Performance of LDL-C by direct method compared to calculation by Friedewald and Sampson equations, to improve provision of lipid lowering therapy

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Overview

- Background
  - Importance of LDL-C
  - LDL-C measurement or calculation
- Aims
- Study
- Results
  - Comparison of results
  - Clinical impact
- Conclusion

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Background: Importance of LDL-C

CVD risk assessment

- **LDL-C is associated with atherosclerosis, CVD development and progression**
- **Global mortality burden**: CVD accounts for 32% of all deaths globally (19.8 million deaths, 2022), exceeding cancer and respiratory disease combined
- **Premature mortality:** 80% of CVD deaths occur in individuals under 70 years old

Background: Importance of LDL-C

Treatment

- **LDL-C is a modifiable risk factor with evidence-based outcomes**: Achieving target LDL-C reduction improves patient outcomes, reduces cardiovascular events and mortality.
- Each 1mmol/L reduction in LDL-C reduces major vascular events by 22% after 1 year.
- Assessment of response requires accurate LDL-C quantification.
- **Healthcare economics**: Achieving LDL-C targets reduction is crucial for addressing NHS £7.4 billion/year CVD burden.
- **Guidelines use LDL-C as target thresholds**: National and international lipid management protocols define treatment goals based on LDL-C levels. LDL-C can be effectively reduced primarily by statins, ezetimibe, bempedoic acid, PCSK9 inhibitors and inclisiran.
- **Guidelines use LDL-C as an eligibility criteria for injectable LLT:** Specific LDL-C thresholds to initiate PCSK9 inhibitors (alirocumab or evolocumab) and inclisiran.

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Background: LDL-C quantification

Beta quantification

- Gold standard method
- Ultracentrifugation separates lipoproteins according to density. Total cholesterol and HDL-C measured in bottom fraction; LDL-C calculated as difference between total cholesterol and HDL-C
- Complex, not appropriate for use in routine patient care. High technical demands, lengthy procedure, expensive

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Background: LDL-C quantification

Direct homogeneous methods

- Chemical regents specifically measure LDL by selective blocking and solubilisation of lipoprotein classes
- Practical for routine laboratory use. Fully automated, easy to use, faster, less expensive than beta quantification
- Decreased accuracy in patients with abnormal lipoproteins in certain dyslipdaemic states. Bias of 13-46% reported between different methods

Background: LDL-C quantification

Calculated methods

- LDL- calculated from components of lipid panel (Cholesterol, triglyceride, HDL-C directly measured) therefore no additional cost beyond standard lipid profile
- Proven correlation with clinical outcomes in trials
- Variety of equations developed, all of which have limitations

Background: LDL-C quantification

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Calculated methods - Friedewald

- Published 1972, derived from 448 individuals, widely adopted
- LDL- C = Total cholesterol – HDL – VLDL. VLDL not measured. VLDL assumed to contain triglyceride:cholesterol 2.26:1. Thus VLDL = TG/2.26

$$LDL = CHOL - HDL - \frac{TG}{2.2}$$

- Easy to calculate therefore commonly used, however significant limitations when triglyceride concentration >4.5 mmol/L, or when LDL-C <1.5 mmol/L
- Calculation assumes patient is fasting and triglyceride $\leq$4.5 mmol/L. Fasting is no longer routinely recommended. Chylomicrons have increased triglyceride mass and increases in the non-fasting state, thus VLDL estimation incorrect and LDL-C is underestimated

Background: LDL-C quantification

Calculated methods - Sampson

- Published 2020[1]. Validated on a set of 8,656 individuals, against beta quantification data

$$LDL = \frac{CHOL}{0.948} - \frac{HDL}{0.971} - \left(\frac{TG}{3.74} + \frac{TG \times Non_{HDL}}{24.16} - \frac{TG^2}{79.36}\right) - 0.244$$

- More complex equation to implement. Used triglyceride and non-HDL as independent variables and multiple least squares regression to develop bivariate quadratic equation for VLDL-C
- More accurate at high triglyceride concentration. Use of continuous variables allows calculation of LDL-C up to a triglyceride concentration of 9 mmol/L
- More accurate at low LDL-C concentrations

Aims

Comparison of methods

Compare LDL-C calculated by Friedewald and Sampson methods with direct LDL-C measurement on the Roche Cobas platform

Evaluation of clinical impact

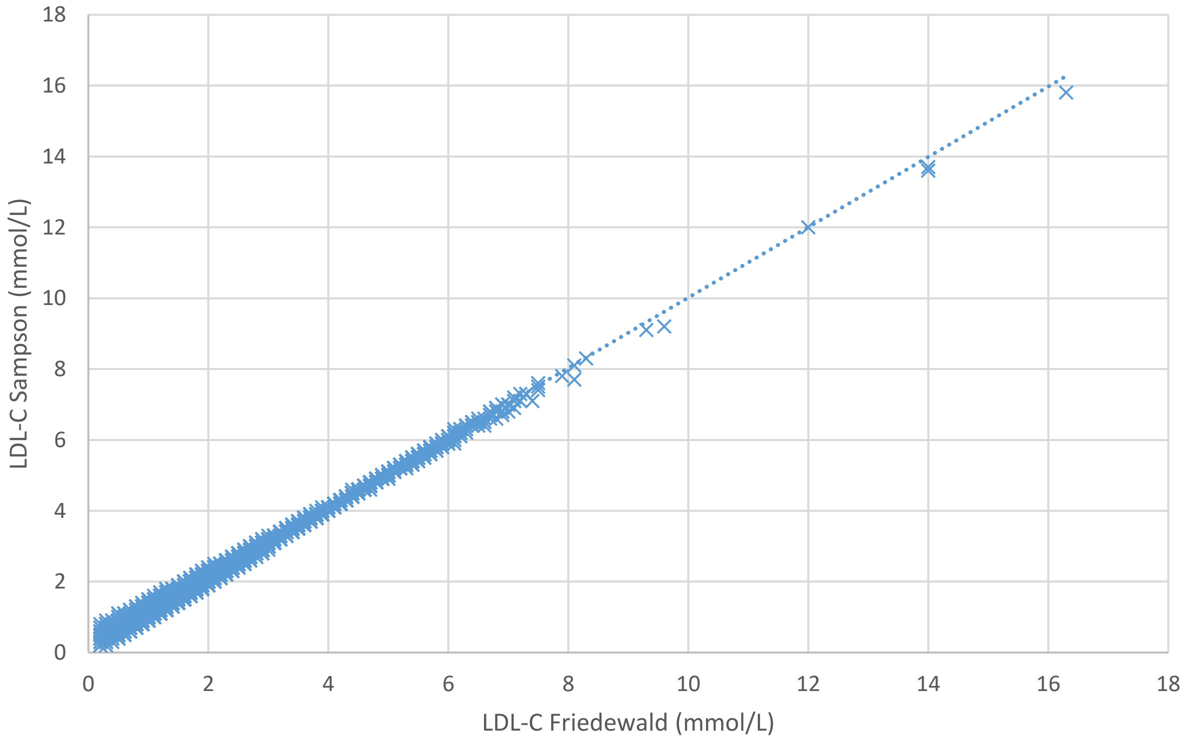
Determine impact of different LDL-C calculations with regard to eligibility for LLT, according to guidelines

Methods

- Routine analysis of patient samples on Roche Cobas 8000 c702 using the Friedewald equation
- Sampson set up as a non-reportable calculation
- Over a 4 month period (Jan to May 2025) 45,915 patient samples were analysed for lipid profiles, including 12 EQA samples over 4 distributions; 48 samples also selected for direct LDL-C measurement
- All data extracted and anonymised. Data analysed by regression analysis, Bland-Altman plots and ANOVA
- Clinical impact determined by calculation of proportion of samples reportable by Sampson but not Friedewald.
- Additional patients meeting criteria for LLT according to guidelines assessed.

Results 1: Correlation between Friedewald and Sampson

Friedewald and Sampson had excellent agreement at triglyceride ≤4.5 mmol/L (n= 39,576 samples)



$y = 0.99x + 0.09$

$R^2 = 0.99$

Results 2: Correlation between Friedewald and Sampson, according to LDL-concentration

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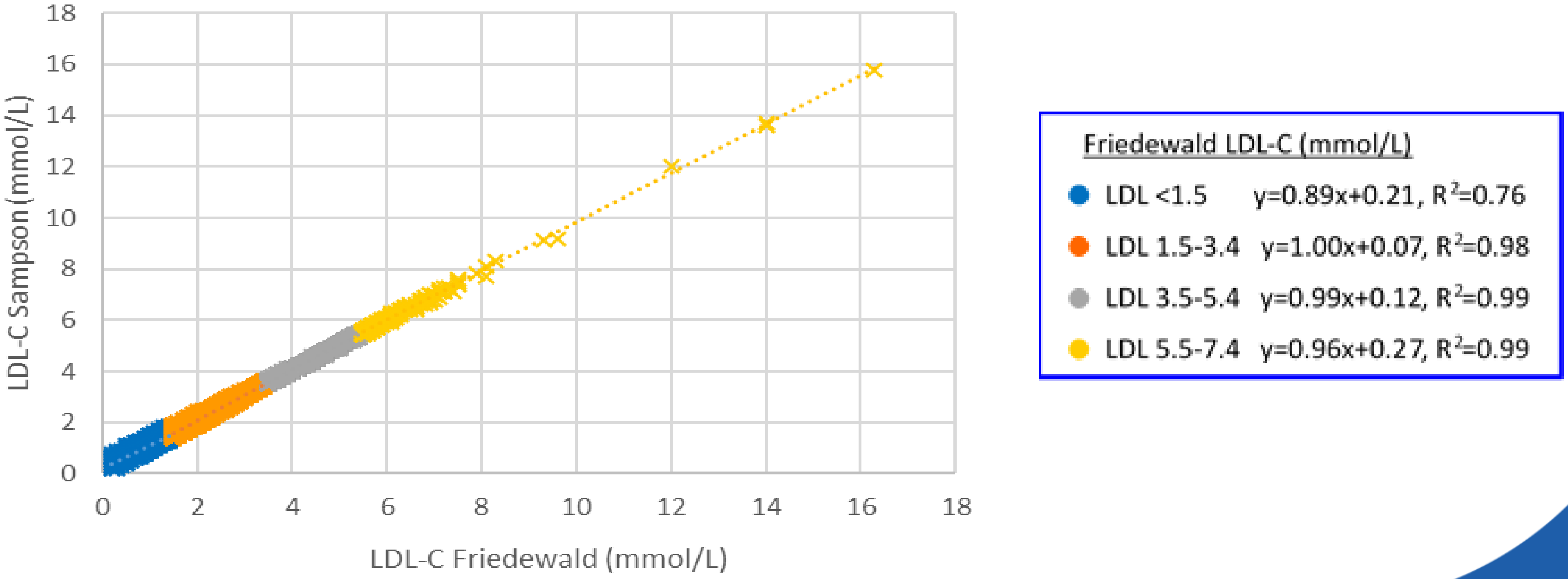
More variation at LDL-C <1.5 mmol/L (R^2 = 0.76) than LDL-C >1.5mmol/L (R^2 = 0.98)

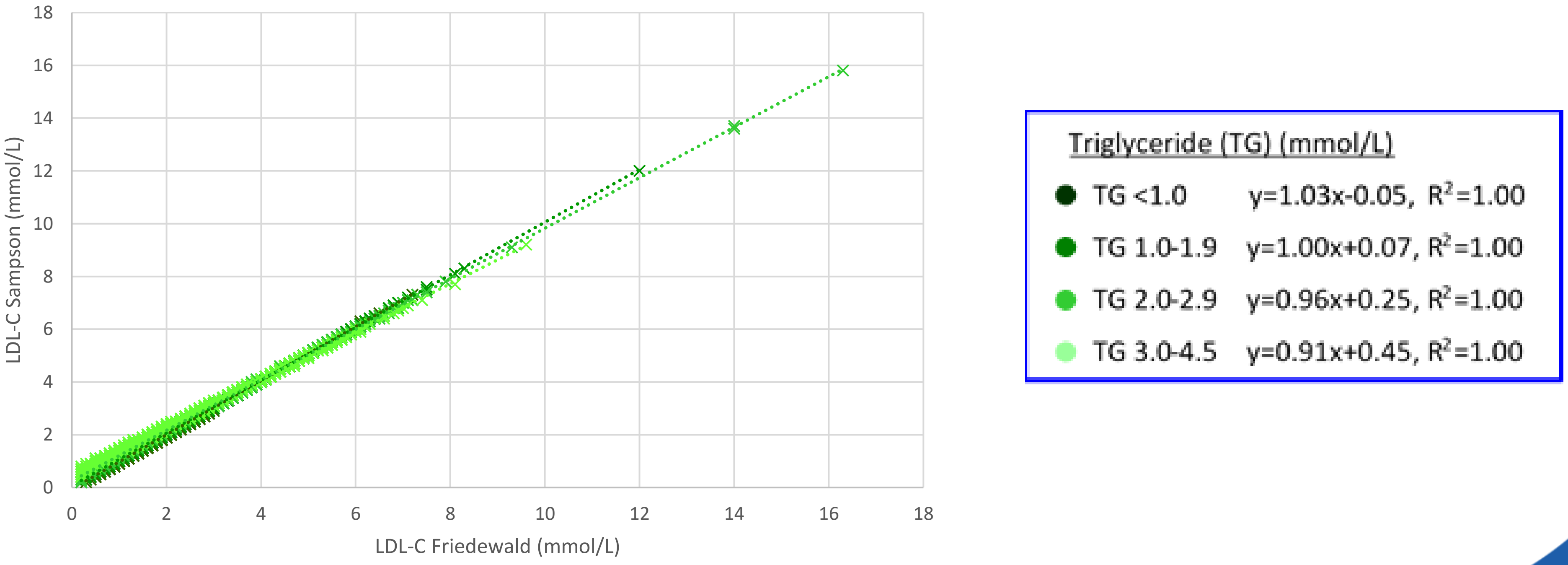
Constant positive bias of 0.2 mmol/L (y=0.89x + 0.21) has a large impact at low LDL-C, causing lower results by Friedewald



Results 3: Correlation between Friedewald and Sampson, according to triglyceride concentration

Association closest with triglyceride ≤3.0 mmol/L

Friedewald overestimates LDL-C at high triglycerides. Sampson LDL-C concentrations 9% lower than Friedewald (y=0.91x + 0.45) at triglyceride 3.0-4.5 mmol/L.

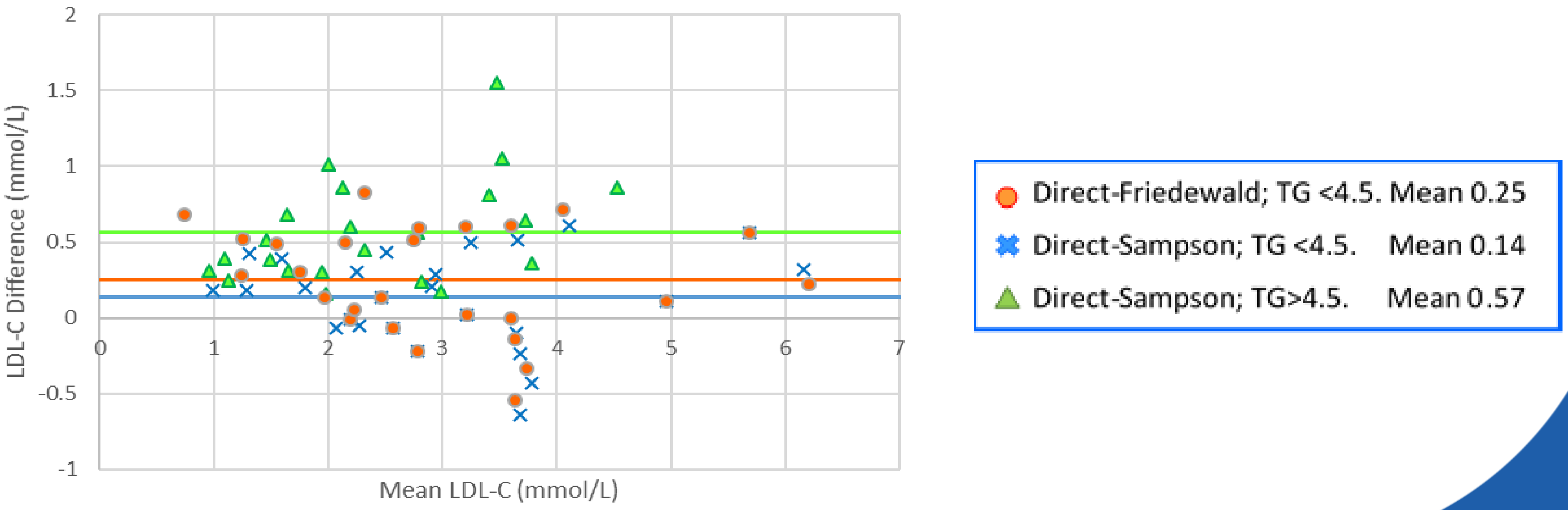


Results 4: Correlation between Friedewald, Sampson and Direct LDL-C

One way ANOVA showed no significant difference between LDL-C by Friedewald, Sampson or direct measurement at triglyceride concentration ≤4.5 mmol/L; $F(2,23) = 0.24$, $p=0.79$

Direct LDL-C was slightly higher than Sampson at triglyceride concentrations 4.6-9.0 mmol/L

Studies in the literature show both calculated and direct LDL-C are affected by hypertriglyceridaemia to some degree, compared to the beta quantification reference method



Results 5: Clinical impact of Sampson on proportion of samples able to report LDL-C

LDL-C was unreportable by Friedewald in 1,042/ 45,915 (2.3%) samples (triglyceride >4.5 mmol/L)

Sampson reduced the number of unreportable LDL-C to 175 (0.4%) (triglyceride >9.0 mmol/L)

Lipid requests	Number (%) of requests
All Triglycerides	45,915
Triglyceride <4.6 mmol/L	44873 (97.7%)
Triglyceride 4.6-9.0 mmol/L	867 (1.9%)
Triglyceride >9.0 mmol/L	175 (0.4%)

Results 6: Clinical impact of Sampson on LLT

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More than half of patients in whom LDL-C is reportable by Sampson but not Friedewald (triglyceride 4.6-9.0 mmol/L), would meet the criteria for lipid lowering therapy for CVD prevention or therapy adjustment:

- 69% exceeded NICE (NG238)[3] target for secondary prevention of CVD
- 33-87% exceeded ESC/EAS target[4] for CVD prevention depending on CVD risk level
- Monoclonal antibodies (PCSK9 inhibitors)[5,6] and inclisiran[7] could be prescribed in 3-16% (depending on CVD risk) and 50%, respectively

Results 6: Clinical impact of Sampson on LLT

Guideline	Threshold treatment (LDL-C mmol/L)	LDL-C reportable by Sampson but not Friedewald above threshold (%)
Secondary prevention of CVD (NICE NG238[3])	>2.0	69
CVD prevention (ESC/EAS Guidelines 2019[4])	CVD risk V.high ≥1.4 High ≥1.8 Moderate ≥2.6 Low ≥3.0	 87 78 50 33
Alirocumab or Evolocumab treatment for secondary prevention (NICE TA393[5], TA394[6])	V.high >3.5 High >4.0	16 8
Alirocumab or Evolocumab treatment for primary heterozygous-familial hypercholesterolaemia (NICE TA393[5], TA394[6])	High/v.high >3.5 None >5.0	16 3
Inclisiran treatment for primary hypercholesterolaemia or mixed dyslipidaemia (NICE TA733[7])	>2.5	50%

Conclusions

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- Friedewald, Sampson and Direct Roche LDL-C methods show good agreement
- Sampson LDL-Calculation gives additional benefit over Friedewald as it allows an extra 1.9% of patients (triglyceride 4.6-9 mmol/L) to have LDL-C reported, half of whom would meet guideline criteria for injectable LLT
- Sampson equation was thus subsequently adopted in our Trust, in line with 2025 Heart UK and Association for Laboratory Medicine guidelines[2]

References

1. Sampson M, Ling C, Sun Q, *et al*. A New equation for calculation of Low-Density Lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *JAMA Cardiol*. 2020;5(5):540-548
2. Kenkre JS, Mazaheri T, Neely RDG, *et al.* Standardising lipid testing and reporting in the United Kingdom; a joint statement by HEART UK and The Association for Laboratory Medicine. *Ann Clin Biochem*. 2025; 62(4): 257-286
3. Cardiovascular disease: risk assessment and reduction, including lipid modification 2023 NICE guideline [NG238]
4. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J*. 2020;41(1):111-188
5. Alirocumab for treating primary hypercholesterolaemia and mixed dyslipidaemia 2016 NICE Technology appraisal guidance TA393
6. Evolocumab for treating primary hypercholesterolaemia and mixed dyslipidaemia 2016 NICE Technology appraisal guidance TA394
7. Inclisiran for treating primary hypercholesterolaemia or mixed dyslipidaemia 2021 NICE Technology appraisal guidance TA733

Further work

Association for Laboratory Medicine National audit led by Prof. Eric Kilpatrick

on lipid testing and prescribing guidance for dyslipidaemia