Keywords Microbiology/Sepsis/Interpretation/Infection

This article has been double-blind peer reviewed

Understanding microbiology tests and interpreting the results

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Abstract Identifying infection, treating it appropriately, and reviewing efficacy of the treatment is a skill that all clinical practitioners should have. All patients receiving antibiotics in an inpatient setting should be reviewed within 48 to 72 hours of treatment commencement, and antimicrobial therapy should be rationalised alongside appropriate culture results to prevent antimicrobial resistance from developing. Close communication with the multidisciplinary team improves patient outcomes. Biomarkers, such as white cell count (also known as white blood cell count), C reactive protein, and procalcitonin, can be useful to identify and monitor the course of infection but should always be correlated with the patient's clinical presentation.

Citation Kempson F (2024) Understanding microbiology tests and interpreting laboratory results. *Nursing Times* [online]; 120: 10.

Advanced practitioners

This series is aimed at nurses and midwives working at or towards advanced practice. Advanced practitioners are educated at masters level and are assessed as competent to make autonomous decisions in assessing, diagnosing and treating patients. Advanced assessment and interpretation is based on a medical model and the role of advanced practitioners is to integrate this into a holistic package of care.

Microbiology in healthcare is the study of organisms that cause infections within the human body. Clinical microbiologists perform various tests on samples taken from a patient to diagnose infection and identify effective treatments appropriate to the identified organism. This area of work is becoming increasingly important as cases of multi-drug resistant (MDR) infections are appearing more frequently across all healthcare settings, with antimicrobial resistance considered one of the biggest threats to public health and development globally (World Health Organization (WHO), 2023).

Patients can develop life-threatening infections in any setting, from primary care and rehabilitation units to the emergency department and intensive care. As the practitioner reviewing these patients, it is important to understand how to interpret the various investigations available to evaluate a patient's infection. This article will explore some of the most commonly used microbiology investigations in the hospital setting: blood cultures, urine cultures, and sputum cultures.

What is causing the problem?

The accurate interpretation of laboratory tests in microbiology is highly dependent on the quality of the samples that are sent, as microorganisms can grow and die very quickly depending on how samples are collected and stored. Ideally, clinical samples should be taken before the administration of systemic antibiotics to reduce the risk of false-negative results, though treatment should not be delayed if the patient is in a critical condition and samples are not easily obtained. All samples sent for culture should be appropriate to the suspected cause and severity of infection (Miller et al, 2024; Peker et al, 2018).

Blood cultures

Blood cultures are a critical component of diagnosing and managing various infections, particularly those suspected of causing systemic illness, such as endocarditis and sepsis. Each blood culture set is defined as one aerobic bottle and one anaerobic bottle (UK Health Security Agency (UKHSA), 2023) to determine which bacteria require oxygen to grow (aerobic) and which do not require oxygen (anaerobic).

Blood cultures should always be obtained in suspected sepsis (UKHSA, 2023). In adults, this ideally consists of two sets of two blood culture bottles, with each bottle containing a minimum of 10ml of blood (NHS England, 2023). For children, the volume of blood collected should be appropriate to the age and weight of the child (Miller et al, 2024).

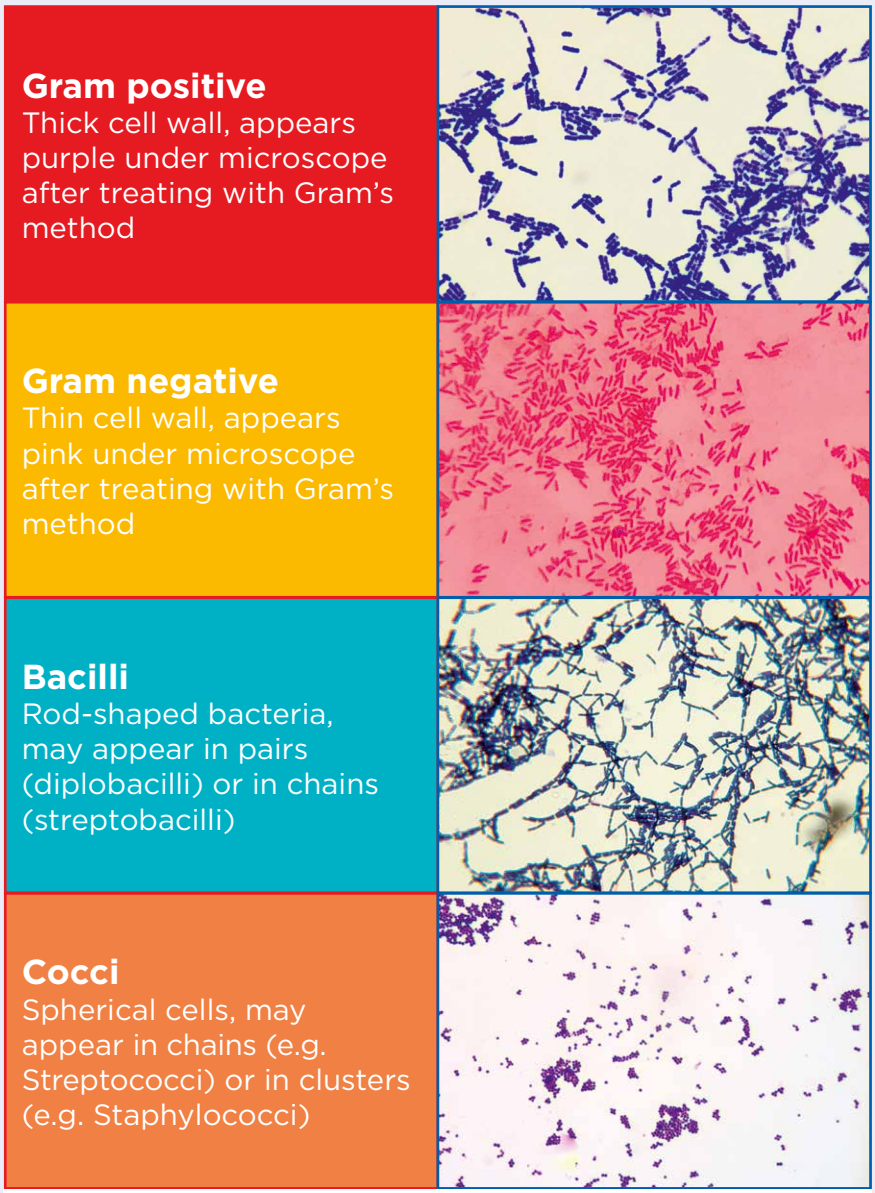
Blood cultures should be placed into the incubator within four hours of phlebotomy. Every hour this is delayed leads to a lower chance of identifying causative organisms, which is an important point to acknowledge if samples need to be transported to a second site for processing (Schwarzenbacher et al, 2019). Blood culture bottles are then placed into an incubator that senses when carbon dioxide levels begin to rise in the bottles and alerts the lab staff if there is a positive result. If the culture remains negative after a certain period of time (usually five days), then the sample is reported as negative. After flagging as positive, the lab staff will then proceed to attempt to identify the causative organism.

Initially, the biomedical scientists will perform a Gram stain to give an early indication of the organism while plated cultures are incubating, and classify bacterial species into either gram-positive or gram-negative (Fig 1). This involves taking a small sample of the blood/broth mixture and applying various dyes to stain the cells (Peker et al, 2018).

Not all bacteria can be grown in culture, so it is best to correlate negative cultures with other diagnostic tests (such as echocardiogram or computed tomography (CT) scan). If there is evidence of endocarditis on a recent echocardiogram, then it is good to bear in mind that cases of blood-culture-negative endocarditis are not uncommon and should still be treated with empirical antibiotics while awaiting review by a specialist (Delgado et al, 2023).

Depending on technique, blood cultures can often be contaminated with

Fig 1. **Classification of bacterial species**



Gram stains are used to identify initial characteristics of organisms while awaiting further information from the culture. This can then be used to guide empirical treatments

skin commensal bacteria (bacteria that are naturally present on the skin, such as *Staphylococci epidermidis*). This can occur at any stage of the test, from obtaining the blood through to applying the sample onto culture plates. It may be easy to dismiss a blood culture containing *S. epidermidis* as a contaminated sample, but there have been many documented cases of endocarditis in patients with either native or prosthetic heart valves, so repeat samples should be taken if clinically indicated (Cordes et al, 2017) and correlated with other appropriate diagnostic tests.

Urine cultures

Urine cultures are only performed if a urine sample has been reviewed under microscopy and deemed to have greater than 40×10^6/L white blood cells. Despite the common misconception, urine is not sterile once it leaves the ureters. Therefore, the presence of bacteria or yeast is not always a cause for concern. Pregnant women are an exception to this rule, as they are at increased risk of infection with group B streptococcus, which may cause asymptomatic bacteriuria initially that later migrates to the reproductive tract (Grey et al, 2023). Microbial colonisation of the urinary tract of older people and those with indwelling urinary catheters is likely to happen, and there is no consensus over the role that these microbes may play in the health of the individual, though if other symptoms of urinary tract infection are present (such as lower back pain, increased frequency of micturition, increased urgency and fever) then empirical treatment should be given in line with local policy (Grey et al, 2023; Miller et al, 2024).

Figure 2 shows a workflow for women under 65 years with an uncomplicated urinary tract infection (UTI) (Grey et al, 2023).

Sputum cultures

Sputum cultures, whether spontaneously expectorated, suctioned from the hypopharynx via the nose (as in children), or acquired as an endotracheal aspirate in ventilated patients, indicate the presence of bacteria or fungi in the respiratory tract. While expectorated sputum is often colonised with normal oropharyngeal flora, it is still valuable for diagnosing infections when clinical signs such as fever, increased sputum production or changes in sputum colour suggest an active infection, even if a chest X-ray is inconclusive.

As with blood cultures, obtaining a high-quality sputum sample before starting antimicrobial therapy is best practice (Ogawa et al, 2023). In cases where tuberculosis is suspected, three separate sputum samples on three consecutive days should be sent for acid-fast bacilli stain testing (Shen and Sergi, 2023).

Polymerase chain reaction (PCR)

PCR is a great tool for identifying organisms that are difficult to culture, such as chlamydia, or viruses like influenza. This process involves amplifying DNA from a clinical sample, such as blood, tissue, or other bodily fluids, which is then compared to a library of clinical samples with known DNA sequences to identify the specific organism present. This used to take multiple days to perform, but is now much accelerated (Espy et al, 2006). In cases of culture-negative endocarditis, tissue samples may be processed for a 16S PCR, which looks for prokaryotic ribosomal DNA to identify organisms that are difficult to culture, such as *Bartonella* and *Tropheryma whippelii*. Similarly, if a fungal infection is suspected, samples can be sent for a 18S PCR. This test is highly sensitive, so the potential for contamination is possible, and any results should be discussed with a clinical microbiologist (Patel et al, 2017).

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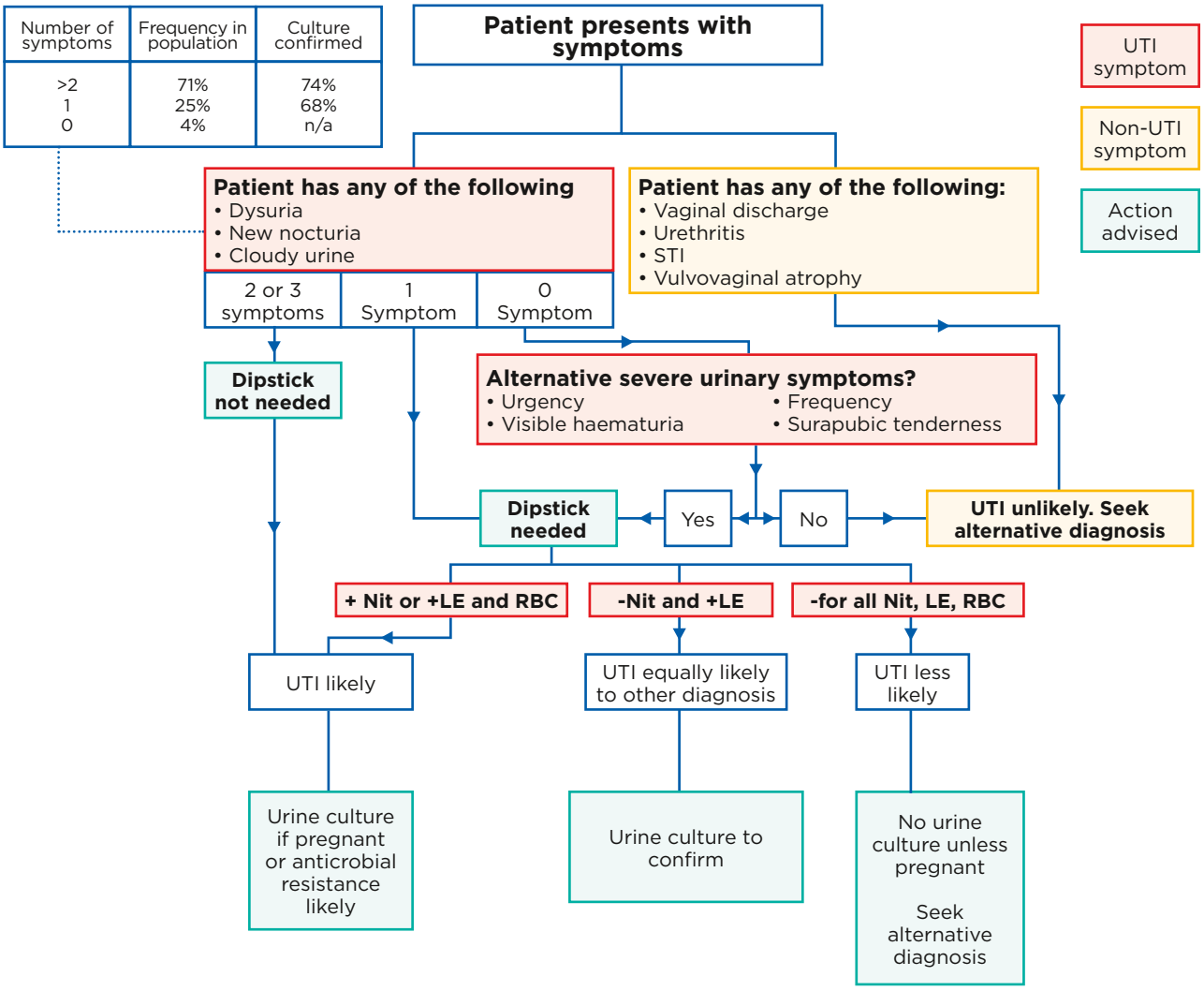
Fig 2. **Workflow for women under 65 years with uncomplicated cystitis**



STI = sexually transmitted infection, UTI = urinary tract infection; Nit = nitrite; LE = leucocyte esterase; RBC = red blood cell.

Source: Grey et al, 2023

Effective first-line treatment

Antibiotics

Antibiotics can be split into 'broad spectrum' and 'narrow spectrum' based on the range of organisms against which they will work. It is good to bear in mind that antibiotics will work against the patient's own commensal bacteria as well as pathogens, meaning that bacteria in the gut will also be depleted. This can have serious implications for the patient, as disrupting the ecosystem of the gut can lead to *Clostridium difficile* filling in the niche left by the eradication of the normal 'friendly' bacteria (Patangia et al, 2022). Antibiotics can disrupt the normal flora of the vaginal tract, leading to the development of thrush, as the pH balance change allows yeast to grow uncontrolled.

Typically, the first-line treatment for a potentially life-threatening infection would be to administer intravenous (IV) broad-spectrum antibiotics, such as piperacillin-tazobactam. Then, once the causative organism has been identified, treatment can be narrowed to target the specific pathogen (for example, narrowing to flucloxacillin if methicillin-sensitive *Staphylococcus aureus* is identified). Local guidelines should always be followed for empirical treatment, as these will have been developed in partnership with the clinical microbiologists to reflect the local resistance rates for specific infections. Always be sure to review this treatment at 48-72 hours, alongside any culture results, to assess whether the treatment has been successful and to ensure the shortest duration of effective treatment is given. (National Institute of Health and Care Excellence, 2015).

Antifungals

Fungal disease is a much larger cause of concern for neonates, people living with asthma (particularly those taking corticosteroid therapies), transplant recipients, cancer patients and individuals living with HIV/AIDS than for the general population (Bongomin et al, 2013). If fungal infection is suspected, initial management usually involves either an azole (such as fluconazole or voriconazole) or an echinocandin (such as caspofungin or anidulafungin) depending on the severity of disease. *Candida* species are the most common cause of invasive fungal infection and can range from

Table 1. **Empirical treatment options before causative organism has been identified**

Infection type	Drug class	Route of administration	Drug and Dosage	Duration
Bacterial	Broad-spectrum antibiotic	IV	**Piperacillin-tazobactam:** ● **Adults** 4.5g every 6-8 hours ● **Neonates and Children** 90 mg/kg every 6-8 hours (max dose 4.5g) ● For people with a penicillin allergy, please see local policy for alternatives	Review within 48-72 hours; adjust length of treatment based on culture results
Fungal	Azole or echinocandin antifungals	IV or oral	**Fluconazole:** ● **Adults** 400mg IV/PO initially then 200-400mg once daily ● **Neonates and children** 6-12 mg/kg IV/PO once daily (max per dose 800mg) **Caspofungin:** ● **Adults** 70mg IV once, followed by 50mg once daily (increased to 70mg once daily for body weight >81kg) ● **Neonates and children** Dosage depends on length of child, see British National Formulary for Children for information	Typically 14-21 days; consult a specialist
Viral	Antiviral	Oral or IV	**Oseltamivir (respiratory viruses)** ● **Adults** 60mg PO twice daily, increased to 75mg if body weight >41kg ● **Neonates and children** Dosage depends on age and weight of child, see BNFC for more information. **Acyclovir (acute encephalitis)** ● **Adults** 5mg/kg IV every 8 hours ● **Neonates and children** Dosage depends on age and weight/length, please consult BNFC for more information.	Duration of treatment depends on severity of disease, consult with a virologist when deciding on treatment duration
Parasitic	No nationally agreed empirical guidelines	N/A	Discuss with microbiology or infectious disease consultant	As advised by a specialist

IV = Intravenous; PO = by mouth (oral)
Dosages may need to be adjusted based on the patient's renal and liver function and local policy.
Always follow local policy for empirical treatment and consult with a specialist when necessary.

simple mucosal infections, such as thrush, to life-threatening invasive disease, such as endocarditis (Pappas et al, 2009). Culture samples should be sent with an indication that fungal infection is suspected, alerting biomedical scientists that fungal cultures should be set up alongside bacterial ones, as this will help guide antifungal treatment.

It is always worth bearing in mind that antifungal agents may be extremely toxic to the patient's kidneys or liver, and resistance is fast emerging as the development of new antifungal drugs lags behind. (Campoy and Adrio, 2017).

Antivirals

The majority of viral illnesses are self-limiting and so management of symptoms is often sufficient treatment. However, as seen with the recent Covid-19 pandemic, viral illnesses can quickly become life-threatening. People more at risk of complicated influenza (that which requires hospital admission, exhibits central nervous system dysfunction, and/or exacerbation of a chronic condition) include:

- Those over 65 years of age;
- Pregnant/recently pregnant women;
- Children under 6 months old;
- Those with a BMI over 40.

"All samples sent for culture should be appropriate to the suspected cause and severity of infection"

Treatment for these patients usually involves a short course of oseltamivir (UKHSA, 2021).

Herpes simplex virus (HSV) is the most common cause of acute encephalitis and is typically identified through the use of PCR testing of cerebrospinal fluid. IV acyclovir is often started before PCR confirmation is received due to the increased mortality associated with delayed treatment. The

typical duration of treatment for viral encephalitis is approximately 14-21 days, though this should always be discussed with a consultant virologist where possible for expert advice (Divyashree et al, 2023; Bradshaw and Venkatesan, 2016).

Antiparasitics

People entering or returning to the UK from abroad with persistent abdominal symptoms (those lasting more than 2 weeks) should always be tested for the presence of parasites in their stool, either through microscopic analysis or PCR testing. While there are no nationally agreed guidelines for empirical treatment of parasitic infection, any suspicion for parasitic infection should be discussed with a consultant in microbiology or infectious diseases (Ross et al, 2013).

Identifying improvements

After commencing treatment (examples of empirical treatment options before the causative organism has been determined can be found in Table 1), it is important to review the patient at regular intervals to identify efficacy. A strong multidisciplinary team made up of surgeons, medics, nurses, pharmacists, radiologists and many more will provide the best possible outcomes for your patients and encourage more effective collaboration (Kempson et al, 2023; Sires et al, 2023).

The trend in white cell count (WCC), or white blood cells (WBC), particularly neutrophils, indicates a response by the bone marrow to an acute infectious or inflammatory response. As treatment progresses, WCC is expected to decrease towards the 'normal' parameters of 4.0-11.0 x 10^9/L. However, if WCC is not trending downwards, further investigations may be needed to identify if there is another source of infection that has not been removed, such as an abscess or empyema. Similarly, C-reactive protein levels are expected to normalise quickly once the source of infection has been treated (Ishimine et al, 2013).

Procalcitonin (PCT) is a protein biomarker that is often used to guide antimicrobial treatment, as it is positively correlated with bacterial infection. However, it does not elevate in viral infections (Assicot et al, 1993), which made it very useful during the Covid-19 pandemic to aid the de-escalation of antimicrobial therapies once PCT levels were less than 0.25µg/L (Peters et al, 2021). Procalcitonin should not be used in isolation to determine treatment courses; the clinical context and severity of illness should always be considered.

Taking serial blood cultures is integral to managing bacteraemias, particularly those caused by *S. aureus*. These help to identify if treatment is working, as well as help identify severity of the disease (multiple positive blood cultures over a period of time may indicate endocarditis, for example) and so further tests such as a transthoracic echocardiogram (TTE) or a CT scan may be required. A single negative culture is not always enough to confirm treatment success though. The 'skip phenomenon' has been identified in some patients, where persistent positive blood cultures are intermittently interrupted with a negative culture. Therefore, it is good practice to wait for multiple consecutive negative cultures before bacterial clearance can be determined (Go et al, 2022).

Conclusion

Microbiology testing and regular review by a dedicated multidisciplinary team are vital to enable effective treatment of infections in accordance with antimicrobial stewardship guidelines. Being able to understand microbiology tests and results allows the clinical practitioner to properly manage the patient and prevent long courses of broad-spectrum antibiotics that will lead to high rates of antimicrobial resistance and treatment failure. However, all test results should be utilised in conjunction with sound clinical judgement and reasoning. **NT**

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Professional responsibilities

This procedure should be undertaken only after approved training, supervised practice and competency assessment, and carried out in accordance with local policies and protocols.