**Direct diagnostic tests (microscopy, culture and susceptibility testing)**

Direct tests have the aim to visualise or recover the fungus directly from patient samples such as blood cultures, BAL, sterile fluids such as cerebrospinal fluid (CSF) or tissue samples. It involve basic microscopy and conventional culture. Special stains can be applied directly to clinical samples to make it easier to see fungal elements. As an example fluorescence quenching dye such as cacofluor can make it much easier to recognise fungal elements such as *Aspergillus* hyphae in a broncho-alveolar washing using a fluorescence microscope (Figure 2.a). Seeing such hyphae in the primary specimen together with clinical information and radiological imaging in high risk patients would make the diagnosis of invasive aspergillosis highly likely. Other fungi such as *Candida* can be seen directly on a Gram stain as purple fungal elements in a positive blood culture (Figure 2.b) confirming the diagnosis of candidaemia. Another classic specimen is CSF fluid which is helpful in the diagnosis of cryptococcal meningitis. India ink microscopy directly from the CSF can be used to demonstrates the presence of yeast with a polysaccharide capsule confirming cryptococcal encephalo-meningitis in high risk patients (HIV, solid organ transplant patients etc.).

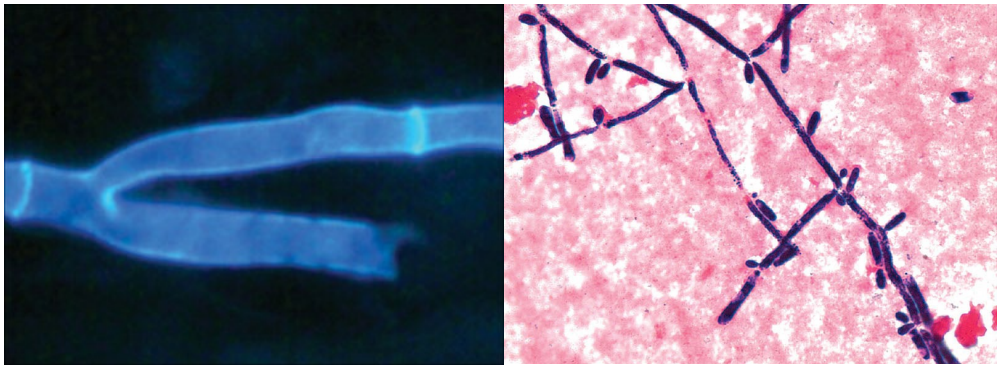

Figure 2. Calcofluor stain (a) in a BAL and Gram stain of yeasts (b) in a blood culture

Clinical samples such a BAL, blood culture, CSF or other sterile fluids are also cultured on a common fungal agar (Sabouraud dextrose agar). Specialist chromogenic agar may be used in addition to help identify some Candida species. Most cultures would be kept for 5 days to assess fungal growth. However, different fungal species can have different growth rates or may be affected by anti-fungal treatments requiring longer culture (incubation) periods. In cases of cryptococcosis this may take up to 21 days in some cases. The temperature is also important and although most pathogenic fungi will grow at 35-37C best practice is to use a number of temperatures (Incubation at 28, 37 and 45 °C).

The benefit of a positive fungal culture is that the isolates can be in many cases speciated on the basis of the macroscopic and microscopic appearance figure 3.

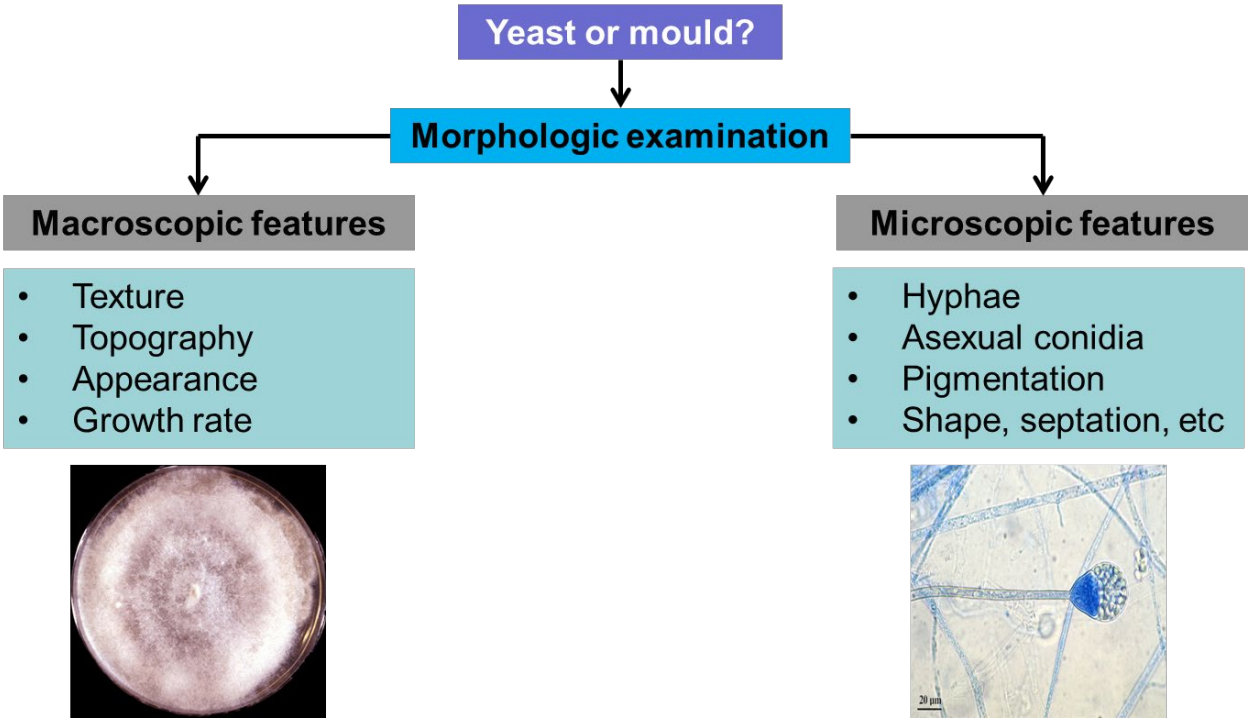


Figure 3. Principles for identifying fungi on the basis of morphological examination.

Other methods such as MALDI-TOF are commonly replacing conventional biochemical tests for the identification of yeasts in the routine laboratory.

Fungal culture is the gold standard but some fungi are not easily cultured or hidden at sites that are not very accessible to sampling (liver, kidney, brain).

Another benefit of conventional fungal culture is the ability to undertake anti-fungal susceptibility testing to commonly used antifungal agents. Antifungal drug resistance has been emerging over the last decade in response to longer term clinical azole use or even use in agriculture. This has led to an increase in azole resistant Aspergillus species. Other multidrug resistance fungi such as *Candida auris* are globally emerging causing severe infections and hospital transmission..

Direct microscopy from tissues remains a vital diagnostic tool in the diagnosis of invasive fungal infection in the immunocompromised and vulnerable patient. The visualisation of fungal elements in tissue is mainly undertaken by histopathology departments. Tissue samples can be fresh frozen or preserved in paraffin blocks and thin slices are cut, placed on glass carrier slides and subjected to special fungal stains (Grocott-Gomori's methenamine silver stain or Periodic acid–Schiff ) which makes it easier to visualise fungal elements. This examination forms the gold standard for confirmed diagnosis of invasive fungal infection and is part the EORTC diagnostic criteria. However, it is important to

remember that microscopy of histology tissue cannot accurately speciate a fungus. Basic fungal morphology can help to distinguish a yeast (single round/oval shape structures) from a mould infection (hyphal structures). When hyphae are seen they may have different morphological appearances (dichotomous branching hyphae are more  in keeping with Aspergillus or if the hyphae do not demonstrate any septae it may point more to a mucoracious mould. Both fungal groups would have a very different implication for which antifungal may be used to treat the patient.