FUNGICHROM®

Identification of the Main Pathogenic Yeasts

25 tests (Cat. N° 44325)

GB-2006-09

CE

1 - INTENDED USE

The FUNGICHROM kit allows the identification of the main human pathogenic yeasts notably via the use of chromogenic substrates (2, 8).

2 - INTRODUCTION

Fungal infections and especially those caused by yeasts have significantly increased over the last ten years (6). Yeasts are opportunistic agents (5). Most of them are saprophytes, but they can become pathogenic when the conditions in the host become favourable.
These conditions are mainly the physiological factors (newborn babies, elderly people, pregnant women), the local factors (chafings, macerations), the pathological factors (cancer, immune deficiency, metabolic disorders...), the therapy-related factors (antibiotics, birth control pills, immuno-suppressive agents, ionising radiations, surgery...).
The clinical features caused by these yeasts are quite varied: cutaneous conditions (intertrigo, onyxis...), mucocutaneous disorders (oral candidiasis, oesophagitis, colitis, vaginitis...) visceral and septicaemic conditions.
Yeasts are unicellular thallus fungi that multiply by budding. The main pathogenic genera are: *Candida, Cryptococcus, Rhodotorula, Saccharomyces, Trichosporon.*
The *Geotrichum* genus is composed of filamentous fungi whose mycelium produces true hyphae that segment into rectangular arthroconidia varying in size and in the roundness of the distal portion.

3 - PRINCIPLE

The identification of yeasts is based on the presence or the absence of various enzymes, visualized by a colour change. The enzymatic activities are revealed by three kinds of reaction.
• **Hydrolysis of chromogenic substrates** (1, 7): The osidase and peptidase activities of the yeasts hydrolyse the chromogenic substrates leading to the release of para-nitroaniline, para-nitrophenol or ortho-nitrophenol, which are characterised by a yellow-coloration (wells GAL, PRO, ONPG, EPA, SGL, GLY).
• **Assimilation of natural substrates**:
-The use of sugars is revealed by the colour change of bromocresol purple (BCP) from violet to yellow or even by the absence of colour (wells GAL-SAC, TRE, MAL, CEL, RAF, LAC)
-The resistance to actidione is revealed by the same principle (well ACT)
-The hydrolysis of urea releases ammonia, which alkalinizes the medium making phenol red (PR) turn to a fuchsia-pink colour (well URE).
• **Oxidation of synthetic substrates**: The activity of phenoloxidase in the presence of caffeic acid produces a brown coloration (well POX) (4).
Each FUNGICHROM tray also includes a positive control well (well T+) which reveals the assimilation of glucose.

4 - REAGENTS

Conditionnement :
Kit contents for 25 tests:
- 1 TC FUNGI Vial
- 27 SUSPENSION FUNGI Vials
- 25 FUNGICHROM Trays

Description :
TC FUNGI : 4-ml vial of barium sulphate solution for turbidity control.
SUSPENSION FUNGI : 4-ml vial of agar solution containing Bacto Agar (0.5 g/L), colimycin (2.5 mg/L) and vancomycin (0.05 g/L).
FUNGICHROM Tray : ready for use 20-well tray
- well 1 = T+: positive control well containing glucose and BCP
- well 2 = GAL: contains a chromogenic substrate for N-acetyl-ß-D galactosaminidase
- well 3 = PRO: contains a chromogenic substrate for L-proline-amidase
- well 4 = ACT: contains actidione, glucose and BCP
- well 5 = ONPG: contains a chromogenic substrate for ortho-nitrophenyl-ß-D-galactosidase
- well 6 = EPA: contains a chromogenic substrate for a peptidase
- well 7 = SGL: contains a chromogenic substrate for a peptidase
- well 8 = GLY: contains a chromogenic susbstrate for glycine-amidase
- well 9 = URE: contains urea and PR
- well 10 = POX: contains a substrate for phenoloxidase
- well 11 = GAL-SAC: contains galactose, sucrose and BCP
- well 12 = TRE: contains trehalose and BCP
- well 13 = MAL: contains maltose and BCP
- well 14 = CEL: contains cellobiose and BCP
- well 15 = RAF: contains raffinose and BCP
- well 16 = LAC: contains lactose and BCP.
- wells 17 to 20 = empty wells

5 - PRECAUTIONS

• The reagents are intended solely for *in vitro* use and must be handled by authorised personnel.
• The samples and inoculated reagents are potentially infectious; they must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
• Certain wells of the FUNGICHROM tray contain raw materials of animal origin which must be handled with caution.
• Do not use reagents after the expiry date.
• Do not use reagents that have been damaged or that have been poorly conserved before use.

6 - SPECIMEN COLLECTION AND TREATMENT

The colonies used for performing the identification of the yeast should be young (24 to 48 hours old) and perfectly isolated on an agar medium in a Petri dish. It is recommended that isolation be made on media that are specific for yeast (3).

7 - REAGENT PREPARATION AND STORAGE

The kit and its contents when stored at 2-8°C in their original state are stable until the expiry date indicated on the box. Reagents are ready for use.
The SUSPENSION FUNGI vials and the trays must be used immediately after opening.

8 - REAGENT AND MATERIALS REQUIRED BUT NOT PROVIDED

• 0.1 N NaOH.
• Incubator at 30°C
• Pasteur pipettes.
• Container for contaminated waste.

9 - PROCEDURE

Allow the reagents to reach room temperature (18-25 °C) before use.

9.1. Examination of the isolated colonies

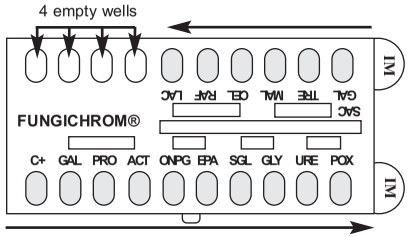
Proceed with the macroscopic and microscopic examination of the colonies before inoculating the tray.

9.2. Preparation of the inoculum

Pick up two or three identical isolated colonies with a wire loop or an occluded Pasteur pipette. Inoculate a vial of SUSPENSION FUNGI with the colonies. Mix well.
The standardization of the inoculum can be performed in various ways:
• **In Relation to the TC FUNGI**
Adjust the opacity of the inoculated medium to that of the TC FUNGI turbidity control with the aid of the black lines printed on the labels of the vial.
If the medium is lighter (insufficient inoculum), the vial should be inoculated again until the observed opacity equals that of the turbidity control.
If the medium is more turbid (inoculum too rich), dilute it with fresh SUSPENSION FUNGI from a newly opened vial until the correct turbidity is obtained.
• **With a Densitometer**
Verify with a densitometer that the turbidity of the inoculated medium is equal to **2 Mac Farland**. If necessary, proceed as above to adjust the turbidity.
• **Enumeration in Malassez Cell**
It is possible to standardize the inoculum by counting the yeast in a Malassez cell. A 10^6 to 10^7 yeast/ml solution should be obtained.

9.3. Inoculation of the tray

Lift the adhesive tape covering the tray. Inoculate the first 16-wells with 2 drops (approx. 100µl) of the inoculated SUSPENSION FUNGI. The last 4 wells do not need to be filled. Reseal the tray with the adhesive tape.



9.4. Incubation

Incubate at a maximum of 30 °C for 24 to 48 hours.
Read the tray when the positive control well has turned from violet to yellow or colourless.
However, for the strains that show only a predominantly proline-amidase positivity at the 24-hour mark, continue the incubation for a further 24 hours. With urea positive strains, incubation can be continued for up to 72 hours.

10 - READING AND INTERPRETATION

10.1 Reading of the tray

Colours are stable for 4 hours on the bench. It is recommended that the "colour chart" included in the kit, be used for the reading of the tray.
Read well T+; if it is still violet, prolong the incubation time.
If well T+ has turned yellow or colourless, **add one drop of 0.1 N NaOH to well GAL (2nd well)** and read the tray referring to the following table:

Wells	Reading of the reaction		Interest
	Negative reaction	*Positive reaction*	
GAL PRO	Colourless	Yellow	Identification of *C. albicans* Orientation of species
ACT	Violet	Yellow to colourless	
ONPG	Colourless	Yellow	
EPA	Colourless	Light yellow	Orientation of main species
SGL GLY	Colourless or very light yellow*	Plain yellow	
URE	Yellow	Red to fuchsia	Orientation *Cryptococcus, Rhodotorula, Trichosporon*
POX	Colourless	Brown	
GAL-SAC TRE, MAL, CEL, RAF, LAC	Violet	Yellow to colourless	Identification of species

*When in doubt, consider the well as negative.

10.2 Identification

The identification of the strains is performed with FUNGICHROM in parallel with the routine analysis of morphological features.
The interpretation of the FUNGICHROM tray is performed either by a coding system or by the identification table. If, at the 24-hour mark, the code obtained is not referenced, continue to incubate for another 24 hours. If, at the 48-hour mark, the code is still not itemized, refer to the identification chart. For a given yeast, all the predominant characteristics mentioned in this table must be definitely positive, except when a positivity percentage is mentioned.
In order to work out the code, the characteristics are grouped in triplets or doublets:
- GAL, PRO, ACT
- ONPG, EPA
- SGL, GLY
- URE, POX
- GAL-SAC, TRE, MAL
- CEL, RAF, LAC.

Each characteristic is ascribed a zero value if the element is negative. If the element is positive, its value depends on its position in the doublet or the triplet:
- 1 for position 1 - 2 for position 2 - 4 for position 3.

In the same doublet or triplet add up the values, a 6 digit number is obtained. Then look for this number on the enclosed list. Example:

GAL	PRO	ACT	ONPG	EPA	SGL	GLY	URE	POX	GAL-SAC	TRE	MAL	CEL	RAF	LAC
+	+	+	-	-	-	-	-	-	+	+	+	-	-	-
1	2	4	0	0	0	0	0	0	1	2	4	0	0	0
	7		0		0		0			7			0	

Code: 70 00 70 ; this code corresponds on the list to *Candida albicans.*

10.3 Differential Diagnosis

Complementary examinations may be necessary for differentiating certain strains:
• ***Candida famata* and *Trichosporon cutaneum*** (code 00 01 73) :
The morphological examination on PCB shows that *Candida famata* does not form filaments, whilst *Trichosporon cutaneum* forms filaments and arthrospores.
• ***Candida krusei* and *Candida rugosa*** (codes 00 10 00 and 00 10 10) :
Candida krusei generally gives rise to colonies with a dry and matt aspect, unlike *Candida rugosa*, but this is not always the case.
• ***Candida inconspicua* and *Candida zeylanoides*** (code 20 00 00) :
Candida inconspicua never forms filaments on PCB, unlike *Candida zeylanoides.*
• ***Candida famata, Rhodotorula rubra* and *Cryptococcus albidus*** (code 20 01 73) :
Unlike *Candida famata*, *Rhodotorula rubra* possesses a red pigment and *Cryptococcus albidus* possesses a capsule.
• ***Candida guilliermondii* and *Candida famata*** (codes 20 00 73 and 60 20 73) :
Candida guilliermondii, unlike *Candida famata* forms filaments on PCB.
• ***Candida lipolytica* and *Geotrichum capitatum*** (code 60 31 00) :
The morphological examination shows that *Candida lipolytica* is a yeast, whilst *Geotrichum capitatum* is a filamentous fungus, that forms arthrospores.

11 - QUALITY CONTROL

It is recommended that the standardization of the method be checked from time to time using the reference strains, *Candida albicans* ATCC 9029 and *Candida glabrata* ATCC 90030.

Strain	***Expected results***														
	GAL	PRO	ACT	ONPG	EPA	SGL	GLY	URE	POX	GAL-SAC	TRE	MAL	CEL	RAF	LAC
C.albicans (ATCC 9029)	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-
C.glabrata (ATCC 90030)	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-

12 - CAUSES OF ERROR

• Handling in a non-sterile environment.
• Preparation of the inoculum from a mixed culture.
• Evaporation of the well contents during incubation as a result of the tray not being properly sealed with the adhesive tape.
• Incubation of the tray at 37 °C instead of 30°C.
• Tray read before the apparition of a colour change of the growth indicator.
• Tray not read after incubation for 24 or 48 hours, even though the growth indicator was positive.
In general the non-respect of the recommendations contained within the instructions.

13 - LIMITATIONS

• The FUNGICHROM method only allows the identification of species present in the enclosed list.
• For certain strains, the analysis of morphological characteristics present in colonies grown on a yeast specific agar medium, may be necessary in order to confirm the diagnosis (§10.3).

14 - PERFORMANCES

The performance evaluation was carried out using 18 reference strains, 227 collection strains and 241 freshly isolated strains from mycological specimens. The comparative study was carried out in parallel with the Auxacolor and API 20C trays (8).
With the FUNGICHROM method (n=486) :
- 85% of the strains were identified within 24 hours of incubation at 30°C;
- the sensitivity was 97,7% after 48 hours of incubation;
- the specificity was 99,8% after 48 hours of incubation and with a morphological examination.

15 - WASTE ELIMINATION

Waste should be disposed of in accordance with the hygiene rules and current regulations for this kind of product in the country of use.

16 - BIBLIOGRAPHY

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