ELIchrom *glabrata*

Rapid test for identification of *Candida glabrata*

40 Tests

(Ref. 44506)

8000050-EN-2025-01

For *in vitro* diagnostic use only, for professional use only.
Single use test.

CE

1 - AIM

ELIchrom *glabrata* is a fast unitary test (20 minutes) for the rapid identification of *Candida glabrata* colonies.
Each kit allows 40 tests to be carried out.

2 - INTRODUCTION

Yeast of the genus *Candida* can be responsible for cutaneous candidiasis, mucosal candidiasis, and candidemias or invasive candidiases.
*Candida*s are usually commensal yeast of the digestive and urogenital mucosas. They become pathogenic only when favourable conditions arise in the host. Among the factors that favour *Candida* infection, there are intrinsic physiological factors or pathologies (advanced age, pregnancy, diabetes, immunological deficiencies and malignant diseases). As for extrinsic factors, these are primarily iatrogenic in nature. The prevalence of candidiasis has increased considerably over the last twenty years, as a consequence of the emergence of pathologies such as AIDS, the generalization of antibiotic treatments and oral contraceptives, the development of therapeutic immunosuppressive agents, parenteral alimentation, and the multiplication of aggressive investigative methods and surgical procedures. For example, the candidemias account for approximately 10% of nosocomial infections; a percentage that can reach 20% according to certain studies. Moreover, the prognosis for the candidiasis is poor, with mortality in affected patients oscillating between 38 and 50%.
C. albicans is the species that is most often isolated. It accounts for 60 to 80% of clinical isolates. However, other species such as *C. dubliniensis* (a species very close to *C. albicans*), *C. glabrata, C. tropicalis, C. krusei, C. lusitaniae, C. parapsilosis,* and *C. lipolytica,* are increasingly being reported as mycotic organisms.
Studies show that *C. glabrata* is implicated in between 5 and 25% of all cases of yeasts isolated in medical mycology. This species is the second most commonly encountered yeast in humans. Its pathogenic nature is no longer disputed, with C. glabrata responsible for 10 to 25% of all the candidemias. In addition, *C. glabrata* is generally less sensitive to azole derivatives, with certain isolates from patients treated with fluconazole or ketoconazole appearing to be resistant to virtually all the azole derivatives.
The isolation and rapid identification of *C. glabrata* is thus essential to the choice of therapeutic regimen. Traditionally, biochemical tests are used in the identification of *C. glabrata*. The disadvantage of the carbon auxanogram (study of the assimilation of monosaccharides as a source of carbon and energy) and the zymogram (study of the use of monosaccharides in anaerobiosis), is that there is a long 24 to 48 hours wait before the tests can be read.
C. glabrata can be identified more quickly with systems that are based upon the detection of preformed enzymes that use chromogenic substrates or alternatively, that are based upon the assimilation of trehalose. **ELIchrom *glabrata*** is a rapid *C. glabrata* identification test based upon the detection of trehalase.

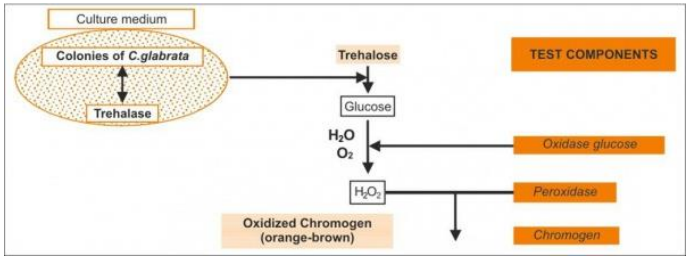
3 - PRINCIPLE

Of the yeast of medical interest, *C. glabrata* is able, under certain conditions, to rapidly hydrolyse trehalose into glucose. The detection of glucose production can therefore be used to positively identify this species. However, in addition to the trehalose test, two controls must also be carried out:

- a maltose control is necessary because certain *C. tropicalis* isolates can also degrade trehalose, but unlike *C. glabrata* they also assimilate maltose;
- a control is carried out in the absence of monosaccharide (reagent base medium control). This is necessary because of the presence of glucose in isolation media. Therefore, if sufficient care is not taken when taking colonies, there could be a risk that the sample is contaminated with agar, which could lead to a false positive reaction.

ELIchrom *glabrata* enables the identification of *C. glabrata* in three stages :

1. Preparation of a distilled water yeast suspension.
2. Incubation of the yeast suspension with the trehalose or the maltose (yeast control) or the base medium (isolation medium control).
3. Detection of the presence of glucose by a revelator, which contains oxidase glucose, peroxidase and a chromogenic substrate.



4 - REAGENTS AND MATERIAL

Description	Quantity
REAG: Vial of lyophilized reagent *(to be reconstituted with 1.3 mL of sterile distilled water)*	3
TEST CARD: Test cards containing 12 wells i.e. 4 identifications **T** wells contain base medium and trehalose **M** wells contain base medium and maltose **B** wells contain base medium	10

5 - PRECAUTIONS

- **ELIchrom *glabrata*** is intended for *in vitro* diagnostic use only and must be handled by authorized personnel. Tests are for a single use only.
- Ensure that the dehydrated media are not altered in any way (the presence of a blue stain in all wells).
- Patient samples 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.
- Do not use the reagents after the expiry date.
- Do not use reagents from different batch numbers.
- Prior to use, allow the reagents to reach room temperature.

REAG: GHS08

Contains oxidase glucose and 3,3-dimethoxibenzidine dihydrochloride.
- H334: May cause allergic or asthma symptoms or breathing difficulties if inhaled.
- H350: May cause cancer.

- P201: Obtain special instructions before use.
- P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
- P280: Wear protective gloves/protective clothing/eye protection/face protection.
- P304+P340: IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing.
- P342+P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
- P308+P313: IF exposed or concerned: Get medical advice/attention.

6 - SAMPLE COLLECTION

ELIchrom *glabrata* can be carried out on yeast isolated from a 24-96 h culture at +37°C or +22°C (Sabouraud media or *Candida* chromogenic media).

7 - CONSERVATION ET PREPARATION DES REACTIFS

Preserve the **TEST CARD** as well as the **REAG** reagent (lyophilized or reconstituted) at 2-8°C and away from the light.
After reconstitution, **REAG** reagent is stable for 2 months at 2-8°C.
The shelf-life of the reconstituted **REAG** reagent can be extended for up to 6 months by freezing (-20°C) 75 µL aliquots (in microtubes with well closed stoppers) immediately after reconstitution.
Defrosted **REAG** reagent cannot be re-frozen.

8 – MATERIAL REQUIRED BUT NOT SUPPLIED

- Automatic pipette(s) with a pipetting volume adapted to the volume that will be measured.
- Haemolysis tubes
- Sterile distilled water
- Contaminated waste containers
- Pasteur pipette or wire loop

9 - METHOD

Allow the reagents to reach **room temperature** before use.

1 Reconstitution of the REAG reagent:
- Open the **REAG** reagent with precaution (vial under negative pressure).
- Add 1.3 mL of sterile distilled water to the vial and shake gently.

2 Realization of the test:
- Remove the number of **TEST CARD**s required according to the number of yeast samples to be tested (In the case of single yeast, cut the card so that there is a **T**, **M** and **B** well present).
- Label a haemolysis tube and add 100 µL of sterile distilled water to it.
- Take great care when harvesting yeast colonies to be identified in the test to avoid contamination. Homogenize the colonies in 100 µL of distilled water in order that the density of the suspension is equivalent to point 5 in the Mc Farland range (3 to 4 colonies if the culture has been carried out on Sabouraud medium and 3 to 8 colonies, according to the size of the colonies, if the culture has been carried out on a chromogenic medium).
- With a micropipette add 25 µL of the prepared yeast suspension to each blue spot in the **T**, **M** and **B** wells.
- Leave the test to incubate for 10 minutes at room temperature (prolongation of the incubation time beyond 10 minutes will not influence in any way the final result).
- With a micropipette add 25 µL of the **REAG** reagent to each of the **T**, **M** and **B** wells.
- Incubate the test for 5 to 10 minutes at room temperature.
- Read the test results.

IMPORTANT: If reading of the test is to be delayed, the reaction can be blocked by the addition of 25µL of H_2SO_4 (3M) to each well. For a positive reaction, the coloration becomes brown/gray and then fuchsia, and in the case of a negative reaction, there is a slightly bluish coloration.

10 - READING

Positive reaction: Appearance of a more or less dark orange coloration
Negative reaction: Absence of any coloration

11 - INTERPRETATION OF RESULTS

RESULTS			CONCLUSION
WELL T (Trehalose)	**WELL M** (Maltose)	**WELL B** (Base medium)	
Positive	Negative	Negative	*C. glabrata.*
Negative	Negative	Negative	Non *C. glabrata* yeast. Continue the identification.
Positive	Positive	Negative	Non *C. glabrata* yeast. Continue the identification.
Negative	Positive	Negative	Non *C. glabrata* yeast. Continue the identification.
Positive	Positive	Positive	Non interpretable. Repeat the tests by carefully sampling the yeast without moving the agar

12 - CAUSES OF ERROR AND TEST LIMITS

Do not remove agar at the same time as the colonies as this can lead to uninterpretable results.
In all cases, it is necessary that the clinical, epidemiologic and biological data are taken fully into consideration before establishing the final diagnosis.

13 - PERFORMANCE

The evaluation results for **ELIchrom *glabrata*** demonstrate, depending upon the isolation medium used, **a sensitivity from 93.8 to 97.9%** (97.9% for the Candida ID, CandiSelect, and Sabouraud media and 93.8% for the CHROMagar candida medium) and **a specificity from 97.6 to 98.8%** (98.8% for the Sabouraud and CHROMagar candida media, 98% for the Candida ID medium and 97.6% for the CandiSelect medium). (Raymond Robert - Laboratoire de Parasitologie/Mycologie, Faculté de Pharmacie, 49100 Angers et Anne-Marie Freydière - Laboratoire de Microbiologie, Hôpital Debrousse, Hospices Civils de Lyon, 69322 Lyon).

14 - 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.
If the reagent is spilled or the work area is contamined by yeast, clean using bleach and absorbent paper.

15 - BIBLIOGRAPHY

1. Abi-said D., Anaissie E., Uzun O., Raad L., Pinzcowski H., and Vartivarian S. 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin. Infect. Dis.* 24:1122-1128.
2. Bouchara J., P., Declerck P., Cimon B., Planchenault C., de Gentile L., Chabasse D. 1996. Routine use of CHROMagar Candida medium for presumptive identification of *Candida* yeast species and detection of mixed fungal populations. *Clin Microbiol Infect* 2:202-208
3. Fenn J. P., Billetdeaux E., Segal H., Skodack-Jones L., Padilla PE., Bale M., Carroll K. 1999. Comparison of four methodologies for rapid and cost-effective identification of *Candida glabrata*. *J Clin Microbiol*. 37:3387-9.
4. Fidel P. L., Vazquez J. A., Sobel J. D. 1999.*Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. *Clin Microbiol Rev*. 12:80-96.
5. Freydiere A. M., Guinet R., and Boiron P. 2001. Yeast identification in the clinical microbiology laboratory: phenotypical methods. *Med. Mycol*. 39:9-33.
6. Lopez J., Dalle F., Mantelin P., Moiroux P., Nierlich A. C., Pacot A., Cuisenier B., Vagner O., and Bonnin A. 2001. Rapid identification of *Candida glabrata* based on trehalose and sucrose assimilation using Rosco diagnostic tablets. *J. Clin. Microbiol.* 39:1172-1174.
7. Parant F., Freydiere A. M., Gille Y., Boiron P., and Odds F. C. 2001. A 'one minute' trehalase detection test for the identification of *Candida glabrata*. *J. Mycol. Med*. 11:26-31
8. Peltroche-Llacsahuanga H., Schnitzsler N., Lütticken R., Haase G. 1999. Rapid identification of *Candida glabrata* by using a Dipstick to detect trehalase-generated glucose. *J Clin Microbiol.* 37:202-5.
9. Pfaller M. A., Diekama D. J., Jones R. N., sader H., S., Fluit A., C., Hollis R., J., Messer S., A. 2001. International surveillance of bloods tream infections due to *Candida* species: frequency of occurrence and *in vitro* susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. *J Clin. Microbiol.* 39:3254-9.
10. Pfaller M. A., Messer S. A., Hollis R. J., Jones R. N., Doern G. V., Brandt M. E., and Hajjeh R. A. 1999. Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. *Diag. Microbiol. Infect*. Dis. 33:217-222.
11. Senet J-M., Robert R. 1995. Physiopathology of candidosis. *J. Mycol. Med*. 5:145-166.
12. Freydiere A-M., Robert R., Ploton C., Marot-Leblond A., Monereau F., Vandenesch F. 2003 Aug. Rapid identification of *Candida glabrata* with a new commercial test, GLABRATA RTT. *J Clin Microbiol*. 41(8) : 3861-3.
13. Freydiere A-M., Perry J-D, Faure O., Willinger B., Tortorano A-M., Nicholson A., Peman J., Verweij P-E. 2004 Oct. Routine use of a commercial test, GLABRATA RTT, for rapid identification of *Candida glabrata* in six laboratories. *J Clin Microbiol*. 42(10) : 4870-2.
14. Peman J., Aparisi N., Garcia-Esteban C., Gobernado M., 2004 Jun. Rapid identification of *Candida glabrata* using a new commercial kit *Rev Iberoam Micol.* 21(2): 82-4.
15. Willinger B., Wein S., Hirschl A-M., Rotter M-L., Manafi M. 2005 Jan. Comparison of a new commercial test, GLABRATA RTT, with a dipstick test for rapid identification of *Candida glabrata*. *J Clin Microbiol*. 43(1) : 499-501.

The changes from the previous version are highlighted in grey.

ELITech MICROBIO
Parc d'activités du Plateau
Allée d'Athènes
83870 SIGNES
FRANCE
☎: +33 (0)4 94 88 55 00
http://www.elitechgroup.com