

Interpretation of microbiological reports

Sensible interpretation of microbiological reports can make a difference to patient management.

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The quality of a laboratory report is influenced by many variables such as the quality of the specimen collected, the time of transporting the specimen to the laboratory and the provision of appropriate and correct information on the laboratory request form to guide the laboratory in processing and interpreting the culture results.

The laboratory procedures are quality controlled to guarantee relevant, reliable, timely, and correctly interpreted reports that will guide patient management. However, poor-quality specimens may lead to misleading results, inappropriate antimicrobial therapy and delay in diagnosis.

Different types of specimens

Sputum

The laboratory can determine the quality of a sputum specimen on microscopy:¹ few or no polymorphic white blood cells (WBCs) and many epithelial cells (derived from mucous membrane of the mouth) indicate that the specimen consists of or contains saliva (Fig. 1). The culture results will therefore not be reliable. Numerous polymorphs with only scanty numbers of epithelial cells, and culture results that correlate with organisms observed on microscopy, indicate a 'good' specimen (Fig. 2). Likely pathogens, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, are always reported, but may also colonise the upper respiratory tract. Other organisms such as *Enterobacteriaceae* group (*Escherichia coli*, *Klebsiella*, etc.) and *Candida* are reported if abundant growth is present, but may reflect upper respiratory overgrowth due to antimicrobial therapy.

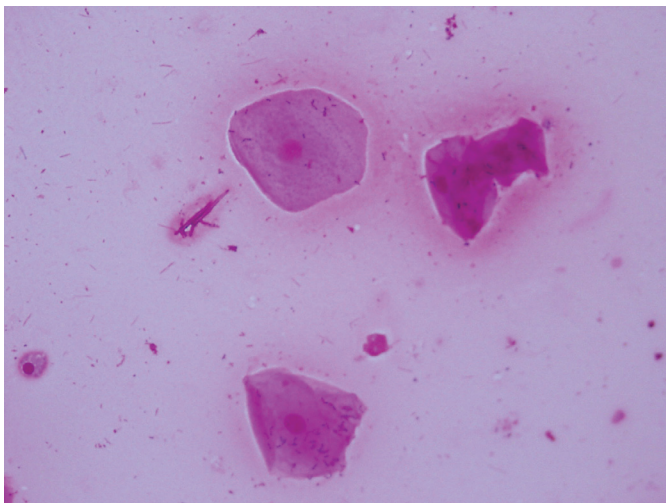


Fig. 1. Gram stain of poorly collected sputum specimen that consists mainly of saliva showing numerous epithelial cells.

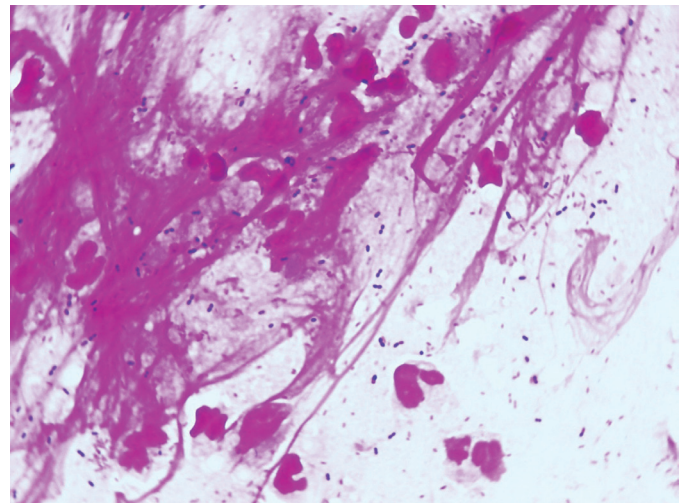


Fig. 2. Gram stain of good sputum specimen showing numerous polymorphs, Gram-positive diplococci presumptive of pneumococci.

Urine

Numerous leucocytes observed on urine microscopy indicate possible urinary tract infection. In addition, a pure growth of one organism of greater than 10^5 colony-forming units (CFU)/ml from a midstream urine specimen (MSU) is regarded as significant in a patient not on any antibiotics (Fig. 3).² A contaminated specimen usually contains scanty or no leucocytes, epithelial cells and yields mixed growth (Fig. 4).

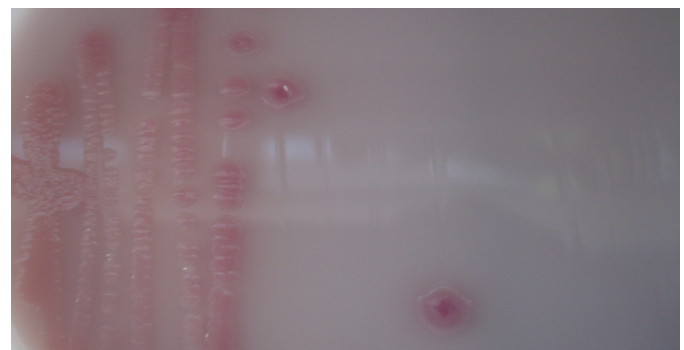


Fig. 3. Urine culture on Uriselect medium showing pure culture of *E. coli*.

In catheter or suprapubic samples if more than one organism is isolated in counts $<10^4$ CFU/ml, the result may still be significant. Note, however, that catheter samples should be collected aseptically from the catheter sampling port and not the collection bag, which may be contaminated.

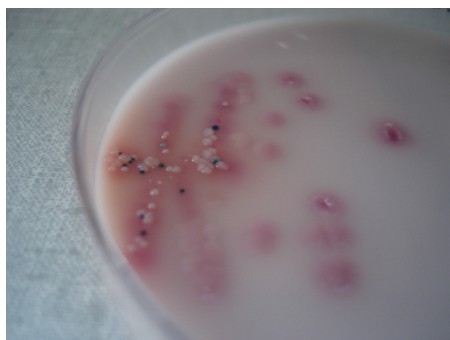


Fig. 4. Urine culture on Uriselect medium showing a mixed growth indicating contamination.

Sterile pyuria (numerous leucocytes but no bacterial growth) may be due to the presence of antibiotics. Also consider possible tuberculosis, vaginitis, non-infective causes such as recent surgery or foreign bodies in the urinary tract, e.g. an indwelling catheter.

The susceptibility of the urinary antiseptics, nalidixic acid and nitrofurantoin, is reported on isolates from urine samples. However, these agents should not be used for systemic infections such as pyelonephritis due to inadequate serum levels.³ Certain antimicrobials, e.g. aminoglycosides and beta-lactams, achieve very high concentrations in the urine and may be clinically efficacious even though these agents test intermediate resistant *in vitro*.

Pus swabs or pus/tissue

It is important to indicate on the request form from which site a specimen was collected, as this facilitates the selection of possible pathogens from a specimen yielding a mixed growth. Also indicate if a specimen was collected from a superficial wound or if pus/tissue was collected during surgery. Superficial wounds are frequently colonised with Gram-negative organisms. Therefore skin disinfection, and wound debridement where necessary, is critical before specimens are collected. Some pathogens will require specific culture media or extended incubation times for isolation. Consult the microbiologist or indicate on the request form if an unusual or fastidious pathogen such as *Actinomyces*, *Corynebacterium diphtheria*, *Neisseria gonorrhoeae* or a fungal cause is suspected.

Swabs are inferior to aspirated pus or a tissue biopsy in the recovery of fastidious and anaerobic organisms, as these organisms may desiccate or die in an aerobic environment. As swabs are frequently used to collect material from the surface of wounds, the culture may often yield mixed growths that are difficult to interpret. Some laboratories will comment that the organisms isolated are of doubtful significance or may represent colonisation or contamination. A repeat specimen will then be recommended if

clinically indicated: pus, tissue or a deep swab of the advancing margin of the lesion after disinfection with sterile saline. Often the laboratory will not be able to identify individual organisms due to mixed growths and will simply report that a mixed growth of questionable significance was obtained. This is particularly the case when more than four different organisms are cultured from a specimen with no predominant growth of a particular organism. Such mixed growths are usually due to a poorly taken specimen, or a heavily contaminated site where empiric therapy will be more cost effective than the separation and individual susceptibility testing of several different organisms, which may take several days.

Stool

Routine processing includes microscopic examination of a wet mount preparation for red and white blood cells and pathogenic parasites (cysts/trophozoites of *Entamoeba histolytica*, *Giardia*, worm ova, etc.), as well as bacterial culture for *Salmonella*, *Shigella* and *Campylobacter*. The diagnosis of typhoid (*Salmonella* Typhi) also requires blood cultures, as stool cultures may be negative. Other pathogens such as *Yersinia*, *Vibrio cholerae*, *E. coli* 0157 (which may be implicated in haemolytic uraemic syndrome) or *Clostridium difficile* require special culture media or tests and should be specifically requested if clinically suspected. Other bacterial causes of gastroenteritis such as the enteropathogenic *E. coli* strains cannot be distinguished from normal gut flora on routine laboratory cultures. A modified acid-fast stain is performed to identify parasites such as *Cryptosporidium* and *Isospora*, causing chronic diarrhoea in immunocompromised patients. Indicate if these investigations are specifically required. Negative results may also indicate a viral gastroenteritis.

Blood culture

Blood cultures are incubated in automated, continuous-monitoring systems. Positive cultures are immediately flagged and, if the system is interfaced with the laboratory computer system, a preliminary report of a positive culture may be immediately available to clinicians. The initial Gram stain on a positive blood culture is useful in determining the possible aetiology of bloodstream infections, e.g. 'Gram-positive cocci in clusters' are presumptive of staphylococcal bacteraemia. Note however that the Gram stain cannot distinguish *S. aureus* from coagulase-negative staphylococci such as *S. epidermidis*. The Gram stain also guides further laboratory testing to determine the identification and susceptibility profile of the organism. Because of the importance of these results, the usual laboratory practice is that

the results of Gram stains are phoned out to the ward or the treating clinician.

Contamination of blood cultures with skin flora (coagulase-negative staphylococci, corynebacteria, etc.) and environmental flora (*Bacillus* species, etc.) is a common problem. Furthermore, these organisms may be significant under certain circumstances: coagulase-negative staphylococci may cause bloodstream infections associated with infection of foreign devices such as intravascular catheters. To establish the clinical significance of these skin commensals, repeated isolation of the same organism from different sterile sites is required. Therefore multiple sets of blood cultures are recommended, with at least one set obtained from the venepuncture site to improve the yield of blood cultures and to determine the clinical significance of isolates.⁴ Intravascular catheter sites are acceptable alternative sites for collecting blood, but should be labelled as such and preferably be combined with blood cultures from a venepuncture site to facilitate the interpretation of clinical significance.

Persistent bloodstream infections (repeated positive blood cultures with same organism) after appropriate antimicrobial treatment may indicate a deep-seated abscess or endovascular source such as endocarditis or intravascular catheter sepsis that requires surgical drainage or removal of the catheter. *Candida* spp. and *S. aureus* are particularly associated with persistent infection, therefore repeat blood cultures after >3 days on adequate treatment is recommended to determine clearance of the blood.⁵

Cerebrospinal fluid (CSF)

Bacterial meningitis is associated with a purulent CSF (>100 polymorphs per mm³), whereas the CSF is clear/slightly turbid with viral or tuberculous meningitis. The normal CSF may contain some lymphocytes.

The most common bacterial pathogens are *N. meningitidis* (Gram-negative diplococci); *S. pneumoniae* (Gram-positive diplococci) and *H. influenzae* (Gram-negative bacilli). *H. influenzae* type b meningitis is now less common due to the routine immunisation of all infants with the Hib vaccine. The key pathogens in the neonate are group B beta-haemolytic streptococcus, *E. coli* and less commonly *Listeria monocytogenes* (Gram-positive bacilli), which may also be a causative agent in the immunocompromised host.

Empiric treatment with cefotaxime/ceftriaxone, targeted at penicillin-resistant pneumococci, will be adequate for all common bacterial pathogens, except *Listeria* which is inherently resistant to the cephalosporins. If *Listeria* is suspected, ampicillin should be added.

Tuberculous and cryptococcal meningitis are characterised by a moderately high white cell count, predominantly lymphocytes, a raised protein and low glucose level. The Indian ink stain is useful to detect the typical capsulated yeasts cells of *Cryptococcus*, but is less sensitive than the cryptococcal antigen latex test. In severely immunocompromised hosts, the cell count may be deceptively normal. With suspected TB meningitis, scanty acid-fast bacilli may be observed on microscopy, but acid-fast stains are often negative and TB culture should be performed (Table I).

Antibiogram

Many laboratories use selective reporting of antibiotics to prevent the inappropriate use of costly or broad-spectrum agents that may select for multi-resistant strains.

Different methods are employed to determine antimicrobial activity, e.g. disk diffusion (measure zone of inhibition), automated broth dilution or E-test method measure minimum inhibitory concentration (MIC).⁷ Susceptibility results are interpreted according to international guidelines, e.g. CLSI⁸ which are based on achievable serum concentrations. Depending on the method, detection of resistance may not be reliable for certain agents, and laboratories will automatically select an appropriate alternative method. For example, for *S. pneumoniae* penicillin susceptibility cannot be reliably tested using the disc diffusion method. Therefore the MIC needs to be determined. The interpretation of susceptibility, however, also depends on the site of infection. For pneumococcal meningitis a lower MIC (≤ 0.064 $\mu\text{g/ml}$) than for respiratory infection (≤ 2 $\mu\text{g/ml}$) is required for the susceptibility category due to poor blood-brain barrier penetration of penicillin. In such cases, clinical information becomes paramount to the correct interpretation of susceptibility results.

Other examples of agents that are inappropriate for treatment despite *in vitro* susceptibility include first- and second-

generation cephalosporins for *Salmonella* infections; cephalosporins for enterococci; and beta-lactams for MRSA. Laboratories will therefore not report such agents for these pathogens, despite their apparent susceptibility *in vitro*.

If the patient does not respond to appropriate antibiotics according to culture and susceptibility results of good-quality specimens, the following possible reasons should be investigated:²

- poor source control – septic foreign material such as intravascular catheter/prosthesis that has not been removed; pus collection not drained or necrotic tissue/bone not debrided
- inadequate therapy – suboptimal antibiotic concentration at site of infection due to poor penetration, e.g. endocarditis or inadequately administered (doses missed, too low dose for patient).
- resistant organism subpopulations not detected with routine susceptibility methods, e.g. subpopulations of *S. aureus* heteroresistant to vancomycin, or that may have been selected on treatment, e.g. resistant mutant populations of *Enterobacter* spp. selected on cephalosporin treatment.

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In a nutshell

- Poor-quality specimens may lead to misleading results, inappropriate antimicrobial therapy and delay in diagnosis.
- The quality of specimens such as sputum and urine is interpreted according to the microscopy and culture findings.
- The site from where the specimen was collected is important to determine the significance of the organisms isolated.
- Organisms from non-sterile sites such as the respiratory tract may be colonisers and not necessarily the cause of infection.
- Swabs are inferior to aspirated pus or tissue specimens in the recovery of pathogens.
- Swabs from non-sterile sites often yield mixed growths due to colonisation or contamination that are generally not clinically significant.
- Contamination of blood cultures with skin and environmental flora or so-called 'contaminants' is a common problem.
- To establish the clinical significance of 'contaminants', repeated isolation of the same organism from different sterile sites is required.
- If the patient does not respond to antibiotic therapy chosen according to the microbiological susceptibility profile, consider: inadequate therapy for the infection site; inadequate dosage; poor source control and the possible selection of resistant mutants.

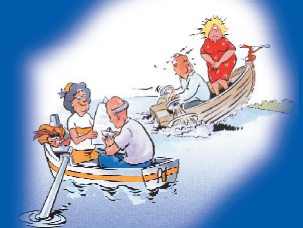
Table I. Characteristic CSF changes in meningitis⁶

Condition	Appearance	Cells, $\times 10^6/\text{l}$ (mm^3)	Protein (g/l)	Glucose (mmol/l)
Normal	Clear	0 - 5 [*] lymphocytes	0.15 - 0.4	2.2 - 3.5 [†]
Bacterial meningitis	Turbid	100 - 2 000 polymorphs	0.5 - 3	0 - 2.2
TB/cryptococcal meningitis	Clear/slightly turbid	30 - 500 lymphocytes + polymorphs	1 - 6	0 - 2.2
Viral meningitis	Clear/slightly turbid	15 - 500 lymphocytes	0.5 - 1	Normal

* In neonates, up to 30×10^6 white blood cells and protein up to 1.5 g/l may be still normal.

† ~60% of blood glucose level.

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