80 – 1.02]	1.65 [1.36 – 1.71]
		P=0.002	**P=0.020**

Data are presented as mean [interquartile range], in mmol/L.

Conclusion: Based on our results lipid profile should be assayed on fresh samples in order to guarantee both laboratory outcomes and reliable results, thus patient safety.