REVIEW

# Best practice in primary care pathology: review 4

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This fourth best practice review examines four series of common primary care questions in laboratory medicine are examined in this review: (1) safety monitoring for three common drugs; (2) use of prostate-specific antigen; (3) investigation of vaginal discharge; and (4) investigation of subfertility. The review is presented in question–answer format, referenced for each question series. The recommendations represent a precis of the 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 them are consensus based rather than evidence based. They will be updated periodically to take account of new information.

This is the fourth in a series of reviews which answers several questions that arise during the use of pathology in primary care.

Each topic is introduced with a brief summary of the type of information found and is handled separately.

Although the individual topics are not related because they cover the disciplines of clinical biochemistry, microbiology, immunology, haematology and cellular pathology, they are designed, once completed, to form a resource that will be indexed and cover a wide range of the most common issues in primary care laboratories, to be made available to users.

In instances where the new UK 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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## SAFETY MONITORING OF COMMON DRUGS (WSAS, IDW, PJC)

Three questions that examine the safety monitoring required for drugs used to treat hyperthyroidism, digoxin and amiodarone are answered here. Further questions on other drugs commonly used in primary care will follow. The complications of treatments with these drugs are well known, and relatively close consensus exists on the safety monitoring required. These should be easy to incorporate into clear pathways and there seems to be good evidence that appropriate monitoring reduces the incidence of adverse effects.

### What safety monitoring is required for a patient receiving carbimazole or propylthiouracil in primary care?

**We recommend:**

- Baseline white cell count before anti-thyroid drug treatment is started.
- Repeat white cell count if patients develop fever, mouth ulcers, sore throat or other symptoms of infection.
- Stop drug and recommend immediate specialist referral if leucocyte count falls to $<1500\times10^6$/l or neutrophil count $<500\times10^6$/l.

Agranulocytosis (absolute neutrophil count $<500\times10^6$/l) is a rare but serious complication of these anti-thyroid drug treatments and also of thyrotoxicosis per se. It is not dose related[1] and is reported as developing in 0.2–0.5% of patients in Asian populations,[2 3] although the incidence in European populations seems to be far lower (0.03%).[4] Extrapolation to the UK setting is hampered because the most common drug used in these studies was methimazole, which is not used in the UK. The most common presenting diagnoses were pharyngitis, tonsillitis, pneumonia or urinary tract infection (8%). Agranulocytosis occurred within the first 3 months of treatment in most patients.[2–4] Elderly people are reported as being at most risk.[3 5] Conversely, a fall in neutrophil count, the absolute count remaining above $2000\times10^6$/l and not progressing to agranulocytosis, occurs in 1–5% of patients, does not seem to be associated with infection and does not require discontinuation of the drug.[6]

Discontinuation of the drug should be considered if the leucocyte count $<1500\times10^6$/l.[7] In view of the sudden onset and morbidity and mortality of agranulocytosis, we recommend that, in patients whose white cell count $<1500\times10^6$/l or neutrophil count $500\times10^6$/l in a primary care context, the drug is stopped and the patient referred for immediate specialist assessment and treatment where applicable.[6]

Opinions are divided on the monitoring of routine blood count when these drugs are started. An article in the *Drugs and Therapeutics*

**Abbreviations:** BNF, *British National Formulary*; DRE, digital rectal examination; FSH, follicular stimulating hormone; GMS, General Medical Services; HVS, high vaginal swab; NHS, National Health Service; NICE, National Institute of Health and Clinical Excellence; PCOS, polycystic ovary syndrome; PID, pelvic inflammatory disease; PSA, prostate-specific antigen; SFA, seminal fluid analysis; STI, sexually transmitted infection; TFT, thyroid function test; TSH, thyroid-stimulating hormone

*Bulletin*[7] recommended that blood count monitoring for agranulocytosis be performed fortnightly during the first 3 months of treatment, analogous to sulphasalazine, for which the incidence of agranulocytosis is similar. This advice was based on a prospective Japanese study,[8] which detected neutrophil counts <1500 in 43 of 55 patients from a cohort of over 15 000 patients with thyrotoxicosis treated with methimazole or propylthiouracil. All the patients recovered when the drug was stopped and 29 patients did not have any infection. The reported incidence of agranulocytosis was, however, far higher than that reported for European populations and the diagnostic threshold was conservative. The Christian Science Monitor[9] did not adopt this recommendation when it reviewed the topic in 1999, but did highlight the need to inform patients of the potential presenting symptoms. These recommendations are restated in the *British National Formulary* (*BNF*).[10]

GMS contract indicator: none.

## What safety monitoring is required in a patient receiving digoxin in primary care?

**We recommend the following during the measurement of plasma digoxin**:

- Toxicity should be diagnosed, or potential toxicity developing from a change in dose, the patient's clinical state or other drug treatment should be monitored.
- Serum potassium and renal function indices should be measured at a frequency depending on the patient's clinical state and other drug treatment.
- Samples for measurement of digoxin should be taken at least 8–12 h after the last dose, and at 8–10 days after any change in dose.
- Plasma monitoring is not necessary in patients who are clinically and biochemically stable.

A limited relationship exists between the level of plasma digoxin and clinical outcome,[11] and a slowing of the heart rate is the clinical indicator of efficacy.[12] The routine measurement of levels of digoxin is not recommended by several sources.[11–15] One expert group, however, recommended routine measurements every 10 months and in the other situations listed below. With these criteria, it found that only 52% of digoxin measurements were appropriate in an outpatient setting, and 16% in an inpatient setting.[16] Most inappropriate outpatient requests were due to early routine monitoring (median repeat time 56 days) in patients in a stable condition.

One meta-analysis, however, suggests that monitoring may reduce toxicity[17] and several documents recommend measurement in specific situations: toxicity, potential toxicity arising from a change in digoxin dose or the patient's clinical state (notably renal function and hydration status), a change in concomitant drugs (notably those influencing renal or hydration status or lowering serum potassium), or to investigate questionable compliance.[12 16 18–20]

Among others, hypokalaemia, hypomagnesaemia and hypothyroidism predispose to the toxic effects of digoxin,[12] and renal dysfunction results in raised concentrations of digoxin for equivalent dose compared with normal renal function.[14] These should be taken into account and appropriate electrolyte monitoring should be carried out in patients predisposed to hypokalaemia (eg, loop diuretics), in dosing in patients with renal dysfunction[14] and in elderly people. No clear guidance was found for monitoring frequency. This would be expected to vary considerably depending on a patient's medical and drug history. Similarly, the *BNF*[14] highlights the risks associated with hypokalaemia, but makes no specific recommendations on monitoring electrolytes. It should be noted, however, that the diagnosis of digoxin toxicity is clinical, and that measured serum concentration inside or outside a target range does not in itself diagnose either toxicity or absence of toxicity.

Although some texts suggest that blood must be withdrawn a minimum of 6 h after a dose, more recent statements recommend 8–12 h[18–20] after a dose of digoxin has been taken, or 7 days after a change in dose because of the long half-life of digoxin[18–20] (up to 21 days may be required to reach steady-state concentrations in patients with renal insufficiency[15]). These recommendations are consistent with the pharmacokinetics of digoxin. In addition to invalidating the result, early measurements may potentially lead to inappropriate change in dosage or other clinical management.

A detailed list of drugs influencing concentrations of digoxin or toxicity is provided in the *BNF*.[21] This provides dosing recommendations, which are of particular importance in renal insufficiency, as one study of patients with chronic kidney disease found excess dosing compared with the recommended doses for degree of kidney failure to occur commonly, digoxin being one of the drugs most frequently incriminated.[22]

GMS contract indicator: none.

## What safety monitoring is required in a patient receiving amiodarone in primary care?

**We recommend the following minimum safety monitoring at baseline and every 6 months on amiodarone if levels are within the population reference range:**

- thyroid profile (thyroid-stimulating hormone (TSH), free thyroxine and free tri-iodothyronine where applicable)
- liver enzymes (aspartate aminotransferase) and electrolytes (urea and electrolytes)
- clinical evaluation.

We recommend that the following assessments be carried out annually:

- chest *x* ray, electrocardiography and clinical assessment.

We recommend additional safety monitoring in patients receiving warfarin:

- prothrombin ratio monitoring weekly during the first 7 weeks of warfarin treatment in a patient receiving amiodarone, adjusted thereafter depending on response.

An American evidence-based review of amiodarone efficacy and safety states that routine monitoring of plasma concentrations of amiodarone is not considered useful in patients who are stable.[22] The recommendations found in all reviews concentrate on safety monitoring with other indicators. Concentrations of total and desethylamiodarone in excess of 255 mg/l have, however, been associated with increased risk of toxicity.[24 25] The ratio of amiodarone to desethylamiodarone can also be used as an indicator of adherence.[26]

One meta-analysis of double-blind trials of amiodarone use[26] disclosed the complication rates of non-cardiac amiodarone toxicity to be 1% for pulmonary toxicity, 0.6% for hepatic toxicity, 0.3% for peripheral neuropathy, 0.9% for hyperthyroidism and 6% for hypothyroidism, annually. Thyroid dysfunction, however, varies depending on environmental iodine content, and the rates for countries with high

environmental iodine such as the UK have been estimated to be 1.7% for hyperthyroidism and 13% for hypothyroidism.[28]

As amiodarone reduces clearance of warfarin, it can produce sudden large rises in the prothrombin ratio (international normalised ratio),[29] the peak effect occurring about 7 weeks after warfarin treatment is started, although the kinetics of the international normalised ratio are altered from the first day.[29] Close measurement of the prothrombin ratio is therefore required during that period, the recommended period being for the first 7 weeks of treatment.[30] Administration of amiodarone also increases levels of digoxin owing to reduced renal digoxin clearance and requires doses of digoxin to be reduced (usually by one half) and concentrations of digoxin to be monitored closely in view of potential toxicity.[31] A literature review of more than 20 sets of published amiodarone guidelines[32] concluded that evidence supporting the guidelines was limited, but constructed minimum quality standards for amiodarone safety monitoring summarised earlier and based principally on the North American Society of Pacing and Electrophysiology.[33] For laboratory tests, these guidelines are consistent with recommendations of the *BNF* and others. The same review found large variations among guidelines drawn from editorials and personal views. No outcome studies were identified and the authors used the minimum ''boundary'' standards to assess clinical practice in a small group of 99 patients over 14 months in one teaching hospital. These are consistent with the *BNF* with respect to laboratory liver and thyroid monitoring[34] and a review for UK Medicines information.[35]

Compliance with these standards was poor, in the region of only 40% for thyroid and liver biochemistry in an American setting, where proportionally far more laboratory tests are ordered. The Committee on Safety of Medicine also recommends regular monitoring of urea and electrolytes (for example, every 6 months in patients taking diuretics).[36]

Other clinical toxic effects to be assessed in a clinical evaluation include referral for ophthalmological evaluation in patients with visual deterioration and assessment for symptoms of gastrointestinal, dermatological or neurological toxicity, which are not considered here.

GMS contract indicator: none.

## UTILITY OF PROSTATE-SPECIFIC ANTIGEN MEASUREMENT (WSAS)

The answers presented here do not deal with the current debate in laboratory medicine about the relative utility of different forms of measurement (free, total, calculated ratios) of prostate-specific antigen (PSA). The use of PSA in the general community has been widely debated on both sides of the Atlantic, although occasionally the implications and limitations of the widespread use of PSA in unselected populations or in those at lower risk has been lost in the emotive and political debate. These answers attempt to put that debate in perspective.

### Should PSA be measured in asymptomatic men?

- PSA screening need not be proactively discussed with asymptomatic men.
- Pending results of studies on PSA screening should not be denied to well-informed men who request it.
- The current implications and limitations of the test should be explained to those who request it.

In England and Wales, the number of cases of prostate cancer diagnosed has more than doubled in a decade, mainly because of the widespread use of PSA testing.[37] Despite being the most common cancer in men, with nearly 27 000 new cases diagnosed in 2002, there is no evidence to support routine PSA screening of prostate cancer in asymptomatic men. Even though screening often detects early ''curable cancers'' in most patients, there is inconclusive evidence from one randomised controlled trial that screening and early detection improves life expectancy or quality of life,[37] and one American study reported no increase in longevity, but increased morbidity in a two-centre comparison of PSA measurement with surgery versus PSA with ''watch-and-wait'' approach.[39]

A randomised study from Sweden has shown a modest survival benefit from radical surgery over watchful waiting.[40] Longitudinal studies in the UK and USA, however, indicate that most men with localised low-grade prostate cancer have only a small risk of dying from prostate cancer and hence are unlikely to benefit from an early diagnosis of their cancer.[41 42] Only about a third of patients with prostate cancer will die from their disease.[42]

Even though up to two thirds of men over the age of 70 are found to have prostate cancer in autopsy series, prostate cancer is responsible for only 3.5% of all deaths in men.[43 44] Screening for PSA need not therefore be proactively discussed with asymptomatic men.

Two large randomised screening trials, the Prostate, Lung, Colorectal and Ovary Cancer Trial[45] in the USA and the European Randomized Screening for Prostate Cancer Trial[46] in Europe are currently assessing the benefits of screening for prostate cancer.

While awaiting the results of the studies on PSA screening, the PSA test should not be denied to men who request it. This should be conducted after proper counselling about the drawbacks of the PSA test (false-positive and false-negative results), the natural history of prostate cancer, the treatment options for cancer, and the physical, psychological and financial implications of detection of a cancer that may never have affected health had it been detected during the lifetime of the person.[47–49]

Prostate cancer is rare in men $<$50 years of age.[44] In asymptomatic men who elect to undergo screening, PSA tests therefore should be started at the age of 50 years (or 45 years in the higher-risk population). Annual or biannual PSA alone, without a rectal examination, is sufficient to identify men who are at high risk of prostate cancer. PSA testing is not usually recommended for asymptomatic men with a life expectancy of $<$10 years.[50 51]

PSA is both a diagnostic and a screening test. UK National Health Service (NHS)[52] guidance recommends that PSA should be measured, if clinically appropriate, in men with refractory lower urinary tract symptoms, erectile dysfunction, hard irregular prostate, haematuria or bone pain with weight loss or other clinical situations compatible with prostate cancer.

Various procedures, activities and diseases can affect the levels of PSA. Hence, the exclusion criteria of a PSA test are:

- an active urinary tract infection
- ejaculation in the previous 48 h
- vigorous exercise in the previous 48 h
- a prostate biopsy or colonoscopy in the previous 6 weeks.

### What action should be taken in a patient with a raised PSA?

If an asymptomatic man has PSA level higher than the age-specific range, he should be counselled about further options, which include a prostate biopsy.

PSA is a protein produced almost exclusively in the epithelial cells of the prostate gland. It is normal for men to have low levels of PSA in their blood, and there is no specific normal or abnormal PSA level. Both prostate cancer and benign conditions can increase the levels of PSA.

An isolated increase in the PSA level should be confirmed several weeks later, before proceeding with further testing, including prostate biopsy.

If practical, the man should have the PSA test before the digital rectal examination (DRE). If not, it is recommended that the PSA test be delayed by 1 week. The blood sample should reach the laboratory (and be separated) <16 h after the sample is taken.[52 53]

Age is an important factor in increasing levels of PSA. For this reason, the NHS Prostate Cancer Risk Management Programme recommends age-adjusted levels of PSA to determine when diagnostic tests are needed. The programme recommends the following cut-off values for referral:

- age 50–59 years, ⩾3.0 μg/l
- age 60–69 years, ⩾4.0 μg/l
- age 70 and over, ⩾5.0 μg/l.

A very high PSA value is strongly suggestive of cancer. A level of PSA >100 μg/l, with an abnormal prostate gland on DRE, is almost certainly caused by prostate cancer. In an elderly man with extensive comorbidities and grossly raised PSA, biopsy is therefore not necessary before starting hormone therapy.[54]

If an asymptomatic man has a level of PSA higher than the age-specific range, he should be counselled about further options, which include:

- Clinical and PSA follow-up without biopsy.
- Clinical and PSA follow-up after ultrasound and biopsy confirmation.
- Active intervention options (surgery, chemotherapy, hormone therapy) depending on the level of PSA, stage of disease once confirmed, comorbidities and life expectancy.

Hopefully, the ongoing NHS Health Technology Assessment-funded ProtecT trial,[55] evaluating the effectiveness of various treatment options, including active surveillance for clinically localised prostate cancer, will benefit future decision making.

### How often should PSA be measured in patients who have been diagnosed as having prostate cancer?

- Fit, treated patients with prostate cancer should have their PSA checked:
  - every 3 months for the first 1–2 years
  - every 6 months for 2 years
  - annually thereafter
  - or if clinical signs or symptoms change between measurements.
- Patients managed on a watch-and-wait basis should have levels measured once every 3–6 months.

Changes in levels of PSA over a 3–6-month period can be prognostic. A PSA that doubles in the 6 months of follow-up after surgery indicates an aggressive cancer and points towards ''incurable'' systemic relapse rather than a ''potentially curable'' local relapse.[56] We therefore recommend PSA checks every 3–6 months in men with prostate cancer.

In general, after radical treatment for prostate cancer, when there are no signs of recurrence, PSA levels should be measured every 3 months for 1–2 years, every 6 months until the fifth year; and after 5 years annual checks should suffice.[57 58] Unlike other cancers, late recurrence and progression can occur even after 10–15 years. Thereafter, it is recommended that periodic measurements of PSA be continued for life.[57 58]

The frequency of PSA checks may be altered in specific cases for various reasons. Elderly or frail men may require PSA levels to be measured less often and only if clinical intervention is practical and realistic.

After radical prostatectomy, PSA should be undetectable and drop to <0.1. Failure to drop to this level is a cause for concern, and further evaluation or treatment may be necessary.[59 60]

After radiation with either seeds (brachytherapy) or external beam or cryotherapy, the PSA will drop slowly, often reaching its nadir after several years. After 18–24 months following radiation, PSA levels are usually <1.0. A slow fall in the levels of PSA is not a cause for concern; paradoxically, the slower the fall, the better the prognosis.[61] It is not uncommon after prostate radiation to see a temporary rise, or ''bounce'', in the level of PSA, which then falls spontaneously. The classic PSA bounce timing is about 1–2 years after treatment and may be seen in up to 30% of patients. Levels of PSA should later fall and remain <1.0.[62]

After hormone therapy alone, nadir levels are quite variable and depend on the extent of the cancer and its aggressiveness. In general, nadir levels of PSA <2.0 indicate good prognosis. After hormone therapy, stable levels are reassuring and any rise in PSA is a cause for concern.[63]

DREs are not necessary during follow-up of patients who have been radically treated. The usefulness of DRE is limited in the follow-up of patients who have undergone radiotherapy.[64 65]

Patients on active surveillance (wait-and-watch policy) should have the PSA levels checked every 3–6 months. If there is a major rise in PSA, the patient should be counselled about treatment options again. It is important to note that the rate of rise in PSA is more important than the absolute levels of PSA.[66 67]

GMS contract indicator: none.

## VAGINAL DISCHARGE AND CHLAMYDIA TESTING (CAMM)

This advice and template has been produced by the Health Protection Agency, Primary Care Research Group (http://www.hpa.org.uk/infections/topics_az/primary_care_guidance/menu.htm). Their website also provides advice on treatment. The guidance is in agreement with other guidance on primary care, including the Scottish Intercollegiate Guidelines Network (www.sign.ac.uk), PRODIGY (www.prodigy.nhs.uk) and British Association for Sexual Health and HIV (www.bashh.org), for which specific references are provided.

### When should I send a vaginal swab from a woman with abnormal vaginal discharge or vaginitis for investigation?

**We recommend that a high vaginal swab (HVS) be sent to the microbiology laboratory to investigate abnormal vaginal discharge or vaginitis in the following circumstances:**

- postnatally
- before and after termination of pregnancy
- before and after gynaecological surgery
- for recurrent discharge[68] (⩾4 cases/year)[i] unresponsive to treatment
- where symptoms are not characteristic of candida or bacterial vaginosis
- for possible sexually transmitted infection (STI)
- for vaginitis

[i]Resistance has not increased with over-the-counter antifungals. Samples should be cultured before embarking on long-term suppressive treatment as only 16% with recurrent symptoms have candidiasis.

- for suspected pelvic inflammatory disease (PID)
- in children.

Also, see national guidelines on the management of STIs in children and young people for more information.[69] These guidelines cover the management of sexual abuse and suspected STI in patients <18 years of age.

The templates are designed so that the recommendations can be changed to suit local service delivery and sampling protocols. Laboratory methods, for example, may influence the samples that are suitable for diagnosis.

Submission of genital swabs to microbiology laboratories varies greatly, from 5 to 40 per 1000 population, and includes many for candidiasis and bacterial vaginosis, which should be diagnosed from clinical symptoms and signs. Bacterial vaginosis is the most common cause of abnormal vaginal discharge and is caused by overgrowth of anaerobic organisms. The UK national guidelines[70] provide extensive evidence-based guidance on the management of genitourinary infections. Trichomoniasis is a less common cause of vaginal discharge in the UK. Candidiasis is also a common cause of abnormal vaginal discharge.[70–72] Most women do not need a swab to be taken in this situation. We suggest that diagnosis can be made in most cases on clinical symptoms, signs and results of a narrow range pH paper (see fig 1). An HVS should be taken if other causes are being considered. These guidelines are summarised in fig 1.

### When should I take an endocervical swab for chlamydia?

**We recommend that testing for *Chlamydia trachomatis* should be carried out in women (particularly those <25 years) with symptoms and signs that may be attributed to *C trachomatis*[76–82]:**

- purulent vaginal discharge
- post-coital or intermenstrual bleeding
- mucopurulent cervicitis
- inflamed or friable cervix (which may bleed on contact)
- urethritis
- PID
- lower abdominal pain in the sexually active
- reactive arthritis in the sexually active.

Specimens should also be taken for chlamydia testing from:

- sexual partners of those with proven or suspected *C trachomatis*[83 84]
- all women undergoing termination of pregnancy[85]
- mothers of infants with chlamydial conjunctivitis or pneumonitis[86]
- semen and egg donors.[87]

The UK Department of Health advocates opportunistic screening in:

- sexually active men and women <25 years attending general practice, with a new sexual partner in the past 12 months[88 89]
- women <25 years having their first cervical smear.

### Do I need to retest for chlamydia after treatment?

**We recommend that:**

- in those patients likely to adhere to treatment and in whom there is no risk of re-infection, a test of cure is unnecessary.[90 91]

Always consider STI and take an endocervical chlamydia HVS in patients at risk: sexually active younger women <25 years, or those >25 years with a new partner for <12 months and no condom use.[70 71 92–94]

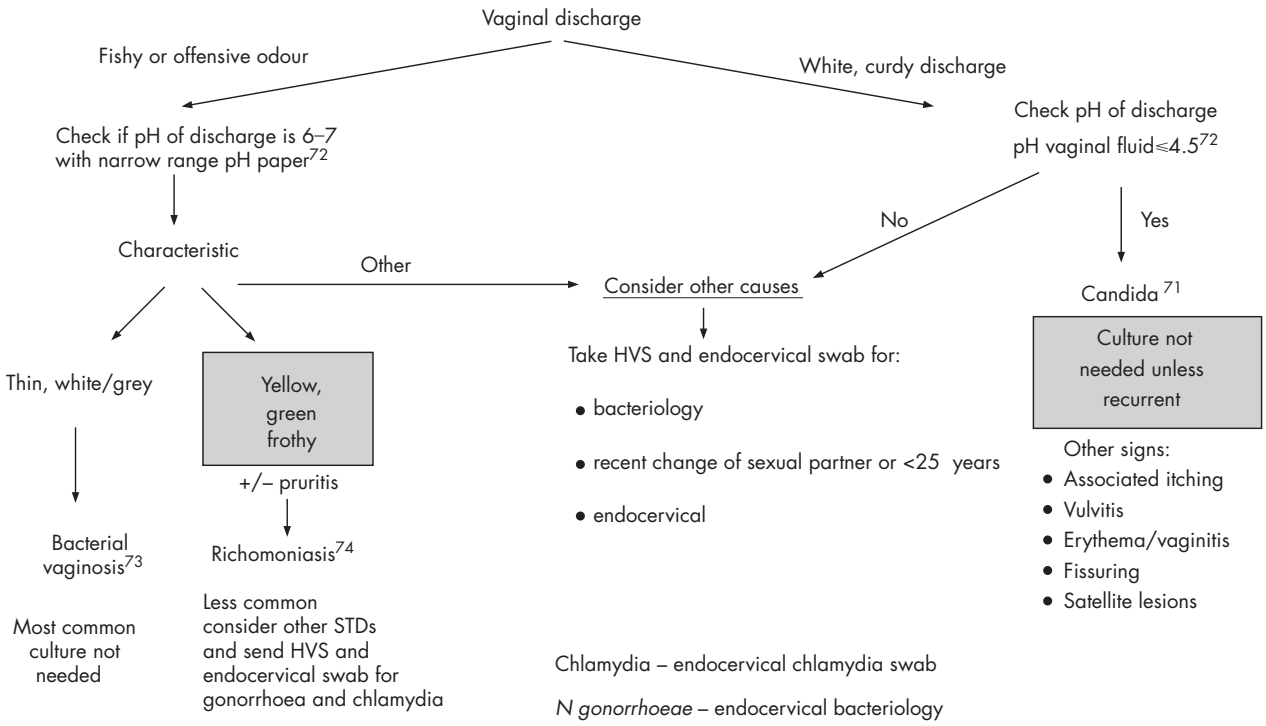


**Figure 1** Diagnosis of candida and bacterial vaginosis by symptoms and signs. STDs, sexually transmitted diseases (Whatman indicator papers pH 4.0–7.0 narrow range. 7 mm×5 m dispenser cat. no. 2600-102A. Available on special order from VWR International (Merck) 0800 22 33 44 £7.98+VAT per reel cat. no. 0080079-91. Whatman website http://www.whatman.plc.uk).

### How often should I repeat a swab?

How often chlamydia swabs should be taken in sexually active young women is a matter for debate. In theory, whenever a person has a new partner and continues to have unprotected intercourse you should retest. Retesting studies are currently in progress.

*C trachomatis* and *Neisseria gonorrhoeae* cause acute pelvic infection with vaginal discharge and intermenstrual bleeding, recent deep dyspareunia, lower abdominal pain, adnexal tenderness and cervical excitation pain.

### How do I obtain a vaginal swab?

- HVSs for bacteriology: obtain discharge present in vagina, place swab in transport medium and transport to the laboratory as soon as possible. Refrigerate in case of any delay.
- For chlamydia testing, a chlamydia collection kit provided by a local laboratory is used. Endocervical cells are required: clean cervical os with large swab, wiping away any purulent discharge, insert other swab into endocervix and rotate. Place in chlamydia transport tube.[70 72 76–95]

If screening a woman, a first-catch urine specimen may be submitted if molecular techniques using nucleic acid amplification are used in the local laboratory.

In men, first-void early-morning urine, which is less traumatic for the patient, or a urethral swab (insert 1–4 cm inside and rotate once) is the sample of choice.[96]

In women with PID or lower abdominal pain, serology should also be carried out.[97]

GMS contract indicator: none.

## INVESTIGATION OF THE SUBFERTILE COUPLE (EK, ES, CC)

The National Institute for Health and Clinical Excellence (NICE) 2004 guidance[98] provided a detailed review on the current advice on subfertility testing. In addition to reviewing the testing approaches for the main different clinical scenarios that may present, these answers distinguish those tests that may reasonably be requested in primary care from those for which secondary care input may be recommended, although this division is not based on published recommendations. This guidance does not include additional testing, which may be dictated by the clinical presentation (eg, anaemia, other systemic diseases).

### Who should be investigated for subfertility?

**We recommend investigation of both partners of couples who have been unable to achieve pregnancy:**

- after 1 year of regular unprotected intercourse
- before 1 year when there is a history of predisposing factors (such as amenorrhoea, oligomenorrhoea, PID or undescended testes)
- before 1 year when a woman is aged $\geqslant$35 years
- before 1 year if attempting pregnancy is associated with other risks (eg, changing epileptic treatment).

### What tests should be used to investigate subfertility in men?

**We recommend clinical examination followed by:**

- seminal fluid analysis (SFA)
- analysis of luteinising hormone, follicular-stimulating hormone (FSH) and testosterone in serum if repeated azospermia is present.

SFA is routinely used to determine male infertility. Although semen analysis lacks specificity, there is no better test at present.[99]

Results of the semen analysis should be compared against the World Health Organization[100] reference values. Successful fertilisation declines as sperm morphology drops below 15%.

Adherence to a strict collection protocol is essential for the accuracy of results. The man must abstain from ejaculation for 2–3 days before the semen sample is collected. The semen specimen should be collected into a wide-mouthed, sterile plastic pot, by masturbation and not by coitus interruptus. It should be protected from extremes of temperature during transportation.[100 101]

If the result of the first SFA is abnormal, a repeat confirmatory test should be offered, ideally 3 months after the initial analysis, to allow time for a new cycle of spermatozoa formation to be completed. But if gross spermatozoa deficiency (azospermia or severe oligospermia) has been detected, the test should be repeated as soon as possible.[98]

Levels of luteinising hormone, FSH and testosterone in serum are useful investigations in repeated azospermia.[102] In non-obstructive azospermia owing to primary testicular dysfunction, FSH is invariably raised.

Further investigation of primary testicular dysfunction showed that reduced levels of serum testosterone and raised levels of serum luteinising hormone are indicative of Leydig cell dysfunction, whereas high levels of serum FSH in the presence of normal serum levels of luteinising hormone and testosterone are indicative of Sertoli cell dysfunction. High serum levels of FSH and luteinising hormone with an inappropriately normal or low level of testosterone confirms testicular failure. In a few cases, low levels of FSH, luteinising hormone and testosterone indicate hypogonadotrophic hypogonadism, and in about 50% of these cases fertility can be restored with exogenous gonadotrophins.[103] Men with hypogonadotrophic and hypergonadotrophic hypogonadism should be referred to an endocrinologist for further evaluation, investigation and treatment.

### Other tests that may be used, normally in a secondary care context

*Anti-sperm antibodies*: Anti-sperm antibodies are present in 5–10% of the infertile population.

Routine screening for anti-sperm antibodies is not recommended, as there is no evidence of effective treatment to improve fertility.[98]

*Karyotyping*: This test is indicated in men found to have non-obstructive azospermia and hypergonadotrophic hypogonadism, as it allows for the detection of problems such as Klinefelter's syndrome (47XXY). Azospermia in association with eunoichoid features suggests a diagnosis of Klinefelter's syndrome.

*Sperm function tests*: The advent of intracytoplasmic sperm injection, used in conjunction with in vitro fertilisation for male factor subfertility, has rendered the various sperm function tests far less relevant.[104]

### What tests should be used to investigate subfertility in women?

- *Test of ovulation*: serum progesterone in the mid-luteal phase (7 days before the expected onset of menses), depending on the length of the cycle (day 21 in a 28-day cycle).
- *Further investigation of ovulatory failure*: when indicated by serum progesterone or in women with amenorrhoea or oligomenorrhoea without suspected androgen excess:
  - FSH, luteinising hormone, oestradiol, prolactin

– thyroid function if suggestive signs or symptoms are present.

- *Additional test if androgen excess suspected (eg, polycystic ovary syndrome)*: testosterone (or free androgen index).

### Test of ovulation in women with regular menstrual cycles

#### Serum progesterone

Women with regular menstrual cycles are likely to be ovulating.[105] Ovulation can, however, be confirmed by measurement of serum progesterone in the mid-luteal phase of the cycle. The sample should be taken 7 days before the expected onset of menses (eg, day 21 of a 28-day cycle or day 24 of a 31-day cycle).[102] A mistimed sample is a common cause of abnormal results.

A concentration of serum progesterone >30 nmol/l is considered proof of adequate ovulation in that cycle.[106]

In women with prolonged and irregular menstrual cycles, serum progesterone may need to be measured later in the cycle (eg, day 28 of a 35-day cycle) and repeated weekly thereafter until the next menstrual cycle starts.[98]

#### Investigation of anovulation or oligomenorrhoea or amenorrhoea without suspected androgen excess

Taken on days 2–4 of the cycle,[107 108] the tests recommended earlier will identify the main causes of ovulatory failure—namely, normogonadotropic anovulation, hyperprolactinaemia, hypogonadotropic hypogonadism and hypergonadotropic hypogonadism. Thyroid function, prolactin and atestosterone can be measured at other times in the cycle, although for practical simplicity the intended tests are best ordered at the same time as FSH or luteinising hormone, which should be taken on days 2–4 of the cycle.

In the absence of menstruation, a markedly raised FSH (>20 mU/ml) is indicative of ovarian failure.

Estimation of serum prolactin should be limited to women who have ovulatory disorders or galactorrhoea.[98]

Estimation of thyroid function should be confined to subfertile women with clinical features of thyroid disease.[98] The routine measurement of thyroid function in an initial investigation for subfertility is not recommended.

#### Investigation of anovulation or oligomenorrhoea or amenorrhoea with suspected androgen excess

Serum testosterone is a reasonable initial investigation in primary care, supplemented as indicated in secondary care by dehydroepiandrosterone sulphate, 17-OH-progesterone and androstenedione.

A leading example of androgen excess as a cause of subfertility is polycystic ovarian syndrome (PCOS).

The level of testosterone is raised in 70% of patients with PCOS,[109 110] with additional corroborative evidence in guiding a request for ovarian ultrasound alongside gonadatrophins and oestradiol.

The level of serum testosterone is also raised in congenital adrenal hyperplasia, adrenal tumours and androgen-producing ovarian tumours. We recommend advice on secondary care in cases of clinical and biochemical androgen excess not explained by PCOS, to exclude congenital adrenal excess, adrenal and ovarian neoplasia.

### When should primary amenorrhoea be investigated and what tests should be used?

**We recommend investigation:**

- in girls aged 14, with no other signs of secondary sexual development or
- by the age of 16, in girls with normal secondary sexual characteristics.[111]

### What tests should be used to investigate primary amenorrhoea?

The following tests should be carried out when secondary sexual characteristics are present:

- exclusion of outflow obstruction (pelvic ultrasound)
- levels of FSH, luteinising hormone, oestradiol and prolactin
- level of testosterone or free androgen index
- thyroid function tests (TFTs), including thyroxine
- pregnancy test (see below).

When secondary sexual characteristics are absent, the following tests are required:

- levels of FSH, luteinising hormone and prolactin.

Primary amenorrhoea is a relatively rare condition (0.3% prevalence[112]) and anatomical abnormalities of the genital tract constitute the most common cause in the presence of normal secondary sexual characteristics. Genital examination and an ultrasound scan of the pelvis are therefore imperative in these cases.[113]

When normal secondary sexual characteristics are present, the appropriate tests for the investigation of primary amenorrhoea should seek to exclude obstruction of the outflow tract.[114]

A pelvic ultrasound scan will determine the presence or absence of a uterus and any obstruction of the outflow tract. In addition, it may aid in the diagnosis of PCOS.[115]

Levels of serum gonadatrophins, oestradiol and prolactin, in the presence of normal pelvic anatomy, normal or low levels of serum luteinising hormone and FSH, may indicate a hypothalamic cause for the amenorrhoea (constitutional delay), the most common cause of primary amenorrhoea.[116] Levels of gonadotrophin will be raised in resistant ovary syndrome and the luteinising hormone is increased in PCOS and associated with raised testosterone (or free androgen index).

Irrespective of the presence or absence of secondary characteristics, persistently raised levels of serum prolactin (in the absence of a history of medication or drugs that may raise the level of prolactin) constitute an indication for referral.[113]

Both hypothyroidism and hyperthyroidism can cause primary amenorrhoea. Estimation of TSH is useful in ruling out subclinical hypothyroidism even in the absence of thyroid-related symptoms.[113]

It is important to exclude pregnancy during investigation of primary amenorrhoea. This is understandably sensitive and should be considered only in appropriate cases (ie, when the teenager is sexually active).

In the absence of secondary sexual characteristics, constitutional delay, Turner's syndrome and gonadotrophin deficiency are the most common causes of primary amenorrhoea.[114]

Karyotyping will normally be requested in conjunction with secondary care to differentiate between premature ovarian failure, resistant ovary syndrome or gonadal agenesis (46XX), testicular feminisation syndrome owing to testicular enzymatic failure (46XY), Turner's syndrome (45XO) or Turner mosaic when gonadotrophins are raised.

### When should secondary amenorrhoea be investigated?

**We recommend investigation:**

- after cessation of menstruation for 6 consecutive months in a woman who previously had regular cycles[117] unless age and symptoms are compatible with menopause
- when pregnancy has been excluded.

### What tests should be used to investigate secondary amenorrhoea?

**We recommend:**

- pregnancy test
- levels of FSH, luteinising hormone, prolactin and thyroid function tests
- testosterone or free androgen index in hirsute women or women with other features of androgen excess.

Pregnancy remains the most common cause of secondary amenorrhoea (with a prevalence about 3%), and a pregnancy test should always be considered.[111]

Secondary amenorrhoea is commonly caused by PCOS, hyperprolactinaemia, premature ovarian failure and hypothalamic dysfunction.[118] The above-mentioned tests will guide further investigations in conjunction with secondary care.

Levels of FSH are raised in ovarian failure. Women <30 years of age with ovarian failure should be told about, or referred to, secondary care to arrange karyotyping to exclude chromosomal abnormalities.[119]

Raised levels of luteinising hormone are suggestive of PCOS. Ratios may be >2,[120–122] particularly when combined with raised testosterone and low oestradiol. No one test is diagnostic, although the predictive value increases when several tests are abnormal. This will guide the decision to request a pelvic ultrasound.[115]

Levels of luteinising hormone and FSH combined with low levels of oestrogen are low in hypothalamic or pituitary dysfunction. This group includes women with amenorrhoea secondary to exercise, low weight, anorexia and stress.

Persistently raised levels of serum prolactin (in the absence of a history of drugs that may raise prolactin) constitute an indication for referral, as magnetic resonance imaging is recommended.[115]

Both hypothyroidism and hyperthyroidism can cause primary and secondary amenorrhoea associated with anovulation. Estimation of TSH is useful in ruling out subclinical hypothyroidism even in the absence of thyroid-related symptoms.[115] In the UK, however, NICE recommends TFTs in the investigation of subfertility only in patients with clinical features of thyroid disease.[98] Although the NICE recommendation is for investigation of infertility and not specifically for investigation of amenorrhoea, the two situations are evidently associated. As the incidence of thyroid disease is extremely low in patients who do not have at least two signs or symptoms of the disease, it would seem reasonable to adopt the NICE approach and not recommend TFTs as an initial investigation unless the patient has other clinical indicator(s).

GMS contract indicator: none.

## CONCLUSION

This review brings to a running total 58 main question and 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 elsewhere.[123–125] They have all used a common search methodology,[126] although where recent systematic reviews have been carried out, the guidance relies heavily also on the findings of these reviews. Authors wishing to consult the UK General Medical Services Contract and related Quality and Outcomes Framework can find these on their respective websites.[127 128]

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