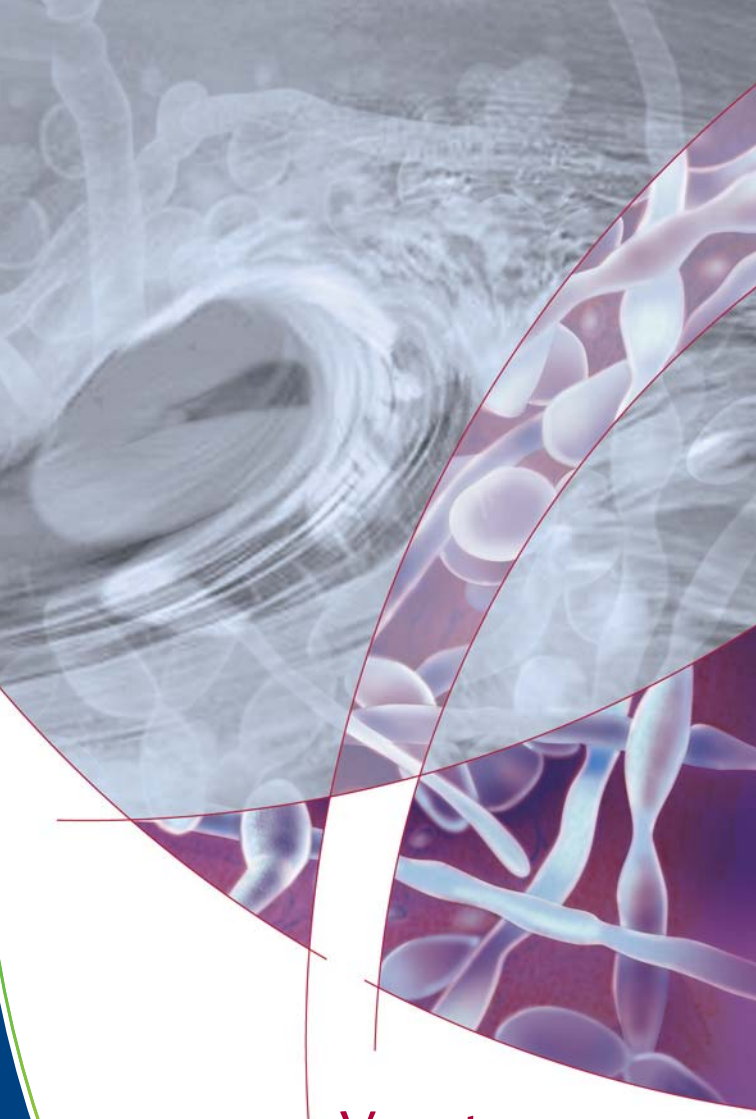
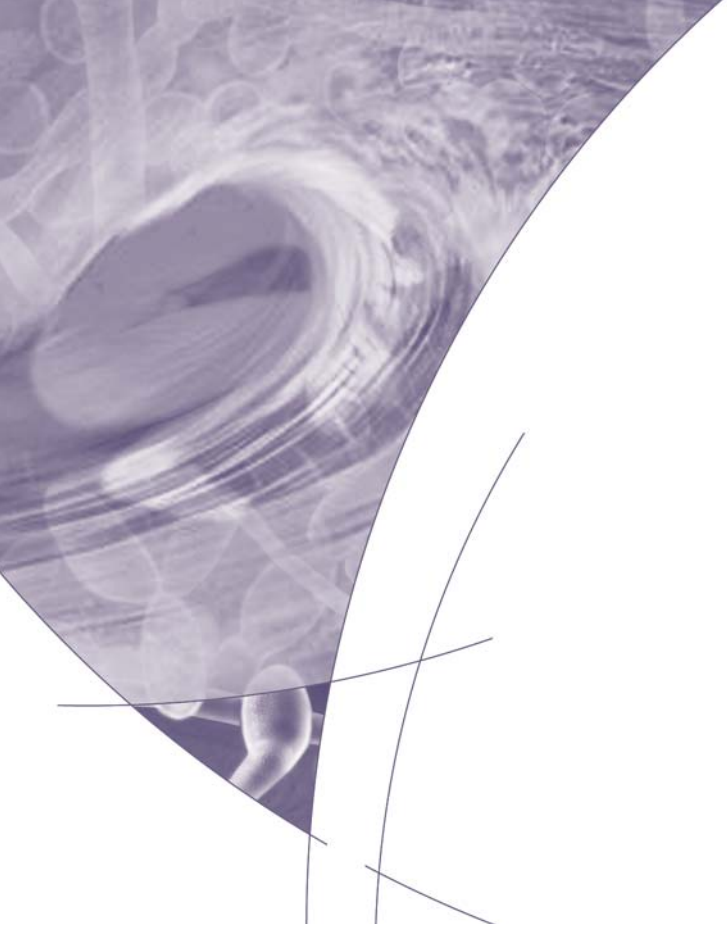


Yeast infections

From specimen collection to antifungal susceptibility testing

from diagnosis,
the seeds of better health

BIOMÉRIEUX



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Mycosis

is a disease that is becoming increasingly common. Its incidence has been on the increase over for the last decades, particularly among intensive care and immunodeficient patients, as well as patients suffering from cancer and neutropenia. Nosocomial infections have a fungal origin in 8% of cases, generally linked to the *Candida* genus [(1)].

While *Candida albicans* and *Cryptococcus neoformans* represent the more commonly pathogenic species of yeasts, the species causing superficial and/or invasive infections diversify with "non-*albicans*" species such as *Candida glabrata*, *Candida tropicalis*, *Candida kefyr*, *Candida parapsilosis*. The *Trichosporon*, *Saccharomyces*, *Rhodotorula genera* are also considered to be the cause of severe infections in some high-risk patients.

Early diagnosis of yeast infections and the implementation of appropriate treatment currently represent major issues for clinicians.

Epidemiology and risk factors

Yeasts

have very varied natural habitats.

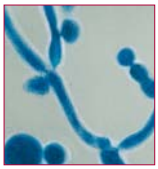
Yeasts are commensal and are widely found in human cavities, mucosa and digestive tracts.

They can become pathogenic and engender opportunistic infections when favourable conditions appear in the host body.

The use of broad-spectrum antibiotics and immunosuppressive agents, invasive surgery, keeping severely weakened patients alive or simply working conditions (long contact with water or detergents) lead to "opportunistic" infections :

- superficial mycosis (skin, hair, mucosa)
  or
- invasive mycosis (septicemic or visceral).

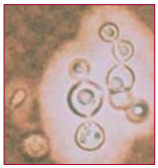
Candida is therefore one of the major types of mycosis, with only around 10 species found to be potentially pathogenic in man.



Candida albicans

- ***Candida albicans*** is the yeast most frequently isolated in clinical practice. This commensal yeast of the digestive mucosa is not normally found in the environment. There is a balance between *Candida albicans* and intestinal bacteria.
  Certain factors (antibiotic therapy, oral contraceptives, immunosuppressive agents, pregnancy, heroin addiction, digestive tract surgery, severe associated conditions, etc) can disrupt this balance, causing proliferation in the digestive tract of *Candida albicans*.

- **Other species** of yeasts are also isolated from the digestive tract but they are more ubiquitous, and are found both on skin and in the environment (water, soil, plants, air). They are less pathogenic, and are associated with a very significant impairment in the host's defenses.



Cryptococcus neoformans

- ***Cryptococcus neoformans***
  Depending on the geographic location, cryptococcosis only concerns 2 to 30% cases of HIV-infection, which is the main factor favouring infection. In AIDS patients, this is an opportunistic infection which occurs at an advanced stage of immunodeficiency.
  This risk can now be well controlled by anti-retroviral tritherapy.
  In people who are not HIV-infected, factors favouring infection are: long-term corticoid therapy, lymphoid hemopathy, organ transplants, and diabetes. Contamination occurs by inhalation.
  The most frequent clinical form is meningo-encephalitis which is fatal without treatment.

Ecology of yeasts and epidemiology of infections

	Natural habitat	*Sites of infection*
Candida albicans	Digestive tract	Mucous membranes Cutaneous extension Invasive sites, blood, urine
Other *Candida* species	Skin, mucous membranes, feces Environment, particularly on flowers, leaves, water, soil	Skin, mucous membranes, nails Invasive sites
Trichosporon	Soil Nails, skin, mouth Animals	Hair (white piedra), skin, numerous invasive sites
Saccharomyces	Humans, mammals, birds, wine, beer, fruit, trees, plants, olives, soil	Colonisation of digestive tube
Rhodotorula	Wet skin, environment : air, soil, fresh water, sea water and dairy products	Blood, CSF, invasive sites
Cryptococcus	Soil contaminated by pigeon or other bird droppings for *Cryptococcus neoformans* var. *neoformans* or tropical trees (eucalyptus) for *Cryptococcus neoformans* var. *gattii*	Lungs (site of entry) Central nervous system, CSF Dissemination possible in the bloodstream, urine, skin, prostate and other sites

Clinical environment

Physiopathology

Yeast infection can be divided into two main groups:
- superficial yeast infections;
- invasive yeast infections (origin is 90% endogenous).

Superficial yeast infections

- Mucosal yeast infections:

oro-pharyngeal (thrush), anal and urogenital.
- Cutaneous yeast infections:

intertrigo, folliculitis, onyxis, perionyxis.

A favourable environment is particularly important e.g. humidity, heat, maceration through perspiration.

Invasive yeast infections

These are septicemic or visceral forms of mycosis which have more varied symptoms (urinary, ocular, meningeal, cardiac, hepatic, renal, pulmonary, osteo-articular and peritoneal infections, etc.).
Invasive forms of yeast infection have two possible sources:
- **endogenous:** in neutropenic patients, mucosa weakened due to chemotherapy and long-term antibiotic therapy favour passage of yeasts that have colonized digestive and/or urogenital sites into the bloodstream and the main organs. Blood culture is the first means of diagnosis for this invasive form. For some cases of invasive candidosis, there is no hematogenous dissemination and consequently it is necessary to associate a sero-immunological approach. *Candida albicans* is the main isolated species among cases of systemic mycosis.

■ **exogenous:** most often of iatrogenic origin (linked to new therapies), often in patients with intravascular catheters (infusion products, etc.), sometimes nosocomial (e.g. via handling by healthcare workers).

Candida species isolated in invasive candidemia: European data [11]

Species	*Frequence*
Candida albicans	43 to 67 %
Candida glabrata	8 to 16 %
Candida parapsilosis	7 to 30 %
Candida tropicalis	2 to 10 %
Candida krusei	0 to 3 %
Candida lusitaniae	0 to 2 %
Candida kefyr	0 to 1.6 %
Candida guilliermondii	0 to 1.6 %

Pathogenic yeast species are increasingly varied.
Some species are resistant to commonly used antifungal agents and require the use of new antifungal molecules.
Identification is systematic notably in the following cases:
■ in immunocompromised patients, regardless of specimen type;
■ or for closed or usually sterile specimens: blood, urine, CSF, synovial fluid, etc.

For invasive yeast infections, clinical symptoms should in no way be considered sufficient to establish diagnosis, since they do not indicate whether the infection is in fact due to yeasts or the type of yeast species involved. Culture, identification and antifungal susceptibility testing are mandatory.

Treatment

Treatment of yeast infections is oriented by:
■ the site of infection
■ the patient
■ the species involved

The antifungal susceptibility test is essential for all yeasts isolated in invasive sites regardless of the patient concerned, and in at risk patients. In cases where the patient has already been treated with an azole antifungal molecule, resistance must be tested for.
In the majority of superficial mycosis cases, topical antifungal agents are highly concentrated and the posologies used are higher than MICs, making the need for antifungal susceptibility testing unjustified.

The treatment of invasive yeast infection is currently based on 4 antifungal agent families corresponding to a limited number of molecules:

■ **Amphotericin B** is the reference because of its fungicidal activity and broad spectrum (yeasts, moulds, dimorphic fungi) but the use of this preparation is limited because of its renal toxicity and patient reactions during infusion. New less toxic and more active lipidic forms have been introduced on the market.

■ In the azole family, **triazoles** (fluconazole, itraconazole) represent, within this context, an important contribution given their safe use and excellent bioavailability.
Due to its spectrum (*Candida albicans*, *Cryptococcus neoformans*), fluconazole has become a first-line molecule against many opportunistic infections linked to HIV-infection, as well as in immunocompromised patients.
There have been recent therapeutic improvements with the emergence, in the azole family, of new molecules such as

voriconazole presenting a broader spectrum of action than fluconazole.
Posaconazole and ravucanazole are also molecules with promising results.

■ **Echinocandins**
Caspofungin is a semi-synthetic derivative of echinocandin used in the treatment of some types of invasive mycosis in patients showing no or a poor response to other treatments.

■ **5 fluorocytosine** is still used despite the hematological toxicity risk and the rapid growth of resistance. This is why it is no longer used as a monotherapy, but in association with amphotericin B in cases of cryptococcocal meningitis.

New strategies based on **associations of antifungal agents**, immunostimulant therapies and growth factors are currently being explored.

The treatment of superficial and mucosal yeast infection requires a wider range of antifungal agents: polyenes (amphotericin B, nystatin) or azole derivates (miconazole, econazole, ketoconazole, clotrimazole, isoconazole, tioconazole, bifonazole).
Terbinafine is used orally in cutaneous candidosis (notably onychomycosis) which resist to local therapy.

Preventive treatment of invasive mycosis
In patients at high risk of invasive candidosis (under treatment leading to neutropenia, in intensive care), numerous prophylactic approaches or preventive therapy can be implemented by clinicians.

Sensitivity of *Candida* species [3]

	Candida albicans	*Candida tropicalis*	*Candida parapsilosis*	*Candida glabrata*	*Candida krusei*	*Candida lusitaniae*
Fluconazole	S	S	S	S-DD to R	R	S
Itraconazole	S	S	S	S-DD to R	S-DD to R	S
Voriconazole	S	S	S	S to I	S to I	S
Flucytosine	S	S	S	S	I to R	S
Amphotericin B	S	S	S	S to I	S to I	S to R
Echinocandins	S	S	S (to I ?)	S	S	S

S sensitive, S-DD sensitive-dose/dependant, I intermediate, R resistant

Biological examination of specimens for the detection of yeasts

Specimen collection

Successful isolation and identification of the causative fungal agent depends on quality of the specimen collection.
Collection should be performed before prescribing any local or systemic antifungal therapy.
If therapy has begun, it must be discontinued for at least eight days or more (three months for nails) before collection.
The collection method varies according to the main site of infection:

site		*Specimen collection*
Skin	Intertrigo, perleche (dry lesion)	Collect using a curette, a vaccinostyle or a bistoury from the periphery of the lesion
	Intertrigo, perleche (weeping lesion)	Sterile swab moistened with physiological saline
	Onychia	Cut off part of the infected nail, after scraping the lower surface of the nail plate with a curette
	Perionyxis	Compress lesion to recover pus or collect scales from sides of nail
Mucosal membrane	Oral thrush, vaginitis, balanitis, anal mucosa	Recover fluids and secretions with moist, sterile swab
Digestive tract	Stools	Collect stools in sterile bottle Rectal swab collection may be performed on children
	Gastric washing	Collect fluid in sterile bottle
Broncho-pulmonary cavity	Sputum	Rinse mouth with an antiseptic to eliminate commensal flora Collect sputum in sterile bottle
	Bronchoscopic aspiration	Collect aspirated fluid in sterile tube or bottle
Viscera	Cerebral Renal	Collect CSF and urine in sterile tubes or bottles
	Septicemia Endocarditis	Carry out blood cultures preferably using selective media
Biopsies		Divide the collection into two: • One specimen for microbiological inoculation in a dry, sterile bottle • The other for anatomopathology (e.g. fixation in Bouin's fluid)

Transport

The specimens are rapidly examined and placed in culture to prevent drying and yeast proliferation in the pathological substance (which would prevent the evaluation of their actual abundance).
The maximum transport time to the laboratory is 24 hours, and 2 hours for lumbar punctures and biopsies.
Blood cultures must be sent to the laboratory immediately.
In the event of deferred culture, semi-liquid specimens (pus, secretions) and swabbed specimens are placed in a suitable transport medium.

Direct examination

Specimen preparation

- Scales and nail fragments: mounting in a 30% potash or lactophenol solution.
- For other specimens (pus, stools, exudates, etc.), perform the examination either fresh between a slide and coverslip or on a stained smear: Gram, Giemsa, cotton blue, methylene blue.

Direct examination represents an important stage in diagnosis which may result in the detection of yeasts under the microscope: 4 to 6 µm, budding oval elements, possibly associated with the presence of mycelian filaments.

For stool, sputum and mucosal specimens, the **abnormal abundance** of yeasts makes it possible to rule out commensalisms. In urine specimens yeast enumeration is recommended.

When the presence of *Cryptococcus neoformans* is suspected, microscopic capsule detection is performed in an India ink-based suspension.

Appearance of yeasts in direct examination [6]

Yeasts	*Diameter of elements*	*Appearance in direct examination*
Candida glabrata	3-6 µm	Blastospores, no filaments
Other *Candida* species	3-10 µm	Blastospores and mycelian filaments
Trichosporon spp.	3-14 µm	Blastospores and arthrosporate filaments
Cryptococcus neoformans	3-20 µm	Round blastospores with capsules of differing thickness seen in negative (India ink)

Culture

The specimens must preferentially be cultured on media allowing a **rapid result** and the **detection of species associations**.
Chromogenic media enable the selective isolation of yeasts and rapid identification of *Candida albicans*.
These media should be associated with conventional media, particularly when screening for yeasts in at risk patients.

Chromogenic culture media

These media contain:

- nutrients enabling microorganism growth,
- antibiotics to inhibit bacteria,
- chromogenic substrates used to detect specific enzymes for certain yeast species.

In this way, it is possible to perform instantaneous identification directly on the culture medium, particularly for *Candida albicans*.

In relation to conventional media, chromogenic media enable:

- easy viewing of species associations,
- identification requiring no confirmation for *Candida albicans*,
- a more rapid result than with the use of conventional media.

Conventional culture media

■ **Isolation medium**

The most commonly used medium is **Sabouraud** medium.
The addition of antibiotics, particularly **Chloramphenicol and/or Gentamicin** increases the selectivity of the medium with respect to bacteria.

■ **Special culture media for morphological observation**

• Morphological tests to detect the genus can be performed by means of culture on a poor medium, such as Rice-Agar-Tween. This medium favours the production of characteristic *Candida albicans* chlamydospores and can also be used to observe a pseudomycelium, characteristic of the *Candida* genus, associated with blastospores. This detection method remains the reference.

• It is possible to differentiate between yeasts using tetrazolium salt reduction. Tetrazolium salt-reducing yeast colonies have a pink to purple colony pigmentation.

Comparison of chromogenic media versus conventional methods

	Chromogenic media	*Conventional methods*
Identification of *Candida albicans* during isolation	Yes Confirmation not required	After isolation and biochemical or other identification tests
Time to result	Over 80% of *C.albicans* are identified in 24 hrs	48 – 96 hrs: 24 – 48 hrs for isolation then 24 – 48 hrs for identification
Visualisation of species associations	Yes, orientation of the identification for *C. tropicalis, C. lusitaniae, C. kefyr*	Selective isolation of fungi
Ease-of-use	Yes	Requires trained personnel for identification

Identification

Identification of *Candida albicans*

■ Using chromogenic media, *Candida albicans* is identified directly on the culture medium by detecting a hexoaminidase-specific enzyme activity.

■ Using Sabouraud culture medium isolates, the most conventional test is the blastesis or serum filamentation test.
Candida albicans is the only species to produce germinative tubes.

Identification of other yeast species

Complete biochemical identification

This involves the study of:

■ sugar assimilation: auxanogram

■ sugar fermentation: zymogram

■ and other tests, if required (tetrazolium chloride reduction, growth in presence of actidione, urease detection).

Identification using immunological tests

Different species can be identified:
Candida albicans, Candida krusei, Candida dubliniensis.

Colony typing, in hospitals for cases of grouped candidemia, can be performed using molecular biology techniques.

Antifungal susceptibility testing

The aim is to define the minimum inhibitory concentration of the antifungal molecule and detect yeast resistance to these molecules. The use of conventional agar diffusion techniques using disks is now on the decline.

Reference protocols have been developed to define a new standardization of the MIC determination method used to obtain satisfactory between-laboratory reproducibility, NCCLS M 27-A2 and EUCAST.

The **NCCLS** defines the reproducibility conditions for *in vitro* susceptibility testing for the three antifungal classes with macro and microdilution.

EUCAST (European Committee on Antimicrobial Susceptibility Testing) describes the protocols to determine the MIC for pathogenic yeasts (essentially *Candida albicans*).

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Web sites

http://www.anaes.fr
http://www.doctorfungus.org/
http://www.medicalmycology.org/
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/candidiasis/

Mycology range

TRANSPORT			
Portagerm®	Ref. 42105	20 tubes	Buffered agar medium for the transport of swabbed specimens at ambient temperature
Portagerm®	Ref. 41995	10 bottles	Buffered agar medium for the transport of liquid specimens at ambient temperature
Portagerm® AMIES agar Swab	Ref. 41999	50 units	Swab for sampling and transporting microorganisms
Mycoline	Ref. 56525	10 slides	Double-sided agar-coated slide for the transport and culture of yeasts and dermatophytes (Sabouraud Gentamicin Chloramphenicol medium on one side and Sabouraud Chloramphenicol Actidione on the other)
CULTURE			
Chromogenic medium*			
Candida ID 2	Ref. 43631 Ref. 43639**	20 plates 100 plates	Chromogenic medium for the direct identification of *Candida albicans* and selective isolation of yeasts by direct inoculation of pathological substances
Other media* : new Sabouraud range			
Sabouraud 2 agar	Ref. 42037** *(replaces ref. 42092)* Ref. 42066** *(replaces ref. 42026)*	20 tubes *(inclined)* 6 x 100 bottles	Culture isolation medium for fungi
Sabouraud liquid medium	Ref. 42108	20 x 9 ml tubes	Culture or subculture medium for yeasts or moulds
Sabouraud Chloramphenicol 2 agar	Ref. 42038** *(replaces ref. 42093)* Ref. 42067** *(replaces ref. 42027)*	20 tubes *(inclined)* 6 x 100 bottles	Selective isolation medium for fungi
Sabouraud Chloramphenicol Actidione agar	Ref. 42094	20 tubes *(inclined)*	Selective culture medium for dermatophytes and other fungi
Sabouraud Gentamicine Chloramphenicol 2 agar	Ref. 43651 *(replaces ref. 43171)* Ref. 43659** *(replaces ref. 43179)* Ref. 42056** *(replaces ref. 42016)* Ref. 42031** *(replaces ref. 42095)*	20 plates 100 plates 6 x 100 bottles 20 tubes *(inclined)*	Culture and selective isolation medium for yeasts and moulds Culture and selective isolation medium for fungi
Sabouraud Tétrazolium Gentamicine Chloramphenicol agar	Ref. 42096	20 tubes *(inclined)*	Selective medium to detect yeast-induced tetrazolium salt reductions
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* *For technical details and product compatibility, refer to the package insert or technical sheet*

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Simplified laboratory testing protocol*

SPECIMEN

Blood culture LCR

Mycoline

DIRECT EXAMINATION

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ISOLATION

24-48 hours at 37° C

Candida ID2

White or pink colonies

Blue colonies

Candida albicans

IDENTIFICATION

24-48 hours at 30° C

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or

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