REVIEW

Best practice in primary care pathology: review 10

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This tenth best practice review examines four series of common primary care questions in laboratory medicine: (i) antenatal testing in pregnant women; (ii) estimated glomerular filtration rate calculation; (iii) safety testing for methotrexate; and (iv) blood glucose measurement 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 are consensus rather than evidence-based. They will be updated periodically to take account of new information.

This is the tenth in a planned series of reviews to answer a number of questions which arise in primary care use of pathology.

Each subject is introduced with a brief summary of the type of information found and is handled separately, with authorship attributed.

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

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.

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ANTENATAL TESTS IN NORMAL PREGNANCY (MFS, JBH AND PRC)

The recommendations for normal pregnancy given in this article are based largely on the guideline entitled ''Antenatal care: routine care for the healthy pregnant woman'' published in October 2003,[1] commissioned by the National Institute for Health and Clinical Excellence (NICE) from the National Collaborating Centre for Women's and Children's Health.

The ethos of the current guideline is that pregnancy is a normal physiological process. Any interventions offered (including laboratory tests) should have known benefits and be acceptable to pregnant women.

The guideline also stresses the importance of communicating the purpose of tests and informing women of all results.

Some women will require additional care because of pre-existing medical conditions or risk factors for complicated pregnancy (see box 1). This article does not address how to identify or manage these individuals. The merits of screening normal, healthy women for a number of conditions are not clearly established and this article highlights some areas where uncertainty remains.

What tests should I perform on a newly pregnant woman (first and subsequent pregnancies)?

We recommend the following:

- *Clinical biochemistry*
  - Down syndrome screening at the first antenatal appointment
  - Urinalysis for protein and blood and blood pressure measurement at each antenatal visit (10 appointments are recommended for a nulliparous woman)
  - No other biochemical tests are necessary systematically
  - Screening for plasma fasting glucose at booking and 28 weeks in women identified to be at higher risk of gestational diabetes mellitus (GDM) (box 1)
  - Systematic (universal) screening at 28 weeks may be beneficial.
- *Haematology*. All the following tests should be offered at the first antenatal appointment and if accepted, arranged before 16 weeks of pregnancy:
  - ABO blood group
  - Rhesus D (RhD) status
  - Atypical red cell alloantibodies
  - Full blood count (FBC)
  - Repeat screening for anaemia and atypical antibodies (regardless of RhD status) should be offered at 28 weeks
  - Haemoglobinopathy screening (unless previous documented result).
- *Microbiology/virology*. All the following tests should be offered at the first antenatal appointment and if accepted, arranged before 16 weeks of pregnancy:
  - Screening for rubella antibody, syphilis, HIV and hepatitis B

- Screening for asymptomatic bacteriuria
- Screening for group B streptococcus (GBS) is not currently recommended in the UK
- Pregnant women should not be offered routine screening for asymptomatic bacterial vaginosis or chlamydia infection
- Pregnant women should not be offered routine screening for cytomegalovirus, toxoplasmosis or hepatitis C.

Biochemical tests

Down syndrome screening

This is offered and explained at the first antenatal visit. If accepted, ultrasound assessment is carried out at 11–14 weeks and serum testing 14–20 weeks. This will not be considered further as it is the subject of individual national screening policies and practices. New developments are being evaluated in the UK, in particular, serum testing with different marker combinations at an earlier stage in pregnancy (11–13 weeks). The current UK policy position may be accessed at the NHS Antenatal and Newborn Screening programme website (www.screening.nhs.uk/downs/procedures.htm).

Urinalysis for proteinuria

Pre-eclampsia is a multi-system disorder affecting 2–10% of pregnancies and results in increased maternal and neonatal morbidity and mortality. It is defined as hypertension new to pregnancy manifesting after 20 weeks of gestation associated with a new onset of proteinuria, which resolves after delivery. Hypertension new to pregnancy without proteinuria and resolving after delivery is termed pregnancy-induced hypertension.

Reagent strip (dipstick) urinalysis is sufficient for initial testing but is prone to observer error. Strips must be used within the expiry date and according to manufacturers' instructions. Automated reading devices significantly reduce both false positive and false negative rates but add to the cost.[2] An initial result of $\geqslant$1+ should be confirmed by laboratory measurement of protein/creatinine ratio on a random urine sample. A protein/creatinine ratio of 30 mg/mmol is regarded as significant.[3 4]

Other biochemical tests

No other biochemical tests are necessary. In particular, universal screening for gestational diabetes mellitus (GDM), including dipstick testing for glycosuria, is not currently recommended by either NICE[1] or the UK National Screening Committee[5] because it meets only some of the well established criteria for such a programme.[6] This field is beset by uncertainty over definitions, diagnostic tests and optimal clinical management. The World Health Organization (WHO) defines GDM as any degree of carbohydrate intolerance with onset or first recognition in pregnancy, applying criteria from non-pregnant subjects to identify ''abnormal'' blood or plasma glucose levels. Unanswered questions remain:

- Is it appropriate to define an abnormality in pregnant women using data from non-pregnant subjects when there is a physiological increase in glucose levels in pregnancy?
- Does intervention improve outcomes for mother and baby at all levels of abnormal glucose tolerance in pregnancy?

The Australian Carbohydrate Intolerance Study (ACHOIS)[7] has reopened the debate in the UK as it provides evidence that active intervention in women with relatively mild impairment of glucose tolerance results in improved perinatal outcomes. The Scottish SIGN guideline[8] recommends screening in pregnancy in urine at every antenatal visit. It recommends a random venous plasma glucose if 2+ glycosuria is detected, and routinely at 28 weeks gestation. It adds that the WHO advises that a 75 g oral glucose tolerance test (OGTT) should be carried out if the blood glucose is $>$5.5 mmol/l two hours or more after food, or $>$7 mmol/l within two hours of food.

In addition the American Diabetes Association guidance[9] recommends that testing may not be needed in women with no risk factors for developing GDM, but testing all others.

Recent guideline development suggest that an approach of screening routinely at 28 weeks with targeted screening of higher risk patients (box 1) at booking or in the first trimester may emerge (Professor R Bilous, personal communication, Feb 2007).

Further valuable data is expected from the Hyperglycaemia and Adverse Pregnancy Outcome Study (HAPO).[10] This is a large, epidemiological study of a heterogeneous, ethnically diverse cohort of 25 000 women designed to clarify the relationship between adverse pregnancy outcomes and various levels of glucose intolerance less severe than overt diabetes mellitus.

There is no consensus on which screening test would be most appropriate for a universal programme and a variety of policies currently prevail in UK obstetric practice.[6] It is to be hoped that a more coherent national approach can be recommended in the anticipated update to NICE guidance which is due to be published in 2007.

It should be noted that in practice urine protein dipstick tests frequently also measure glucose and that this level of screening facility is probably therefore in widespread use although is insensitive compared to fasting plasma glucose or OGTT.

Box 1: Higher risk women who may justify screening at booking or in first trimester

- Severe overweight (body mass index $>$30 kg/m^2)
- Past history of poor pregnancy outcome
- First degree family history of diabetes
- Previous history of disorder of glucose metabolism
- High risk ethnic origin
- Possible, older women

Haematological tests

ABO blood group, RhD status and atypical red cell alloantibodies

This is to identify any possible transfusion problems which might arise, anticipate the need for anti-D prophylaxis in RhD negative women and to determine the risk of other types of haemolytic disease of the newborn.

FBC

FBC will detect anaemia (low haemoglobin), but also less commonly, white cell and platelet abnormalities. The normal UK haemoglobin in pregnancy is accepted as $\geqslant$110 g/l at up to 12 weeks, and $\geqslant$105 g/l at 28–30 weeks. A significantly abnormal result will dictate further investigations, for example ferritin if haemoglobin and mean cell volume (MCV) are reduced. Serum ferritin is the most sensitive single screening test to detect adequate iron stores, with a sensitivity of 90% at a cut-off of 30 μg/l.

Haemoglobinopathy (principally thalassaemia and sickle cell anaemia)

An NHS Sickle Cell and Thalassaemia screening programme for pregnant women commenced in April 2005.[5 11] All pregnant

women in Trusts defined as high prevalence (sickle cell disease expected to affect more than 1.5 per 10 000 pregnancies) should be offered antenatal screening. Women in low prevalence Trusts (sickle cell disease expected in less than 1.5 per 10 000 pregnancies) should, from April 2006, be offered screening based on family origin and formal inspection of blood count indices.

Microbiological tests

Screening for rubella, syphilis, HIV and hepatitis B

Testing for rubella susceptibility identifies women at risk of contracting infection and who need vaccination in the postnatal period to protect future pregnancies. Although syphilis is relatively rare in the UK, treatment as early as possible is beneficial to mother and fetus. Antenatal intervention in HIV positive women and postnatal intervention in hepatitis B positive women reduces mother to child transmission. Tests for all four of these infectious diseases can be carried out on a single blood sample, and there are well-established pathways for dealing with positive screening results of

serological tests

Screening for asymptomatic bacteriuria (first and subsequent pregnancies)

All pregnant women should be offered routine screening for asymptomatic bacteriuria by midstream urine culture at their first antenatal visit. It may be helpful to repeat samples if contamination is suspected. If, however, there is a pure or predominant growth of 10^5 organisms per ml, the woman should be treated with an appropriate antimicrobial agent.[1] A further urine culture should be performed as a test of cure and again at regular intervals (monthly) for the remainder of gestation.

The prevalence of asymptomatic bacteriuria in pregnancy is about 5%, the same as in non-pregnant women. During pregnancy there is a dilatation of the ureters and renal pelvices with decreased ureteric peristalsis, changes beginning as early as the seventh week of gestation.[12] This predisposes to infection, and about a third of pregnant women with untreated bacteriuria develop acute pyelonephritis.[13] Infection may also be complicated by low birth weight and prematurity, pre-eclampsia, maternal anaemia, amnionitis and intrauterine death.[1] More controversial is whether screening is cost effective. This depends partly on the nature of the population being screened (affluent populations have a lower prevalence of bacteriuria). The two most widely used strategies for diagnosing bacteriuria are the use of leucocyte esterase-nitrite dipsticks and quantitative urine culture. Urine culture is more expensive, but has a higher sensitivity and specificity and is therefore the recommended test.[1 14]

Based on the original work of Kass in 1957,[15] bacteriuria has traditionally been diagnosed on the basis of a pure or predominant growth of 10^5 organisms (enterobacteria, ''coliforms'') per ml of a properly taken clean catch or midstream specimen of urine.[13] Kass recommended that positive cultures should be repeated for confirmation on the basis that two positive results increase the post-test probability of true bacteriuria from 80% to 95%. However, workers have relied on single samples,[13] and NICE currently recommends treatment based on the result of a single screening test.[1]

A midstream urine culture should be requested routinely at first antenatal visit. A urine culture should be performed 7 days after antibiotic treatment as a test of cure, and at regular intervals (monthly) for the remainder of gestation, as women whose bacteriuria fails to respond to treatment are at highest risk of developing symptomatic infection.[12]

Screening for group B haemolytic streptococcus (*Streptococcus agalactiae*)

Antenatal screening for group B Streptococcus (GBS) is not currently recommended in the UK. Pregnant women with risk factors for early-onset neonatal GBS disease may be offered intrapartum chemoprophylaxis; therefore GBS cultured incidentally from antenatal samples, including urine samples, should be reported by the laboratory.

GBS is the most frequent cause of severe early-onset neonatal infection in the UK, causing septicaemia, pneumonia and meningitis. The incidence is estimated to be 0.5/1000 births and approximately 10% of infections are fatal.[1 16] GBS may also cause maternal infections such as amnionitis, endometritis or septicaemia. Risk factors for early-onset GBS disease are intrapartum fever, prolonged rupture of membranes, delivery at <37 weeks gestation and a previous infant with GBS disease. GBS is carried asymptomatically by approximately 25% of pregnant women in the UK. In the USA, where the incidence of GBS disease was previously three times that in the UK, interventions have been associated with a reduction in GBS disease.[17] In the USA, a universal culture-based screening programme is used to detect GBS carriage, using both vaginal and rectal swabs at 35–37 weeks gestation. Intrapartum antimicrobial prophylaxis is given to GBS-colonised women and those with risk factors for GBS disease, supported by the use of algorithms.[17] The effectiveness of screening in preventing early-onset neonatal GBS disease has been estimated at 50–80% based on observational studies.[1 16] Since the introduction of universal screening in the USA in the 1990s, the incidence of early-onset GBS disease has fallen to a similar level to that seen in the UK.

In the UK, NICE has recommended that women should not be offered routine antenatal screening for GBS because evidence for clinical and cost effectiveness remains uncertain.[1]

Box 2: Pregnant women who may need additional care

- Conditions such as hypertension, cardiac or renal disease, endocrine, psychiatric or haematological disorders, epilepsy, diabetes, autoimmune diseases, cancer, HIV
- Factors that make the woman vulnerable, such as lack of social support
- Age $\geqslant$40 years or $\leqslant$18 years
- Body mass index greater $\geqslant$35 or $<$18
- Previous caesarean section
- Severe pre-eclampsia, HELLP (haemolysis, elevated liver enzymes, low platelets) or eclampsia
- Previous pre-eclampsia or eclampsia
- Three or more miscarriages
- Previous preterm birth or mid-trimester loss
- Previous psychiatric illness or puerperal psychosis
- Previous neonatal death or stillbirth
- Previous baby with congenital abnormality
- Previous small-for-gestational-age or large-for-gestational-age infant
- Family history of genetic disorder

Box 2 is adapted from the practice algorithm in NICE Clinical Guideline 6.[1]

Screening and prophylaxis strategies have not demonstrated an overall effect on all-cause neonatal sepsis or neonatal mortality.[16] However, since approximately 60% of early-onset GBS cases have risk factors for GBS, the Royal College of Obstetricians & Gynaecologists currently recommends that pregnant women should be considered for intrapartum prophylaxis on the basis of risk factors, rather than culture-based screening.[16]

Screening for bacterial vaginosis

Bacterial vaginosis (BV) is the most common cause of vaginal discharge and malodour. BV results from a change in the normal flora of the vagina with a relative overgrowth of anaerobic bacteria. The condition is not sexually transmitted, but is associated with sexual activity. The presence of BV during pregnancy is around 10–20%, and 50% of these women are asymptomatic. BV is associated with pre-term birth: women with BV are twice likely to deliver pre-term than women without BV. There is no evidence that screening and treating asymptomatic women who are not at high risk improves outcomes such as pre-term labour or birth.[1 18] Further studies are required to define the role of screening and treating for BV in pregnant women who have experienced previous pre-term delivery, as treatment may reduce pre-term birth in these women.

Conclusion

In conclusion, a limited number of investigations are recommended for a newly pregnant woman who is healthy (box 2). These should be offered and explained at the first antenatal visit.

GMS contract indicator: none.

CALCULATION OF ESTIMATED GLOMERULAR FILTRATION RATE IN ADULTS (PJT)

This question considers the recommended method used to calculate estimated glomerular filtration rate (eGFR) and frequency of measurement in patients with chronic kidney disease. Different methods are available in textbooks and on the internet for rapid calculation of eGFR; they may, however, produce different results and confuse serial interpretation of renal function testing. The answers do not consider further testing which may be appropriate in patients with chronic kidney disease, for which the reader is referred to the joint Renal Association/Royal College of Physicians 2005 guidance.[19]

Box 3: Recommended antenatal tests: summary

- Laboratory tests to be arranged early in pregnancy (before 16 weeks gestation)
  - ABO blood group, rhesus D (RhD) status and atypical red cell alloantibodies
  - full blood count (to screen for anaemia)
  - haemoglobinopathy (sickle cell and thalassaemia)
  - hepatitis B surface antigen
  - HIV 1/2 antibody
  - rubella IgG antibody
  - syphilis serology
  - midstream urine culture for asymptomatic bacteriuria
  - urinalysis for protein
  - serum screening for Down syndrome at 14-20 weeks
- Laboratory tests to be repeated during pregnancy
  - urinalysis for protein, along with blood pressure measurement at each antenatal visit
  - repeat full blood count and atypical red cell alloantibodies at 28 weeks

Box 3 is adapted from the practice algorithm in NICE Clinical Guideline 6.[1]

How should I estimate glomerular filtration rate?

We recommend:

- Using the MDRD 4 equation adjusted for laboratory method, either as calculated by the laboratory performing the tests or using the equation provided by that laboratory.
- Other calculations (particularly Cockcroft and Gault) will not produce parallel results and should not be used to classify CKD along current guidelines.

The principal function of the glomeruli is to filter water and low molecular weight components of the blood while retaining cells and high molecular weight components. The glomerular filtration rate (GFR) is thus the rate at which substances are filtered through the glomeruli and cannot be directly measured. It plays an important role in the diagnosis and treatment of chronic kidney disease (CKD) as it precedes clinical symptoms in all forms of progressive kidney disease. It is thus a marker of progression, and a predictor for the onset and complications of renal disease. It must be noted that while eGFR is the pillar of CKD diagnosis, staging and management it is only one component of clinical assessment.

Gold standard methods for the assessment of GFR are impractical for widespread use; creatinine is an insensitive marker of CKD, while measured creatinine clearance using a 24 hour urine sample is unreliable. Formula based estimations of glomerular filtration rate (eGFR) are more sensitive than creatinine for detecting moderate CKD. Accordingly, the National Service Framework (NSF) for Renal Services recommended the use of eGFR[20] in adults. Subsequently, the use of the isotope dilution mass spectrometry (ID-MS) creatinine traceable version of the four-variable modification of diet in renal disease (MDRD) formula was recommended[21] for the estimation of glomerular filtration rate (eGFR) by clinical biochemistry laboratories:

$$\text{GFR (ml/min/1.73 m}^2) = 175 \times [\text{serum creatinine } (\mu\text{mol/l})/88.4]^{-1.154} \times (\text{age})^{-0.203} \times [1.212 \text{ if black}] \times [0.742 \text{ if female}]$$

This version differs from the initial four-variable MDRD formula.[22] If the patient is not Caucasian or African-Caribbean, an assumption of Caucasian ethnicity can be made as there currently is no evidence to suggest that eGFR is invalid in these groups.[21] If the ethnic origin of the patient is unknown, then the patient should be assumed to be Caucasian until the ethnicity is confirmed. While the equation has not been validated for other ethnic groups or in those >70 years, eGFR is still recommended as it is more accurate than serum creatinine alone.

To improve inter-laboratory agreement, laboratories should apply method specific correction factors in order to improve alignment between creatinine assays.[23] This takes into account the slope and intercept of the method when compared to ID-MS measured creatinine. It is important that such laboratories utilise the assays as intended by their manufacturers.[23] The recommended method for laboratories to use is:

$$\text{GFR (ml/min/1.73 m}^2) = 175 \times ((\text{method-specific intercept} + [\text{serum creatinine } (\mu\text{mol/l})]/\text{slope})/88.4)^{-1.154} \times (\text{age})^{-0.203} \times [1.212 \text{ if black}] \times [0.742 \text{ if female}]$$

It should be noted that eGFR is an estimate that assumes that the patient is representative, for weight and height, of their age, gender and race; accordingly, it should be employed in conjunction with clinical judgement. In particular, it has not been validated for use in patients with pregnancy, malnourishment, amputations, muscle wasting disorders, oedematous states and acute renal impairment. Situations of proportionately low muscle bulk will tend to overestimate eGFR and those of increased muscle mass to underestimate it. All adult serum or plasma creatinine requests received by laboratories should have eGFR reported, bearing in mind the above issues.[23]

The US National Kidney Foundation has derived five stages of CKD based on eGFR (see table 1). CKD is defined as either kidney damage or eGFR <60 ml/min/1.73 m^2 for ⩾3 months, with kidney damage being defined as pathological abnormalities or markers of damage including abnormalities in blood, urine or imaging tests. These conditions include persistent microalbuminuria, persistent proteinuria, persistent haematuria (non-urological), renal structural abnormalities such as polycystic kidney disease or reflux nephropathy, and biopsy-proven chronic glomerulonephritis. Accordingly, eGFR values ⩾60 ml/min/1.73 m^2 (stages 1 and 2) are normal unless other evidence of kidney damage exists. Values of eGFR <60 ml/min/1.73 m^2 must be confirmed after 3 months to classify the patient as having CKD.

It should be noted that many pharmacy computations for drug dosage adjustment are based on the e GFR calculated by the Cockcroft and Gault equation, on which the original computations were based. This produces different results and is adjusted for body surface area. This is a current source of potential confusion.

How often should I measure eGFR?

We recommend the following.

CKD stages 1–3

- At least 12 monthly if changing by <15% between successive measurements.
- 6 monthly if changing by >15% between successive measurements.

CKD stages 4 and 5

- At least 6 monthly if changing by <15% between successive measurements.
- At least 3 monthly if changing by >15% between successive measurements.

Patients with CKD 3 should have a repeat eGFR at least every 12 months if eGFR is stable and 6 monthly if not.[19] The definition of stable is a relative eGFR change of <15%[24] over 6 months; for example, a change from 59 ml/min/1.73 m^2 to 55 ml/min/1.73 m^2 is a change of (59–55) × 100/59 or 2.4%, which is consistent with being stable, whereas a change from 59 ml/min/1.73 m^2 to 49 ml/min/1.73 m^2 is a change of (59–49) × 100/59 or 16.9%, which is not consistent with being stable. Patients with CKD 4 and 5 should have a repeat eGFR at least every 6 months if eGFR is stable, and 3 monthly if not.[19] Patients with eGFRs consistent with CKD 1 and 2 with diabetes, vascular disease, heart failure, hypertension, urinary tract obstruction, neurogenic bladder or surgical urinary diversion, people taking diuretics, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers and people with a family history or genetic risk of kidney disease should have a repeat eGFR at least every 12 months.[25]

Table 1 Stages of chronic kidney disease (CKD)

CKD stage	Description	eGFR (ml/min/1.73 m^2)
1	Normal or increased eGFR*	⩾90
2	Mildly decreased eGFR*	60–89
3	Moderately decreased eGFR	30–59
4	Severely decreased eGFR	15–29
5	Kidney failure	<15 (or dialysis)

eGFR, estimated glomerular filtration rate.
*Normal unless other evidence of kidney damage exists.

Independent of the individual clinical profile, what tests should I carry out in addition to eGFR testing?

CKD stages 1–2

We do not recommend any additional tests.

CKD stage 3

Haemoglobin, serum cholesterol, potassium, calcium, phosphate and urine dipstick measurement for proteinuria (microalbuminuria in diabetic patients) are recommended annually. If the urine dipstick for proteinuria is positive, laboratory quantification of the protein creatinine ratio is advised. Fasting plasma glucose is recommended every 5 years.

CKD stages 4 and 5

- Haemoglobin, serum potassium, calcium, and phosphate measurement are recommended at least 6 monthly if eGFR changes by <15% between successive measurements.
- Haemoglobin, serum potassium, calcium and phosphate measurement are recommended at least 3 monthly if eGFR changes by >15% between successive measurements.
- Annual serum cholesterol and urine dipstick measurement for proteinuria (microalbuminuria in diabetic patients) are recommended. If the urine dipstick for proteinuria is positive, laboratory quantification of the protein creatinine ratio is advised.
- These patients will normally be under the (joint) care of a nephrology service.

The majority of patients with CKD are at increased risk of cardiovascular disease. However, independent of established vascular disease, diabetes or hypertension, there is no agreement whether such patients should receive primary or secondary vascular prevention.

While a very small minority of patients with CKD progress to end stage kidney disease, it is important to identify these patients. Persistent proteinuria (protein:creatinine ratio >100 mg/mmol) is the best indicator of this risk, although microalbuminuria should be used in diabetic patients.

Clinically significant bone mineral disorders due to kidney impairment are uncommon in people with stage 3 CKD. Though biochemical abnormalities can develop, routine requests for parathyroid hormone and vitamin D assays are not recommended when inorganic phosphate is not elevated, or the patient is in primary care.

Anaemia due to kidney disease is uncommon in stage 3 CKD, except in those with diabetes mellitus or an eGFR of <45 ml/min/1.73 m^2. All patients with anaemia should be investigated for alternative causes before ascribing this to CKD.

GMS contract indicator: register of CKD patients stages 3–5.

SAFETY MONITORING IN THE USE OF LOW DOSE METHOTREXATE (AMK, JPN)

This answer considers the monitoring recommended for patients being treated in shared care, with specialist supervision, with low dose methotrexate for inflammatory disease (rheumatoid arthritis, psoriasis). It does not apply to use in

malignant disease and other occasional unlicensed indications, for which individual guidance from the specialist concerned should be observed. Serious incidents have occurred frequently with methotrexate, a significant proportion of which derive from failure to monitor according to guidelines or to act appropriately on monitoring results.

What safety monitoring is required for methotrexate used in non-malignant disease?

We recommend:

- Baseline full blood count (FBC), serum creatinine and liver function tests (LFTs) before methotrexate therapy is commenced.
- FBC, serum creatinine and LFTs should be monitored every week until the dose of methotrexate is stable.
- Once the dose is stable, FBC, serum creatinine and LFTs should be checked every 1–2 months.
- For patients taking methotrexate for psoriasis, monitoring of serum amino-terminal peptide of type III procollagen (PIIINP), a marker of hepatic fibrosis, is also recommended if available, every 2–3 months.

The recommendations in this article refer to the use of methotrexate in low dose as a disease-modifying agent as opposed to its use in high dose as a chemotherapeutic agent. Methotrexate is licensed for use in moderate to severe rheumatoid arthritis and in severe recalcitrant psoriasis, which is unresponsive to conventional therapy.[26] It is unusual as it is given only once weekly, either orally or parenterally. Common side effects include nausea, diarrhoea, mucositis and abnormal LFTs.[27] The most serious toxicities are hepatic fibrosis and cirrhosis, pneumonitis and myelosuppression.[28] Weekly folic acid treatment reduces the risk of gastrointestinal and mucosal side effects.[29]

The National Patient Safety Agency (NPSA) reviewed 137 incidents related to oral methotrexate use in the UK.[30] Eight out of 25 reported deaths and 2 out of 26 incidents of serious harm, requiring hospitalisation, were due to failure of monitoring procedures, including: failure to perform regular blood tests, delays in receiving results and failure to alter treatment after receiving abnormal results. Despite the safety report, incidents have continued to be reported to the NPSA.[31]

The incidence of cytopenia (neutropenia, thrombocytopenia or pancytopenia) is not high, estimated at 1% in patients with rheumatoid arthritis receiving low-dose methotrexate.[32] However, this toxicity may be fatal in up to 15–20% of cases.[32] Although the cytopenia can occur at any time during treatment,[32] neutropenia with low-dose methotrexate occurs late with a median delay of around 16 months, which suggests a cumulative dose effect as the likely mechanism.[33] Earlier onset or a more severe degree of myelosuppression can occur and are thought to be due to elevated levels of methotrexate as a result of renal impairment, drug interactions, hypoalbuminaemia and drug errors.[32 34] However, severe pancytopenia can occur suddenly, without warning signs and independent of dose of methotrexate; this complication very likely represents an idiosyncratic drug reaction.[35]

For practical reasons, the recommendation for monitoring haematological toxicity of low-dose methotrexate therapy is to check the blood counts prior to starting, then weekly until the dose is stable and subsequently every 1–2 months. While such monitoring will help clinicians to identify patients who have developed subclinical or asymptomatic haematological toxicity, it is important that patients are also well educated in recognising symptoms or signs of cytopenia (eg, fever, sore throat, bleeding) and seek urgent medical attention to have their blood counts checked sooner than planned; this is because cytopenia can occur in an unpredictable fashion in some groups of patients.[32 35]

All the guidelines reviewed[26 30 36–38] recommend pretreatment assessment of renal function with serum urea, electrolytes and creatinine. Methotrexate is concentrated in the kidney and 60–95% of a dose is renally excreted. Therefore, methotrexate is contraindicated in patients with significant renal impairment.[39] Monitoring recommendations, during dose stabilisation, vary from weekly[26 38] to every two weeks.[30 37] In the stable patient opinion is divided as to the frequency of monitoring required, from 4–6 weekly,[28 37] 2–3 monthly[26 30 38] or 6–12 monthly.[36] Abnormal renal function increases the risk of acute myelosuppression,[38] so the dose of methotrexate should be reduced if there is a significant deterioration in renal function.[36 38]

All the recently published guidelines agree that liver function tests, including serum albumin and alanine aminotransferase (ALT) or aspartate aminotransferase (AST), should be checked before commencing methotrexate therapy. There is a lack of consensus on the frequency of monitoring required. The Committee on Safety of Medicines[26] and the British Association of Dermatologists[38] recommend weekly LFTs until the methotrexate dose is stable. Monitoring every two weeks, during dose stabilisation, is recommended by the British Society of Rheumatologists,[34] the Royal College of Nurses[37] and the NPSA.[30] The recommended frequency of monitoring of stable patients varies from monthly[26 28 34 35] to two or three monthly.[24 36] The likelihood of finding a raised serum transaminase (ALT or AST) is several-fold higher in patients with rheumatoid arthritis taking methotrexate, compared to those taking placebo. There is only one published case of clinically significant liver disease occurring in a patient monitored every 4–8 weeks, with appropriate methotrexate adjustment.[39]

Methotrexate therapy should not be commenced if baseline LFTs are abnormal.[26] Patients with serum transaminases greater than twice the upper limit of normal or with an unexplained fall in the serum albumin should be discussed with the rheumatologist[29 36] and consideration given to withholding treatment. Abnormal LFTs may be more common in patients with psoriasis.[40 41] The amino terminal peptide of type III procollagen (PIIINP) is produced during the synthesis of type III collagen. Serum PIIINP levels correlate with the degree of hepatic fibrosis and are sensitive in detecting hepatic fibrosis.[42] Studies, in patient receiving long-term methotrexate therapy for psoriasis, have shown that if PIIINP levels are consistently normal, liver biopsy can be avoided.[41 43] This marker has been in routine use in the Greater Manchester area in the UK since 1996.[41] It is recommended that serum PIIINP levels are checked in patients with psoriasis before commencing methotrexate, and 2–3 monthly thereafter.[36] Abnormal results should be discussed with the responsible secondary care specialist. PIIINP is a recent marker and recommendations on its use may change in coming years.

Methotrexate is a safe drug if monitored according to the guidelines and the dose is adjusted accordingly.[30] However for safe prescribing, clarity is needed on the monitoring and prescribing responsibilities between primary and secondary care.[44] The NPSA has produced a patient information leaflet emphasising the importance of regular monitoring[45] and a patient-held blood monitoring and dosage record booklet.[46]

GMS contract indicator: none.

BLOOD GLUCOSE TESTING IN THE DIAGNOSIS AND MONITORING OF TYPE 2 DIABETES (WSAS)

The question and answer sets below attempt to summarise the content of the set of national guidance documents found, and highlight any major differences. Overall, apart from differences between the American Diabetes Association guidance of 1997,[47]

reaffirmed in 2005[9] and 2006,[48] and other consistent American documents such as the Michigan[50] and Veterans' Affairs[51] guidance, the other guidance documents found broadly agree, although minor qualitative differences exist, mostly relating to the specificity of the wording. Some guidance documents are interrelated (for example in the UK, the PRODIGY, NICE and UK Department of Health National Service Framework documents frequently cross-refer).

Who should I screen for diabetes?

We recommend an active (rather than opportunistic) screening, which can be combined with screening of cardiovascular risk in:

- Patients>65 years old.
- Patients >45 years old with one or more risk factors (box 1).
- Patients <45 years old with two or more risk factors or otherwise suspected to be at high risk.
- Repeating the test annually in those considered at high risk to 3–5 yearly in those considered at low risk.
- For convenience the assessment should be incorporated into protocols for interval cardiovascular risk assessment and other active monitoring programmes.
- Active (rather than opportunistic) screening is considered preferable by several guideline sources.

Absolute consensus on screening for diabetes does not exist. The American Diabetes Association[49] advocates considering screening in all adults >45 years old (particularly those with body mass index 25 or above and in younger patients with risk factors). Population screening has however been reported as not being cost effective,[50] and other American guidance[50 53] states that there is limited evidence to support mass population screening of asymptomatic adults.

Most of the other national guidance documents[54–61] specify active but targeted approach to those with risk factors. The risk factors quoted vary slightly between guidance documents; box 4 provides an overview of the at-risk groups listed in the various guidelines.

The question of active versus opportunistic screening is resource-dependent. The International Diabetes Federation guideline[62] refers to opportunistic screening of high risk people only as a minimal care standard. A practical approach would be to incorporate screening of people in the above groups into protocols for other active screening systems (hypertension, cardiovascular risk, well-woman) which would capture a significant proportion of the target population and reduce the resource implication for a separate active screening programme were this to be used.

As the prevalence of risk factors is high in the general Western populations over the age of 45 years, the practical difference between targeted and population screening may not be that great.

One guideline[58] provides a pragmatic approach of screening all those over 65 years old, those over 45 years old with one risk factor, or those 18–45 years old with more than one risk factor.

A reasonable policy may therefore be to test all those over 65 years old, those over 45 years old with one or more of the above risk factors, and those under 45 years old with two or more risk factors or thought otherwise to be at particular risk (severe hypertriglyceridaemia, gross obesity). Particular attention is recommended for patients with multiple risk factors, as some factors in isolation (such as age alone) may have a low yield.[63]

The guidelines are consistent in recommending annual measurement for those considered at high risk of developing diabetes and less frequently, eg 3 yearly, in those at low risk. For practical purposes this test would combine easily with the annual or 3–5 year cardiovascular risk assessment recommendations. There is no clear distinction between high and low risk, and in the absence of clear guidance we recommend that retest intervals be decided prospectively with the patient on an individual patient basis, based on the number and severity of risk factors.

What test should I use?

We recommend:

- Measurement of random or preferably fasting plasma glucose.
- Confirmation of a diagnostic result by a second, fasting, plasma glucose test, unless the patient has unequivocal symptoms of hyperglycaemia.
- Whole/capillary blood glucose meters are not recommended for diagnosis—if used in screening they must be confirmed by two plasma laboratory glucose measurements.
- Urine testing for diagnosis is not recommended.

Box 5 and table 2 (plasma values) show the diagnostic criteria for impaired fasting glycaemia, impaired glucose tolerance and diabetes.

There is good consensus among all of the guidance that the diagnosis of diabetes requires two plasma measurements unless unequivocal symptoms of hyperglycaemia are present.

Few guidance documents specifically refer to the type or place of measurement.

However, with very few exceptions the guidance documents refer to measurement of plasma glucose for diagnosis of diabetes, which would implicitly indicate measurement on a laboratory instrument rather than capillary blood glucose meter, and the Singapore guideline[60] refers specifically to measurement in an accredited laboratory as the preferred method. The Flemish guideline[58] specifically refers to meters not being recommended for screening, and the International Diabetes Federation guideline[63] includes capillary blood glucose as an option (although fasting laboratory glucose is preferred) in its minimal care scenario. Urine testing is not recommended for diagnosis unless blood glucose measurement is not available.

While capillary measurements are convenient to perform in local surgeries by point of care testing, the potential confusion that may arise between diagnostic criteria for plasma and whole venous or capillary blood results combined with the quality control and audit trail of results held by analytical laboratories provide a strong argument for formal laboratory analysis to make the diagnosis of diabetes. We would therefore recommend that if a point of care testing (POCT) blood glucose meter is used in initial screening, a diagnostic POCT result should be confirmed by one (if unequivocal symptoms are present) or two formal laboratory plasma glucose measurements.

There is consensus as to the thresholds which define diabetes, based on WHO criteria,[64] but there are different approaches to the ''pre-diabetes'' states and the type of diagnostic plasma glucose test to use.

Most guidance advocate a fasting plasma glucose or OGTT as the confirmatory test preceded by either a random or fasting measurement. The ADA in 1997 adopted FPG as the preferred test,[47] and confirmed this in the most recent subsequent position statement.[48]

None of the guidelines currently recommend use of HbA1c in the diagnosis of diabetes.

When should I perform an oral glucose tolerance test (OGTT)?

We recommend:

Box 4: Prevalent risk factors for type 2 diabetes

- Hypertension
- Family history in first degree relative
- Overweight (body mass index >25 kg/m^2) or girth >88 cm (women) or 102 cm (men)
- Vascular disease
- Hyperlipidaemia, notably low HDL (<0.9 mmol/l) or raised triglycerides (>2.3 mmol/l)
- Recognised higher risk ethnic groups, eg Asian origin
- Polycystic ovarian syndrome
- Previous gestational diabetes or large baby (>4 kg)
- Drugs known to be diabetogenic (for example, corticosteroids, or atypical anti-psychotics, eg clozapine)
- Drugs known to be diabetogenic (for example, corticosteroids, atypical anti-psychotics, eg clozapine, or β blockers)
- Other autoimmune disease, eg thyroid disease, pernicious anaemia

Box 5: Diagnosis of diabetes mellitus (from Canadian diabetes Association[54])

- FPG ⩾7.0 mmol/l

Fasting = no caloric intake for at least 8 hours

- Casual PG ⩾11.1 mmol/l + symptoms of diabetes

Casual = any time of the day, without regard to the interval since the last meal
Classic symptoms of diabetes = polyuria, polydipsia and unexplained weight loss

- 2hPG in a 75-g OGTT ⩾11.1 mmol/l

A confirmatory laboratory glucose test (an FPG, casual PG, or a 2hPG in a 75 g OGTT) must be done in all cases on another day in the absence of unequivocal hyperglycaemia accompanied by acute metabolic decompensation.

PG, plasma glucose; 2hPG, 2-hour plasma glucose; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test.

  - Oral glucose tolerance testing may be helpful in patients in whom a fasting result is non-diagnostic (5.6–6.9 mmol/l), but either
  - diabetes is strongly suspected on clinical or laboratory grounds from risk factors for diabetes; or
  - impaired glucose tolerance is suspected in a patient without impaired fasting blood (5.6 > plasma glucose < 6.1 mmol/l) from risk factors for diabetes.

- Systematic use of OGTT in all patients with impaired fasting glucose (IFG) poses considerable logistic difficulties and would be of uncertain benefit in light of the annual testing recommendations in at-risk patients.

Guidance on the use of the 75 g OGTT varies. While all offer OGTT as a means of diagnosing diabetes, there is little clear guidance about when it should be used. The American Diabetes Association recommendation for measurement of fasting glucose alone, created two separate categories of impaired glucose metabolism, impaired fasting glucose and impaired glucose tolerance. More end-point data are available on the latter although both appear to be associated with increased risk of developing diabetes and adverse cardiovascular outcome.[65–69] However many people without IFG have impaired glucose tolerance (IGT) and vice versa.[70–73] While simpler to perform than a glucose load, a fasting plasma glucose measurement diagnostic of IFG will fail to identify a significant number of people with IGT who are at risk of diabetes and cardiovascular disease.

The upper limit for fasting plasma glucose of 6.1 mmol/l therefore appears to miss a proportion of patients with IGT. The 2005 American Diabetes Association follow-up report defined IFG as plasma glucose >5.6 mmol/l, and the International Diabetes Federation recommends OGTT if the measured plasma glucose is 5.6–7.0 mmol/l. Whilst the current UK guidance cites an upper limit of 6.1 for fasting plasma glucose,[56] consistent with earlier American guidance, it would appear reasonable to consider OGTT to detect impaired glucose tolerance in patients with plasma fasting glucose below 6.1 but above 5.6 mmol/and clinically suspected of having IGT because of clinical risk factors or other laboratory results (eg hypertriglyceridaemia). The benefit of systematic OGTT in all those with fasting plasma glucose >5.6 mmol/l is unclear in light of the logistic burden this would impose. Several of the guideline documents acknowledge the logistic and cost difficulties surrounding widespread use of the OGTT. In addition, despite the OGTT, being a defining tool for diagnosis is itself poorly reproducible over time, particularly in the ''pre-diabetes'' phase and ''early'' stage of diabetes.[74] This difficulty is partly circumvented by the consensus emerging from the guidelines that an annual test be performed in high risk people and in those with IGT or IFG, compared to 3-yearly in patients over 45 years old at lower risk

The question of how frequently to retest patients over 65 years old is not addressed, and it would seem reasonable to recommend annual testing in patients with IFG or IGT, as in younger patients, and in those with normal fasting plasma glucose but any additional risk factors.

When should I measure blood glucose in a diabetic patient?

We recommend:

- ''Routine'' glucose measurement in a primary care clinic setting is of limited if any value.
- Measurement to confirm suspected hypoglycaemia or hyperglycaemia in unwell patients.
- Intermittent (eg, annual) measurement, either via a laboratory plasma glucose or clinic POCT capillary glucose to validate patient and meter technical results in patients using self-monitoring of blood glucose concentrations (SMBG), or if incorrect meter results are suspected.

Table 2 Diagnostic plasma glucose levels for the diagnosis of IFG, IGT and diabetes mellitus (from Engelau *et al*[52])

	FPG (mmol/l)		2hPG in a 75 g OGTT (mmol/l)
IFG	6.1–6.9		NA
IFG (isolated)	6.1–6.9	and	<7.8
IGT (isolated)	<6.1	and	7.8–11.0
IFG and IGT	6.1–6.9	and	7.8–11.0
Diabetes	⩾7.0	or	⩾11.1

2hPG, 2-hour plasma glucose; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NA, not applicable; OGTT, oral glucose tolerance test; PG, plasma glucose.

The guidance documents found do not recommend routine blood glucose measurement in diabetic patients other than in situations in which a clear clinical indication exists, notably in patients who are unwell, in order to detect or confirm suspected hyperglycaemia or hypoglycaemia.

One guideline specifically advocates comparison with a paired laboratory glucose sample[75] to check technical performance, or if meter records are inconsistent with other (clinical or laboratory) indicators of glycaemic control. Others are less specific in their guidance, but recommending intermittent evaluation of patient meter use appears consistently in the different guidelines. There appears to be limited evidence at present to support any particular strategy to monitor patient meter use.

Which patients should perform SMBG?

We recommend SMBG in:

- Patients being treated with insulin or preparing to start insulin.
- Patients on oral treatments in conjunction with an integrated educational programme to identify glucose excursions due to lifestyle changes or intercurrent illness.
- Patients who are intensifying glycaemic control for medical reasons, eg complications.
- Patients in whom HbA1c results are thought to be clinically inconsistent with the patient's state.

However:

- The value of SMBG in well controlled, well motivated patients with stable HbA1c is uncertain.
- SMBG use and frequency should be tailored to its specific purpose, and the utility and frequency of SMBG in individual patients should be assessed regularly (eg annually).

There is good consensus that SMBG is required in type 1 or 2 patients receiving insulin, and this will not be further discussed.

In patients with type 2 diabetes managed on diet or oral hypoglycaemic agents, SMBG is considered helpful and is associated with lower HbA1c when used as part of a structured diabetes educational programme but not as a stand-alone intervention.[48 60] It would appear logical to tailor the frequency and use of self-monitoring to individual patients depending on need and purpose. There would appear to be questionable merit in the systematic use of SMBG in well-controlled stable diabetic patients with a good understanding of their diabetes management and consistently low HbA1c, except during intercurrent illness.

GMS contract indicator: none relating to these specific questions.

CONCLUSION

This tenth review brings us to a running total of approximately 113 question and answer sets written in order to provide an overview of current advice in use of laboratory tests in primary care. Answers to the first nine question–answer sets can be found elsewhere.[76–84] They have all used a common search methodology,[85] although where recent systematic reviews have been performed, the guidance relies heavily also on the findings of these reviews. For authors wishing to consult the UK General Medical Services Contract and related Quality and Outcomes Framework, these can be found elsewhere.[86–88]

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