REVIEW

Best practice in primary care pathology: review 3

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This best practice review examines four series of common primary care questions in laboratory medicine: (i) ''minor'' blood platelet count and haemoglobin abnormalities; (ii) diagnosis and monitoring of anaemia caused by iron deficiency; (iii) secondary hyperlipidaemia and hypertriglyceridaemia; and (iv) glycated haemoglobin and microalbumin use in diabetes. The review is presented in question–answer format, referenced for each question series. The recommendations represent a précis of guidance found using a standardised literature search of national and international guidance notes, consensus statements, health policy documents and evidence-based medicine reviews, supplemented by Medline Embase searches to identify relevant primary research documents. They are not standards, but form a guide to be set in the clinical context. Most of the recommendations are based on consensus rather than evidence. They will be updated periodically to take account of new information.

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This is the third in a planned series of reviews to answer several questions that arise during the use of pathology in primary care.

Each subject is introduced with a brief summary of the type of information found and is handled separately with its own reference list.

Although the individual subjects are not related, as they cover the disciplines of clinical biochemistry, microbiology, immunology, haematology and cellular pathology, they are designed to form a resource, which will be indexed and cover a wide range of the most common primary care laboratory issues.

In instances where the new United Kingdom General Medical Services (GMS) contracts make specific reference to a laboratory test, the indicator or target is appended at the end of the answer.

''MINOR'' ABNORMALITIES IN BLOOD COUNT (DB AND ECML)

Clear diagnostic results (eg, acute leukaemia, agranulocytosis) arising from a full blood count rarely pose serious diagnostic consequences in primary care. The boundaries between the statistically abnormal and clinically relevant abnormalities and the further investigations recommended in these situations are, however, less clear. The following series of questions examines raised and low platelet counts and haemoglobin and attempts to identify situations in which further monitoring or investigation is required and those in which immediate secondary care attention is appropriate.

When and how should I investigate a low platelet count?

Table 1 shows the recommended actions for various values of platelet count threshold.

The lower end of the UK population reference range for blood platelets is 140×10^9/l,[1] although one review has recommended 150×10^9/l (or 120×10^9/l during late pregnancy) as the threshold to trigger a repeat test in a Caucasian population.[2] Platelet counts of 120×10^9/l may, however, be seen in healthy people of African origin and 110×10^9/l in those of Afro-Caribbean origin.

Symptoms of a low platelet count are bruising and ecchymoses, muco-cutaneous bleeding and prolonged bleeding after trauma or lacerations. Very low platelet counts cause petechiae.

History

- Bleeding, bruising, alcohol
- Drug history—for example, co-trimoxazole, quinine, thiazide diuretics and heparin (for a complete list of drugs, see http://moon.ouhsc.edu/jgeorge).[3]

Examination

- Bruising or bleeding, petechiae
- Blood blisters in mouth
- Lymphadenopathy, hepatosplenomegaly, jaundice, fever.[4]

Investigations and management

- Platelet count may be falsely low if there is difficulty bleeding the patient or owing to EDTA-related pseudothrombocytopenia (anticoagulant contained in blood count tubes). Repeat the full blood count with blood film.[5] Some laboratories may request a repeat sample in an alternative anticoagulant. No recommendation for a specific timing was found and we would suggest a repeat test in 4 days.
- Consider alcohol or current drugs. Platelet counts will recover 5–7 days after stopping the incriminated drug.[4]
- If platelet count $<30\times10^9$/l, stop any antiplatelet drug—for example, aspirin.[6]

Abbreviations: ADA, American Diabetes Association; CVD, cardiovascular disease; DCCT, Diabetes Control and Complications Trial; GMS, General Medical Services; HbA_{1c}, glycated haemoglobin; LDL, low-density lipoprotein; MCV, mean cell volume; NICE, National Institute for Health and Clinical Excellence

Table 1 Recommended actions for various values of platelet count threshold

Platelet count threshold	Action
$<30\times10^9$/l or <70/l with symptoms	Repeat the test with blood film to exclude artefact and myelodysplasia, marrow infiltration or other problems. If confirmed, urgently refer to a haematologist
$<70\times10^9$/l	Confirm immediately with repeat test and refer if not chronically thrombocytopenic
$<110\times10^9$/l	Recheck monthly for 3 months. Refer if falling
$<150\times10^9$/l	Recheck in 2 months to ensure not falling

- Examine for splenomegaly, and measure bilirubin and liver enzymes as indicators of possible liver disease or portal hypertension causing hypersplenism.[7]
- If platelet count $>60\times10^9$/l, consider myelodysplasia.[8]
- Thrombocytopenia may be an early sign of serious marrow disease, thrombotic thrombocytopenic purpura or the haemolytic uraemic syndrome.[9]
- Consider HIV infection in patients with risk factors.

Platelet counts $>30\times10^9$/l rarely cause symptoms and receive no urgent treatment.[10–13] Antiplatelet treatment should, however, be avoided or stopped, depending on the relative risks of haemorrhage or thrombosis.[10] Although no specific referral threshold was found, we recommend referral to a haematologist if the platelet count falls to $<70\times10^9$/l or is $<110\times10^9$/l and falling on 4-weekly checks.

It is now recommended that children with immune thrombocytopenia do not receive treatment even for severe thrombocytopenia ($<10\times10^9$/l), in the absence of clinically important bleeding.[13–15] A further guideline recommends that hospital admission is not warranted in idiopathic thrombocytopenia if the platelet count is $>20\times10^9$/l and no treatment if it is $>50\times10^9$/l. For practical purposes, these patients would have been referred under the above recommendations and the decision on further action would be taken in secondary care.

Low platelet count in pregnancy excludes hypertensive disorders and gestational thrombocytopenia.[16] (Gestational thrombocytopenia requires no intervention and is defined by five criteria: mild and asymptomatic thrombocytopenia; no history of thrombocytopenia (except possibly during a previous pregnancy); occurrence during late gestation; no association with fetal thrombocytopenia; and spontaneous resolution after delivery.)

No excessive bleeding was detected in the mother at delivery with platelet counts $>50\times10^9$/l.[17]

Excessive complications were not detected when epidural punctures were carried out with platelet counts $>70\times10^9$/l.[18] Therefore, we adopted 70/l as a threshold for seeking haematological advice in asymptomatic people. Children have not had complications from lumbar punctures carried out with platelet counts $>10\times10^9$/l.[19]

For a slightly reduced platelet count (between 110 and 150×10^9/l), the national guidelines clearing house recommends rechecking ''every few months'' to ensure that the count is not falling.[1] We recommend checking every 2 months. No guidance is given for counts $<100\times10^9$/l.

What is the relevance of a high platelet count?

We recommend that if asymptomatic, in the case of a raised platelet count, the test should be repeated after an 8-week period so that any cause of reactive thrombocytosis settles or becomes overt.

Table 2 shows the suggested thresholds on confirmation of a rise in platelet count.[20 21] The categories of a high platelet count ($>450\times10^9$/l) are primary, secondary and redistribution.

- *Primary*: diagnosis is often arrived at by exclusion of secondary causes.
- *Secondary*: to conditions which result in a reactive marrow, such as infections, inflammation, trauma, malignancy or iron deficiency.
- *Redistribution*: because of splenectomy. Platelet count would not be >600 and requires no action.

Symptoms occurring with primary thrombocythaemia

- *Major complications*: Transient ischaemic attack, cerebrovascular accident, digital gangrene, venous thrombosis. Haemorrhagic problems can occur with high platelet counts ($>1000\times10^9$/l).
- *Minor complications*: Headaches, dizziness, tinnitus, visual disturbances, erythromelalgia (burning pain in the hands or feet associated with erythema and warmth), paraesthesis, leg pain, digital cyanosis.[23 24]

Patients can have thrombotic problems even when the platelet count is in, or just above, the normal range.[25] If the patient is below 60 years of age and has no other risk factors, then primary thrombocythaemia should be considered (if in doubt, seek advice from a haematologist).

Reactive thrombocytosis

- Even platelet counts $>1000\times10$/l are not associated with major thrombosis or haemorrhage.[26]

Investigations

Clinical evaluation—signs of inflammation (eg, infection, rheumatoid arthritis) or bleeding

- Temperature, C reactive protein/plasma viscosity or erythrocyte sedimentation rate, if raised, would indicate an infectious, inflammatory or malignant cause.[27]
- Ferritin or transferrin saturation, if low, would indicate a rise secondary to iron deficiency.
- Check previous blood counts if available. If platelet count is previously raised in the absence of chronic illness, this would suggest a primary cause.

What is the relevance of high haemoglobin?

We recommend firstly repeating the measurement. Consider referral if a patient has a persistently raised venous haematocrit (>0.52 for males, >0.48 for females for >2 months).[28]

Table 2 Suggested thresholds on confirmation of a rise in platelet count ($\times 10^9$/l)

<450	No further action required
450–600	If symptoms, refer If asymptomatic, investigate
600–1000	If no secondary cause, refer Consider aspirin*
>1000	Refer. Avoid aspirin unless thrombotic complications present

*Aspirin has been shown to be efficacious in primary thrombocythaemia, but the level at which it should be initiated is unknown.[22]

Clinical assessment

- *History*: Pruritis, especially after a hot bath, or splenomegaly can be a sign of primary polycythaemia. Raised WBC and platelets are suggestive of primary polycythaemia. Heavy smoking and lung disease (which can also cause a moderate increase in platelet and white cell count) may indicate apparent or secondary polycythaemia.
- *Examination*: Blood pressure, chest disease, splenomegaly.
- *Main investigations*: Chest *x* ray (if indicated).

The major pathophysiology of a raised haemoglobin level is increasing haematocrit causing an increase in whole blood viscosity. The incidence of large-vessel occlusion increases with haematocrits >0.45.[29]

High haemoglobin levels can occur with a normal whole-body red cell mass—this is called apparent erythrocytosis.[30] True polycythaemia can be assumed to be present when the haematocrit is >0.6 in males and 0.56 in females.[31]

A high haemoglobin level can be caused by a secondary polycythaemia such as hypoxic lung disease or even by chronic high levels of carboxyhaemoglobin owing to cigarette smoking. Other much less common causes include erythropoietin release from renal cysts and tumours, liver and parathyroid tumours, and uterine fibroids.[28]

A high haemoglobin level can be caused by a primary polycythaemia (incidence 2:100 000) in which the WBC and platelets may also be raised. These people are at increased risk of thrombosis even with haematocrits near, and occasionally in, the normal range, possibly caused by the panmyeloproliferative element of the condition.[32] Aspirin reduces the incidence of thrombosis in primary polycythaemia,[33] suggesting a key role for platelets in thromboembolic events.

Studies have shown that a haematocrit level in the upper normal range, or slightly raised, may be associated with an increase in thrombotic events and cardiovascular mortality compared with those with an haematocrit level in the middle or lower part of the normal range.[34] That the increase in mortality associated with apparent erythrocytosis is because of the high haematocrit level is not clear; nor are there randomised studies to show that reducing the haematocrit level in apparent erythrocytosis reduces morbidity or mortality.

Serial measurements of the haematocrit level in untreated patients with apparent erythrocytosis show that the haematocrit level returns to within the normal range in up to 30% of patients.[35]

Modifications in the factors that are associated with apparent or secondary erythrocytosis, such as obesity, smoking and hypertension, may lead to a reduction in haematocrit level.[36] In those with severe hypoxic pulmonary disease, supplementary oxygenation may be required and a review by a specialist respiratory physician should be considered. In addition, a clinically relevant minority of patients with erythrocytosis has nocturnal oxygen desaturation because of obstructive sleep apnoea,[37] and such patients should be referred for appropriate investigation.[38]

ANAEMIA CAUSED BY IRON DEFICIENCY (MJG, DB)

Tests used in the diagnosis of anaemia caused by iron deficiency have changed in recent years, and there remains some variability between the tests that laboratories offer and recommend. Their interpretation can also be complicated in the presence of acute or chronic inflammation. The guideline base is relatively limited, although reasonable consensus exists and laboratories are recommended to review the tests they offer and recommend for the investigation of iron deficiency.

In which patients with anaemia should iron deficiency be assessed and what tests should be used?

We propose that in patients with anaemia, as the mean cell volume (MCV) falls, the probability of iron deficiency increases, although no specific cut-off can be used. Even in patients with MCV <75 fl, only 68% will have iron deficiency.[39] In patients with anaemia and MCV >95 fl, there is a low probability of iron deficiency being present. Other causes should be investigated initially. Ferritin is superior to iron and iron-binding capacity or transferrin saturation.[40]

Pretest assessment

All patients with anaemia should be assessed to estimate the presence of iron deficiency. The history should include:

- review of diet
- history of bleeding
- ingestion of gastric irritant drugs or other causes of iron deficiency.

Similarly, other causes of anaemia, such as chronic inflammation or underlying malignancy, can also be assessed before testing.

Post-test assessment

A serum ferritin concentration of <15 ng/ml confirms a diagnosis of iron deficiency and a ferritin concentration of >100 ng/ml rules out the diagnosis.[40] For patients with chronic iron deficiency, ferritin concentrations between 15 and 40 ng/ml provide sufficient evidence for the diagnosis to be made and treatment to begin. For patients with chronic inflammation, the same conclusion can be reached for ferritins up to the concentration of 70 ng/ml. For ferritins above this level, however, further assessment is required before a diagnosis can be reached, as ferritin levels rise with acute or chronic inflammation. A response to iron replacement treatment is definite confirmation of deficiency.

How should iron deficiency be monitored in patients who have received replacement treatment?

We recommend that remeasurement of ferritin is not necessary. Recovery of anaemia caused by iron deficiency is assessed from haemoglobin levels, after 3 weeks, to confirm response, and after 9 weeks, to confirm recovery once the source of iron deficiency has been identified and corrected.

Once oral iron replacement treatment has been started, the haemoglobin level should rise by about 2 g/dl every 3 weeks. Therefore, it is suggested that a blood count be repeated after 2–4 weeks of treatment to make sure that there is a response.[41 42] A further full blood count should be performed at 2–4 months[42] to ensure that the haemoglobin level has returned to normal. Iron replacement should then be continued for 3–6 months once the haemoglobin level has normalised, to replenish iron stores (3 months, 4–6 months[42]). Once the diagnosis of iron deficiency has been confirmed, its cause will need to be established.[43]

When should I screen for secondary hyperlipidaemia and what investigations are required?

We recommend screening for the causes of secondary hyperlipidaemia in all patients in whom lipid-lowering treatment is being considered.

The following investigations are recommended:

- dietary, alcohol and drug history
- urine dipstick testing for protein
- serum creatinine
- liver enzymes (alkaline phosphatase and a transaminase, preferably alanine aminotransferase) and bilirubin

- bone marrow, or laboratory blood glucose if diabetes is suspected
- thyroid-stimulating hormone, if the total cholesterol level is >8 mmol/l, unless thyroid disease is suspected clinically, and free thyroxine, if there is any suggestion of pituitary hypothyroidism.

One study of 1190 people referred from primary care to a hospital lipid clinic reported a prevalence of secondary hyperlipidaemia of 1.8% when diagnosed by laboratory tests alone, despite 17% of the people having at least one abnormality of the four screening tests used (although the abnormality was not believed to be related to an underlying secondary hyperlipidaemia).[44] Although the prevalence of secondary causes would therefore appear to be low overall, the prevalence of laboratory abnormalities that may be relevant to lipid management is far greater. The prevalence of secondary causes appears to be considerably greater as lipid parameters rise. This is considered below.

Alcohol overuse and liver disease are common conditions that present with dyslipidaemia, notably hypertriglyceridaemia.[45–48]

Raised triglyceride concentrations are typically seen when people are first diagnosed with type 2 diabetes[49] and may be in excess of 20 mmol/l. Hypertriglyceridaemia is also common in type 2 diabetes in the metabolic syndrome,[50] even when blood glucose levels are well controlled.

Average cholesterol levels rise as the thyroid-stimulating hormone increases outside the reference range.[51] One study that screened 2250 Scottish people found 90 people with a total cholesterol concentration >8 mmol/l, and 12% of these had biochemical evidence of hypothyroidism.[52] Hypopituitarism should also be considered.[53]

Specific patterns of dyslipidaemia are associated with certain conditions (table 3), but these patterns are not found consistently and the associated conditions may not be clinically apparent.

Table 3 Important causes of secondary hyperlipidaemia

Lipid abnormality	Cause
Hypercholesterolaemia	Hypothyroidism Cholestatic jaundice Anorexia nervosa Nephrotic syndrome Drugs*: ciclosporin
Hypertriglyceridaemia	Hepatitis, hepatobiliary disease Alcohol misuse Diabetes mellitus Drugs*: isotretinoin, oral contraceptives (oestrogens), high doses of β-blockers, anion-exchange resins, antiretroviral drugs Pregnancy Obesity Renal failure
Combined hypertriglyceridaemia and hypercholesterolaemia	Drugs*: corticosteroids, high-dose thiazides, atypical antipsychotic agents Pregnancy Multiple myeloma Conditions that predominately cause hypertriglyceridaemia can also result in combined hyperlipidaemia in some people (eg, those with type 2 diabetes mellitus, obesity)

Secondary causes should (usually) be dealt with first and then the need for specific lipid-lowering treatment reassessed.
*If a drug is thought to be the cause of clinically relevant hyperlipidaemia, review the indications for the drug and consider alternative treatments or reduction of dose. Consider also the addition of a lipid-lowering diet, with or without lipid-lowering drug treatment.

Adapted from PRODIGY and Durrington

Widespread consensus exists about which investigations to perform to screen for the causes of secondary hyperlipidaemia,[45–48 54 55] although the International Task Force also recommends specialised tests (eg, to diagnose polycystic ovary syndrome) in specific situations. These investigations are recommended to exclude the most common conditions that cause hyperlipidaemia, irrespective of the pattern of the dyslipidaemia.

Hyperlipidaemia is rare as a presenting feature of nephrosis, but urine testing is a simple, non-invasive and inexpensive means of screening for this.

No evidence or clear expert consensus exists on when to screen for the causes of secondary hyperlipidaemia, and most guidelines do not provide absolute screening thresholds. PRODIGY[46] recommends excluding secondary causes, in particular in people with cholesterol >6.5 mmol/l or triglycerides >8.0 mmol/l. The European Atherosclerosis Society[56] and Scottish Intercollegiate Guideline Network[45] recommend exclusion of secondary causes before initiating lipid-lowering treatment.

When and why should I measure triglycerides at the same time as I measure cholesterol?

We recommend that triglycerides are measured

- in all people being assessed for cardiovascular disease (CVD) risk;
- in all people being considered for lipid-lowering treatment;[57 58]
- preferably if the initial cholesterol concentration was >2.3 mmol/l and definitely if it was >4.5 mmol/l; and
- before starting drugs known to increase triglycerides.

Triglycerides should be measured at the same time as cholesterol in certain circumstances, because raised levels

- can affect the accuracy of standard methods of calculating low-density lipoprotein (LDL) cholesterol;
- are associated with increased CVD risk;
- can indicate an undiagnosed secondary cause of hyperlipidaemia.

Triglyceride concentrations can be described as raised if >2.3 mmol/l and greatly raised if >5.6 mmol/l,[59] although thresholds for these descriptions vary.

Raised triglyceride levels are common in the general population. The Munster Heart study[60] reported a prevalence of hypertriglyceridaemia (triglycerides >2.3 mmol/l) of 5% in 20-year-old men, rising to 20% at age 45, and 2% in 20-year-old women, rising to 7% at age 60. Triglyceride concentrations >4 mmol/l are more unusual in the general population (the 95th centile in men from UK being approximately 3.8 mmol/l).[61] A raised value should be rechecked to take account of biological variability. Non-fasting values >mmol/l would appear to justify rechecking on the basis of increased cardiovascular risk in this group regardless of fasting status,[62] although a local evidence-based UK guideline has recommended a threshold of 4.5 mmol/l.[58] There would not appear to be a clear evidence-based answer to this question, although, as 4.5 approximates to the threshold for triglyceride treatment (see below), retesting on a fasting sample would appear logical.

Many laboratories offer measurement of cholesterol without simultaneous measurement of triglycerides. For reasons set out below, however, in certain circumstances we recommend measuring the triglyceride level simultaneously.[63]

Triglyceride levels have several implications for the measurement and treatment of cholesterol. Concentrations >2.3 mmol/l reduce the accuracy of methods used by many laboratories to calculate LDL cholesterol. When concentrations of triglycerides are >4.5 mmol/l, the method used to calculate LDL becomes unreliable and target total cholesterol levels cannot be used as treatment goals.[64] The NCEP recommends the use of non-HDL cholesterol as a different treatment goal if triglyceride concentrations are >2.3 mmol/l.[59]

Although it is difficult to separate triglycerides from other risk variables, raised triglycerides are associated with increased coronary risk,[64] which is reduced by treatment increasing HDL and lowering triglycerides.[65] Therefore, we recommend measuring triglycerides in all people being assessed for CVD risk. Opinions are divided on the inclusion of triglycerides as formal targets in coronary prevention.

Hypertriglyceridaemia is also a frequent finding in undiagnosed secondary dyslipidaemia, notably in diabetes,[50] and in association with high alcohol intake and certain drugs (see secondary hyperlipidaemia above). Values may rise exponentially in these situations, owing to saturation of the triglyceride-removal system.[66] Therefore, we recommend measuring triglycerides during the assessment of all people being considered for lipid-lowering treatment.

Triglyceride concentrations can fluctuate considerably over time and with diet.[57 67] The European Task Force therefore recommends that raised fasting values (>2 mmol/l) should prompt repeat measurements.

What triglyceride levels are associated with a risk of pancreatitis and require treatment on this basis?

Serum triglycerides of

- 5 mmol/l carry a probable increased risk of pancreatitis;
- 10 mmol/l carry a high risk of pancreatitis; and
- 20 mmol/l carry a very high risk of pancreatitis.

We recommend the following:

- Underlying causes, particularly diabetes, alcohol and drugs (eg, tamoxifen, oestrogens), are looked for and managed appropriately when present.
- People with triglyceride concentrations >5 mmol/l are treated.
- People with triglyceride concentrations >10 mmol/l are treated with increasing urgency the higher the triglyceride concentration.

Very high levels of triglycerides carry a risk of pancreatitis. No absolute risk threshold exists for this and thresholds for increased risk described in recent guidelines vary—6.5–13 mmol/l,[68] 10 mmol/l[69] or even 20 mmol/l.[70]

One retrospective study in 56 women with hypertriglyceridaemia (triglyceride concentrations >5.2 mmol/l, median 16.5 mmol/l) found that 30% of the women had an episode of pancreatitis during the previous 3-year period.[71] An earlier study of severe hypertriglyceridaemia (concentrations >9.8 mmol/l) associated with oestrogen replacement treatment reported that four of seven women with triglyceride concentrations >19.5 mmol/l had experienced acute pancreatitis and a further two women had acute abdominal pain thought to be pancreatitis over a period of 2.75 years.[72] Triglyceride levels that were previously regarded as being associated with an increased risk of pancreatitis are probably too conservative (C Glueck, personal communication relating to Goldenberg *et al*[71] and Glueck *et al*[72], 2005). Additionally, on the basis of the limited evidence available from these small studies, there seems to be a very high risk of pancreatitis for people with triglyceride concentrations >19.5 mmol/l; a smaller but still high risk for people with triglyceride concentrations >9.8 mmol/l; and a probable increased risk for people with triglyceride concentrations >5 mmol/l, as risk probably increases incrementally.

Most guidance recommends treatment of raised triglyceride concentrations >5 mmol/l regardless of other factors[59 68 69 73–75] and consideration of secondary referral for concentrations >10 mmol/l,[75] because of the increased likelihood of underlying medical causes and familial hyperlipidaemias.

TYPE 1 DIABETES (PT, TMR, CW)

Extensive guidelines exist for the management of diabetes, many of which have been extrapolated from the large landmark trials that are cited. This is a series of questions principally on glycated haemoglobin (HbA_{1C}) and its use in the diagnosis and monitoring of diabetes, with an additional question on microalbumin monitoring in patients with diabetes.

How often should HbA_{1c} be measured in patients with diabetes?

We recommend

- a minimum of 15-monthly HbA_{1c} measurements in all patients with diabetes;
- ideally, two measurements per year in patients who are meeting treatment goals and who have stable glycaemic control; and
- more frequent measurements, up to a maximum of four to six per year, in patients whose treatment has changed or who are not meeting treatment goals.

HbA_{1c} values enable clinicians to identify patients with poor glycaemic control, a task that is often difficult when using clinical judgement alone. Approximately half the glycation occurs in the preceding 30 days: 40% between 31 and 90 days and 10% after 90 days.[76–79] Combined with the lifespan of red cells, which have a mean survival of 120 days, HbA_{1c} value reflects a weighted mean glycaemia over the preceding 2–3 months in the absence of blood loss or haemolysis.

No consensus exists as the optimal frequency for measuring HbA_{1c} has not been well established. More frequent testing is recommended when treatment goals are not being met or when a treatment regimen is changed.

One study recommended that no more than four to six HbA_{1c} assays be carried out each year for type 1 diabetes and approximately every 6 months for type 2 diabetes.[76] Consequently, a UK health technology assessment[80] recommended 6-monthly HbA_{1c} testing in stable patients with type 2 diabetes and 3-monthly testing in those with type 1 diabetes or unstable type 2 diabetes. UK national guidelines[81 82] have generally kept to the spirit of this health technology assessment by recommending more frequent testing in type 1 diabetes mellitus, with the minimum period being 2 months since the last measurement.

The American Diabetes Association (ADA)[83] and the National Academy of Clinical Biochemistry[84] recommend HbA_{1c} testing at least twice per year in patients who are meeting treatment goals (and who have stable glycaemic control) and quarterly in patients whose treatment has been changed or who are not meeting glycaemic goals. The American Association of Clinical Endocrinologists and American College of Endocrinology[85] recommend a minimum of 3-monthly HbA_{1c} assessments. Neither the ADA, National Academy of Clinical Biochemistry nor the American Association of Clinical Endocrinologists/American College of Endocrinology differentiate between patients with type 1 and type 2 diabetes.

Despite the above recommendations, US quality assurance programmes[86] are based on at least one HbA_{1c} determination during the preceding year (GMS contract indicator greater than at least one HbA_{1c} during the previous 15 months).

When should HbA_{1c} be used in the diagnosis of diabetes or in patients without diabetes?

We do not recommend that HbA_{1c} be used in the diagnosis of diabetes or in patients without the disease.

HbA_{1c} is not a diagnostic criterion for diabetes; no national or international guideline recommends that HbA_{1c} be used in the diagnosis of diabetes or in patients without diabetes. Conversely, several authors and advisory documents specifically state that HbA_{1c} does not have a role in the diagnosis of diabetes.[83 87–89]

It is also a poor screening test for diabetes. Kilpatrick *et al*[90] evaluated the index of variability in people without diabetes and found this to be 0.27 (the ideal value being >1.4 for a screening test; index of variability <0.6 implies that a test is of little value for screening purposes).

How often should microalbumin be measured in patients with diabetes?

We recommend microalbumin measurement once in every 12–15 months in all adult patients with diabetes.

Before designating a patient as having microalbuminuria, at least two of three samples tested in a 3–6-month period should contain raised levels of microalbumin.

Diabetic nephropathy is the single leading cause of end-stage renal disease. Persistent microalbuminuria is the earliest stage of diabetic nephropathy and is also a marker of increased CVD risk.[91] The term ''microalbumin'' is technically used incorrectly in most situations, as it refers to the use of albumin testing methods that detect small (or micro) quantities of urinary albumin below the levels detected by conventional urine test strips. As the term microalbumin is widely used, however, it will be adopted in this answer.

In type 1 diabetes, the cumulative incidence of microalbuminuria at disease duration of 30 years is approximately 40%, and these patients have a relative risk of developing proteinuria of 9.3 compared with patients with normoalbuminuria. Approximately 20% of patients with type 1 diabetes develop proteinuria after a disease duration of 25 years.

In type 2 diabetes, the cumulative incidence of microalbuminuria at a disease duration of 10 years is approximately 20–25%. One fifth of these patients who survive for 10 years develop proteinuria. The prevalence of proteinuria in patients with type 2 diabetes is approximately 15%.

UK and American guidelines recommend annual microalbumin measurements in adult patients with diabetes.[83] First-morning samples are generally recommended, but this issue is not dealt with by the ADA.[83] There is agreement that urinary microalbumin assessment should be performed from diagnosis onwards in patients with type 2 diabetes. Slight divergences, however, are observed in the starting point for monitoring type 1 diabetes—the Scottish Intercollegiate Guideline Network[92] recommends beginning at the age of 12, whereas the ADA[83] recommends that the child is 10 years of age and has had diabetes for 5 years. This has recently been reviewed by the National Institute for Health and Clinical Excellence (NICE).[81]

Before designating persistent microalbuminuria, it is important to confirm the result, because of the inherent variability in microalbuminuria results (usually one more positive test of two additional tests is sufficient). The NICE[81] type 1 guidelines specifically refer to the absence of proteinuria/urinary tract infections: proteinuria detectable by urine stick testing represents a stage beyond microalbuminuria, and urinary tract infection causes proteinuria unrelated to diabetic renal disease, which resolves on treatment. No consensus exists on the length of the confirmation period, with repeat tests to be performed ''without delay'',[92] ''within one month where possible'' (NICE type 2[93]), ''at least every three to four months'' (NICE type 1[81]) or ''within a 6-month period''.[83]

The role of regular microalbuminuria assessment after the designation of persistent microalbuminuria and the beginning of treatment has not been fully dealt with by clinical trials and thus is not specifically considered by the various guidelines. Many doctors continue surveillance to assess the response to treatment and progression of the disease (GMS contract indicator: at least one microalbumin measurement in the preceding 15 months).

How are HbA_{1c} values interpreted?

As the absolute risks and benefits of lower targets are currently unknown, we recommend a general HbA_{1c} target of <7.5% aligned by the Diabetes Control and Complications Trial (DCCT) Research Group (and an ideal target of <6.5–7.0%), which should be individualised for each patient, noting their life expectancy and age, the incidence of hypoglycaemia, comorbid conditions and the potential for marked interindividual differences between mean blood glucose values and HbA_{1c} levels.

Glycaemic control is fundamental to the management of diabetes mellitus. Prospective randomised clinical trials such as DCCT[94] and United Kingdom Prospective Diabetes[95] showed that more intensive hypoglycaemic regimens, and thus lower HbA_{1c} values aligned by the DCCT, are associated with decreased rates of microvascular end points. These trials, however, showed that intensive glycaemic control is associated with severe hypoglycaemia and weight gain.

Laboratories should report DCCT-aligned HbA_{1c} values[82] until the International Federation for Clinical Chemistry calibration is fully implemented. Samples for HbA_{1c} assays do not require any specialist timing relative to food.

The optimal target for HbA_{1c} is yet to be established and, accordingly, there is no consensus between guidelines. All, however, acknowledge that targets need to be individualised with the clinical risk of diabetic complications balanced against the risk of severe hypoglycaemic episodes. Assuming a non-diabetic reference interval of 4.0–6.0%, HbA_{1c} targets include <6.5% (International Diabetes Federation,[96] American College of Endocrinology,[97] NICE type 1—high cardiovascular risk[81]), 6.5–7.5% (NICE type 2[82]), <7.0% (ADA[83] and NSF after the first year[99]) and <7.5% (NICE type 1—not high cardiovascular risk[81]).

Although no optimal HbA_{1c} target currently exists, goals should be individualised to achieve as normal glucose values as possible. Interestingly, although there may be no population HbA_{1c} threshold for cardiovascular end points, the relationship was not linear in DCCT. Of the macrovascular benefit seen when mean population HbA_{1c} values decreased from 9.0% to 7.0%, approximately 50% and 70% of the decrease in events occurred at mean HbA_{1c} values of 8.6% and 8.0%, respectively.[99]

Before alignment of HbA_{1c} by DCCT, it was difficult to meaningfully compare different HbA_{1c} methods. DCCT alignment and International Federation for Clinical Chemistry calibration, however, do not overcome method-specific issues. As proper interpretation of HbA_{1c} results requires an understanding of the assay method, including its reference interval, precision, known interferences and specificities, such as abnormal haemoglobins or haemoglobinopathies (independent of any altered erythrocyte lifespan), laboratories should provide the healthcare professional of this information.

Proper interpretation also requires knowledge of the kinetics of glycation and the relationship between HbA_{1c} and mean plasma glucose. Large intraindividual differences exist in the relationship between mean plasma glucose levels of the population and HbA_{1c}. DCCT[100] showed that HbA_{1c} values ranged between 6% and 11% in patients with a mean plasma glucose concentration of 10 mmol/l. Aiming for the same HbA_{1c} in all patients therefore implies that the target will be unrealistically low in some (with the potential risk of hypoglycaemia) and unrealistically high in others, providing false reassurance.[101] HbA_{1c} results can be affected by conditions other than diabetes. Some of these can be method dependent—for example, pregnancy, increased erythrocyte turnover, anaemia caused by iron deficiency and blood transfusion. Quoted target values cannot be used in such patients, in whom more emphasis may also be needed on the use of blood glucose self-monitoring results to assess the relationship between HbA_{1c} and mean blood glucose.

Although the glycosylation kinetics, in theory, allow for frequent HbA_{1c} monitoring, small changes in values during a short time period may reflect biological variation or imprecision of assay. Statistically, a total coefficient of variation of 2.5% around a result of 7% would result in a 95% confidence interval of 6.6–7.4%. Care must therefore be taken in interpreting small changes in HbA_{1c} (GMS indicator HbA_{1C} proportion of registered diabetic patients $\leqslant$7.4%).

CONCLUSION

This review brings to a running total of 42 question–answer sets written to provide an overview of current advice in the use of laboratory tests in primary care. Answers to the first two question–answer sets can be found in Smellie *et al*.[102] All of them have used a common search method,[103] although the guidance relies heavily also on the findings of recent systematic reviews. Authors wishing to consult the UK General Medical Services contract and related Quality and Outcomes Framework can refer to the General Medical Services contract[104] and Quality and Outcomes Framework,[105] respectively.

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Take-home messages

- Guidance on the use and interpretation of laboratory tests in primary care is fragmented. This review series examines series of clinical scenarios and summarises available guidance on test use and interpretation.
- The first of the scenarios examined is ''abnormal'' haemoglobin and platelet counts.
- The second scenario considers diagnosis and monitoring of anaemia caused by iron deficiency.
- The third scenario considers testing for secondary causes of hyperlipidaemia and hypertriglyceridaemia.
- The fourth scenario considers glycated haemoglobin and microalbumin measurements in patients with diabetes.

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