



NATIONAL LABORATORY HANDBOOK

Recommendations for the testing and reporting of lipids in clinical diagnostic laboratories within the Republic of Ireland.

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Background

The testing and reporting of lipids play an important part in cardiovascular disease (CVD) risk assessment. Chemical Pathologists are uniquely trained in both the laboratory and clinical aspects of lipidology. Accordingly, the Chemical Pathology subgroup of the Faculty of Pathology, Royal College of Physicians of Ireland (RCPI) has produced the first Irish guidelines for lipid testing and reporting.

Scope

The aim is to provide guidelines for lipid testing and reporting for use by clinicians and clinical laboratories. It follows the laboratory pathway that a sample takes: pre-analytical, analytical and post-analytical and makes recommendations relating to each of these phases.

Abbreviations

HDL-C	HDL cholesterol
LDL-C	LDL cholesterol
TG	Triglycerides

Key Recommendations

1. The standard lipid profile should contain serum/plasma total cholesterol, HDL cholesterol (HDL-C), non HDL cholesterol (non HDL-C), triglycerides (TG), and LDL cholesterol (LDL-C).
2. LDL-C can be reported as either "calculated LDL cholesterol" using the Friedewald equation or analysed by a "direct LDL cholesterol" assay.
3. Each laboratory should state clearly which method of LDL-C is being reported.
4. This standard profile should be provided whenever the following tests are requested to ensure a standard approach for patients: "cholesterol", "fats", "lipids", "lipid profile", "cholesterol profile", and so forth.
5. Fasting is not required for the analysis of the standard lipid profile.
6. Serum and plasma separated within 12 hours are the usual specimen types. Analysis of separated specimens may occur within 7 days if stored at 4-8°C until analysis.
7. The measurement of triglycerides should be considered on the first lipaemic sample in a healthcare episode to help determine the degree of the dyslipidaemia.
8. The acute phase response is known to affect lipid and lipoprotein concentrations and thus lipids and lipoproteins should ideally be requested when a person is well. In general,

lipids and lipoproteins should not be measured during a fever or major infection or after major surgery. Ideally, lipids and lipoproteins should be requested as soon as possible within the first 24 hours post an acute coronary syndrome or a stroke. Otherwise measurement should not be done for at least 6 weeks following such clinical events.

9. In general, it is not recommended that lipids or lipoproteins are routinely measured in pregnancy, in the first 6 weeks of puerperium or immediately after excessive alcohol intake unless pancreatitis is suspected.
10. It is recommended that there should be at least one public hospital laboratory able to analyse the following specialist lipid assays for specialist lipid management:
 - a. Familial Hypercholesterolaemia (FH) mutation detection
 - b. Serum/Plasma Lipoprotein (a)
 - c. Serum/Plasma Apolipoprotein B
 - d. Serum/Plasma Apolipoprotein AI
 - e. Apolipoprotein E genotyping
 - f. Lipoprotein electrophoresis
 - g. Beta quantification/ultracentrifugation.

Epidemiology

Raised total cholesterol (≥ 5.0 mmol/L) is a major cause of vascular disease burden in Ireland. With the prevalence of raised total cholesterol in adults of both genders being $\geq 60\%$, Ireland is amongst the countries with the greatest hypercholesterolaemia burden in the world. The global prevalence of raised total cholesterol among adults is 37% for males and 40% for females and the European prevalence is 54% for both genders. In Ireland, a 30% reduction in the heart disease death rate has been attributed to a 4.6% reduction of the population mean for total cholesterol. Average total cholesterol worldwide has decreased by less than 0.1 mmol/L between 1980 and 2008 per decade in men and women.

Testing

Pre-analytical phase

The pre-analytical stage is about doing the right test on the right patient at the right time and is where most errors occur. This is especially the case with lipids in view of the use of calculated values. It also has the potential to be rate limiting especially in primary care if the fasting state is only employed.

1. The recommended standard serum/plasma lipid profile provided by laboratories should contain total cholesterol, HDL cholesterol, non-HDL cholesterol¹⁻², triglycerides and LDL cholesterol. It is important to note that LDL cholesterol can be reported as either a "calculated LDL Cholesterol" using the Friedewald equation (see point 18 for equation) or analysed by a "direct LDL Cholesterol" assay, and therefore each laboratory should state clearly which LDL-C method is being reported. Furthermore, it is recommended that this profile is provided whenever the following tests are requested by laboratory service users to ensure a standard approach for patients: "cholesterol", "fats", "lipids", "lipid profile", "cholesterol profile", and so forth.
2. Fasting is not required for the analysis of the standard lipid profile and a non-fasting sample is fully acceptable for CVD risk estimation.^{3-5, 16}
 - a. The maximal mean changes between fasting and non-fasting states is not clinically significant at +0.3 mmol/L for triglycerides, -0.2 mmol/L for cholesterol, and there is no change in HDL-C²⁰.

- b. Fasting is beneficial where there is a known elevated triglyceride (e.g. > 4.5 mmol/L, which will also affect the LDL-C calculation) and where another fasting blood test is required such as a fasting glucose.
 - c. Non HDL cholesterol is a strong CVD risk indicator and can be calculated in a non-fasting specimen (by subtracting HDL cholesterol from the total cholesterol) and thus can be reported even when triglycerides are high and calculated LDL cholesterol not possible.
3. Serum and plasma separated within 12 hours are the usual specimen types and are assumed unless otherwise stated. Analysis of separated specimens is valid within 7 days if stored at 4-8°C until analysis⁶.
 4. The measurement of triglycerides should be considered on the first lipaemic sample in a healthcare episode to help determine the degree of the dyslipidaemia. This may be by means of an automated process based on the lipaemic index or other procedure⁷.
 5. The acute phase response is known to affect lipid and lipoprotein concentrations and thus lipids and lipoproteins should ideally be requested when a person is well or six weeks following a clinical event. Lipids and lipoproteins should not be measured during a fever or major infection or after major surgery. Ideally, lipids and lipoproteins should be requested as soon as possible within the first 24 hours post an acute coronary syndrome or a stroke, or six weeks should elapse from onset of such clinical events⁸.
 6. In general, it is not recommended that lipids or lipoproteins are routinely measured in pregnancy, in the first 6 weeks after delivery or following excessive alcohol intake unless pancreatitis is suspected⁹.

Who to Test

7. As ≥60% of the Irish population has hypercholesterolaemia, lipids should be requested during opportunistic and planned cardiovascular disease (CVD) risk assessment. Patients presenting with new onset CVD should be checked upon admission.

Who to Re-Test and Re-Testing Intervals

8. As ≥60% of the Irish population has hypercholesterolaemia, lipids should be requested during opportunistic and planned cardiovascular disease (CVD) risk assessment. Patients presenting with new onset CVD should be checked upon admission.
9. The dose of LDL cholesterol lowering medications should be individualised according to baseline LDL-C levels, the goal of therapy, and patient response. Accordingly, patients on LDL cholesterol lowering medications will require lipid testing based on their individual clinical condition.
10. Re-testing intervals must not only reflect the analyte being requested, but also how it is being used - thus they must also reflect local protocols. Clearly, clinicians can decide otherwise if they feel that it is clinically appropriate to request a test more or less frequently based on the patient's clinical condition. This is especially the case in CVD risk prevention due to the large biological variability of lipids¹⁷. Retesting annually in patients within target values is sufficient. The efficacy of therapy changes should be checked in 2 months. Retesting may encourage patient awareness and participation in the management of their CVD risk. The availability of all previously reported laboratory results at or before the time of requesting a new test should greatly assist the clinician in deciding whether a test is appropriate.

Who Not to Test

11. In general, it is not recommended that lipids or lipoproteins are routinely measured in pregnancy or the puerperium or immediately after excessive alcohol intake unless pancreatitis is suspected.
12. The maximum therapeutic response for HMGCoA reductase inhibitors (statins) is usually achieved within 4 weeks. Similarly, the adjustment of statin dose should be made at intervals of 4 weeks or more. Accordingly, there is little benefit in rechecking within 4 weeks.

How to Test Analytical phase

13. All labs analysing lipids, lipoproteins and a lipaemic index should perform Internal Quality Control using third party materials¹⁰.
14. Laboratories should participate in an External Quality Assurance (EQA) programme for lipids and lipoproteins that is designed and overseen by appropriately competent professionals including clinical oversight. The distribution frequency should be monthly with values that cover the whole clinical range. EQA samples should be treated as much as possible as if they were patient samples. The EQA programme must be an accredited programme and should participate in post marketing vigilance with the appropriate national competent authority for in vitro diagnostics¹¹.
15. Triglyceride assays that measure triglyceride associated glycerol and not total glycerol are ideally recommended¹² to avoid pseudo-hypertriglyceridaemia¹³.
16. Total error in HDL-C assays in relation to the effect of interference at various triglyceride concentrations should be established to relate to National Cholesterol Education Program (NCEP) and European total allowable error limits¹⁴.
17. For laboratories providing direct LDL-C assays, the potential effect of raised triglycerides on the quality of the results produced should be ascertained.
18. For calculated LDL-C, the recommended Friedewald equation calculation that should be employed is the original equation¹⁵:

$$\text{Calculated LDL-C} = \text{total cholesterol} - \text{HDL cholesterol} - \text{triglycerides} / 2.2$$

19. The maximum recommended triglyceride concentration above which the calculated LDL-C should not be provided is 4.5 mmol/L¹⁵
20. The use of calibrators that have assigned values that are traceable to the United States Centres for Disease Control (US CDC) reference methods are recommended.
21. SI units are recommended for reporting of results, that is, mmol/L.
22. Total cholesterol, HDL-C, triglycerides and LDL-C should be reported to a single decimal place⁴.
23. It is recommended that there should be at least one public hospital laboratory able to analyse the following specialist lipid assays for specialist lipid management:
 - a. Familial Hypercholesterolaemia (FH) mutation detection
 - b. Serum/Plasma Lipoprotein (a)
 - c. Serum/Plasma Apolipoprotein B
 - d. Serum/Plasma Apolipoprotein AI
 - e. Apolipoprotein E genotyping
 - f. Lipoprotein electrophoresis
 - g. Beta quantification/ultracentrifugation.

Hospitals that currently provide such tests include:

Test	Laboratory
FH mutation detection	Biochemistry Department, St. James's Hospital
Apolipoprotein E genotyping	Biochemistry Department, St. James's Hospital
Lp(a)	Tallaght Hospital. St. Vincent's University Hospital (from 2017)
Apo B	Tallaght Hospital, St. Vincent's University Hospital (from 2017)
Apo AI	Tallaght Hospital, St. Vincent's University Hospital (from 2017)
Lipoprotein electrophoresis	Tallaght Hospital (Quantimetrix system) City Hospital Nottingham NG5 1PB, or St Thomas' Hospital, London SE1 7EH
Beta quantification / ultracentrifugation	Glasgow Royal Infirmary, Glasgow G4 0SF St Thomas' Hospital, London SE1 7EH

24. A completed standard laboratory test request form must be sent with all samples.

Information required on the referral form

25. The request form must include detailed patient and clinical information including:

- **Patient demographics**
 - Patient's Name
 - Patient's Date of Birth
 - Medical Record Number
 - Name of Referring Clinician
 - Name of Referring Hospital
 - Order number / external laboratory number (if applicable to external agencies only).
- **Request details**
 - Example - Clinical indication for testing (see list above)
 - Example - Details of any medications.

Requests received with inadequate patient demographics may not be analysed

26. Full clinical information should accompany all requests. In the event a request for a specialised lipid investigation is received which does not have the required data (above) or does not have adequate clinical details the laboratory may:

- a. Issue a report to the requesting doctor, requesting additional clinical details and/or advise that the case is discussed with the local Laboratory Medicine Consultant, and advising that the sample will be discarded after 7 days if there is no reply
- b. Store the sample for up to 7 days awaiting further communication from the referring clinician
- c. Samples can be discarded after 7 days if the referring clinician has not provided the required details or if it is determined that testing is not indicated.

Interpretation of tests

27. When a triglyceride concentration is ≥ 4.5 mmol/L, it is recommended that a comment is appended to the report advising the requester to consider a repeat fasting profile if the initial request related to a non-fasting sample.
28. When a triglyceride concentration is > 10 mmol/L, a comment should be appended to the report¹⁶, *“High Triglycerides > 10 mmol/L – risk of pancreatitis”*.
29. It is recommended that patients at high risk of CVD, including those at high risk of Familial Hypercholesterolaemia due to their LDL-Cholesterol concentration being ≥ 5.0 mmol/L, are highlighted to laboratory service users by means of an automated laboratory comment appended to the report or by another process. An example of such a comment is *“Significantly elevated LDL-cholesterol – patient at high risk of CVD. If there is a personal or family history of premature vascular disease then this patient may have Familial Hypercholesterolaemia”*.
30. The following European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) clinical action points are recommended¹⁶:
 - a. For patients with very high CVD risk, the treatment target for LDL-C is < 1.8 mmol/L (non HDL-C < 2.6 mmol/L) or a reduction of at least 50% if the baseline LDL-C is between 1.8 and 3.5 mmol/L is recommended;
 - b. For patients with a high CVD risk, an LDL-C level of < 2.6 mmol/L (non HDL-C < 3.4 mmol/L) or a reduction of at least 50% if the baseline LDL-C is between 2.6 and 5.2 mmol/L is recommended;
 - c. For patients at moderate CVD risk, an LDL-C target of < 3.0 mmol/L (non HDL-C < 3.8 mmol/L) is recommended.
31. Non-HDL-cholesterol cut-offs are generally 0.8 mmol/L higher than the respective LDL-cholesterol cut-offs.
32. Appropriate age-related acceptable limits for children with calculated LDL-Cholesterol using the Friedewald equation are provided by the National Heart Lung and Blood Institute (NHLBI) Guidelines¹⁹.
33. It is good practice to do an annual audit of the performance of laboratory calculations to verify that the equations remain valid. This also applies to calculated LDL-C, non HDL-C and similarly derived indices.
34. Recommended cut-points for reporting of lipid results are shown in the following table²⁰, along with CVS guidelines cut-points, and “extremely abnormal “ cut-points for flagging and commenting:

Recommended cut-points for flagging lipid results

Standard Lipid Profile Result	Non-Fasting	Fasting	Very abnormal concentrations requiring specific commenting (regardless of whether fasting or non-fasting) and discussion with a lipidologist
Triglycerides	≤ 2.0 mmol/L	≤ 1.7 mmol/L	≥ 10 mmol/L (risk of pancreatitis)
Total cholesterol	≤ 5.0 mmol/L	≤ 5.0 mmol/L	≥ 7.5 mmol/L especially if family history, - risk of FH (22)
HDL cholesterol (gender-specific cut-points are also available)	≤ 1.0 mmol/L	≥ 1.0 mmol/L	≤ 0.3 mmol/L (possibility of genetic causes)
LDL cholesterol	≤ 3.0 mmol/L	≤ 3.0 mmol/L	≥ 5 mmol/L (may indicate heterozygous FH with increase CVD risk) ≤ 0.3 mmol/L (possibility of genetic

			causes)
Non HDL cholesterol	≤ 3.8 mmol/L	≤ 3.8 mmol/L	

Cardiovascular Disease Risk Assessment Tools

There are a number of well validated cardiovascular disease risk assessment systems available that are recommended as part of different guidelines. The 2016 European Guidelines on cardiovascular disease prevention in clinical practice¹⁸ provide a list of commonly used tools and the authorities recommending them. There is no consensus recommendation on which of these systems should be used, but it is agreed that these tools can enhance clinical decision making in the primary prevention of cardiovascular disease.

Implications for GP ICT Systems and MedLIS

User-friendly electronic ordering and reporting for lipids should be developed and implemented at the point of ordering/reporting in GP or Hospital Information Systems. The information required for requesting and reporting lipids is as stated above, and a user-friendly screen should be developed to allow selection of one or more of the relevant clinical indications and to indicate relevant drug therapy.

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Quick Reference Card for Lipid Testing

Recommended cut-points for flagging lipid results

Standard Lipid Profile Result	Non-Fasting	Fasting	Very abnormal concentrations requiring specific commenting (regardless of whether fasting or non-fasting) and discussion with a lipidologist
Triglycerides	≤ 2.0 mmol/L	≤ 1.7 mmol/L	≥10 mmol/L (risk of pancreatitis)
Total cholesterol	≤ 5.0 mmol/L	≤ 5.0 mmol/L	≥ 7.5 mmol/L especially if family history, - risk of FH (22)
HDL cholesterol <i>(gender-specific cutpoints are also available)</i>	≤ 1.0 mmol/L	≤ 1.0 mmol/L	≤ 0.3 mmol/L (possibility of genetic causes)
LDL cholesterol	≤ 3.0 mmol/L	≤ 3.0 mmol/L	≥5 mmol/L (may indicate heterozygous FH with increase CVD risk) ≤ 0.3 mmol/L (possibility of genetic causes)
Non HDL cholesterol	≤ 3.8 mmol/L	≤ 3.8 mmol/L	

Recommended EAS/ESC Clinical Action Points

Patients with very high CVD risk	The treatment target for LDL-C is <1.8 mmol/L (non HDL-C <2.6 mmol/L) or a reduction of at least 50% if the baseline LDL-C is between 1.8 and 3.5 mmol/L is recommended;
Patients with a high CVD risk	An LDL-C level of <2.6 mmol/L (non HDL-C <3.4 mmol/L) or a reduction of at least 50% if the baseline LDL-C is between 2.6 and 5.2 mmol/L is recommended
Patients at moderate CVD risk	An LDL-C target of <3.0 mmol/L (non HDL-C <3.8 mmol/L) should be considered.