CANDIFAST

Identification of yeasts and antifungal resistance test

30 tests (Cat. 44030)

CANDIFAST ES *TWIN*

Antifungal resistance test for yeasts

30 tests (Cat. 44130)

CPB 0038_EN-2024-10

For in vitro diagnostic use only, for professional use only

Single use in vitro diagnostic test

CE

1 - INTENDED USE

The CANDIFAST kit allows the identification of the main human pathogenic yeasts as well as the testing of their resistance to various antifungal agents.

The CANDIFAST ES *Twin* kit is intended for testing the resistance of yeasts to various antifungal agents.

2 - INTRODUCTION

Fungal infections and especially those caused by yeasts have significantly increased over the last ten years (5). Yeasts are opportunistic agents. Most of them are saprophytes, but they can become pathogenic when the conditions in the host become favorable. 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, ionizing radiation, surgery...).The clinical features caused by these yeasts are quite varied: cutaneous conditions (intertrigo, onyxis ...), mucocutaneous disorders (oral candidiasis, esophagitis, colitis, vaginitis...) visceral and septicemic conditions.

The increase in the number of available antifungal agents and the appearance of treatment-resistant mycoses justify testing the response of the yeasts in the presence of antifungal agents (1, 2).

3 - PRINCIPLE

The identification of the yeast is based on:

- the susceptibility of the strain being tested to actidione which is visualized by a colour change of the indicator either to yellow, to orangey-yellow or to fuchsia;
- the fermentation of seven sugars which is visualized by a colour change of the indicator to yellow or to orangey-yellow due to the acidification of the medium,
- the demonstration of urease activity, which produces an alkalinization of the medium, resulting in the indicator changing to a fuchsia colour.

The determination of the resistance of yeasts to antifungal agents is based on the growth or the absence of growth of these yeasts in the presence of various antifungal agents. Growth is demonstrated by a colour change of the medium:

- the fermentation of glucose by the yeasts leads to acidification of the phenol red containing medium, making it change to a yellow or to an orangey-yellow colour,
- the hydrolysis of urea by the urease-positive yeasts releases ammonia which alkalinizes the phenol red containing medium, making it change to a fuchsia-pink colour.

4 – REAGENTS

Packaging

Reagent	**Quantity #44030**	**#44130**
CANDIFAST	30	-
CANDIFAST ES *Twin*	-	15
R1 : Vial of Reagent 1	35	35
R2 : Vial of Reagent 2	30	30
TC : Vial of Turbidity Control	1	1

Description

CANDIFAST : 20-well tray, ready for use

Each tray allows the testing of one sample (identification + resistance test)

Row for Identification	**Row for Resistance Test**
Well 1 contains phenol red, actidione (ACT) and glucose.	Well 1 is a growth control well (C+)
Well 2 to 8 contain phenol red and a different sugar as follows:	Wells 2 to 8 contain a different antifungal agent as follows:
well 2 (GLU): glucose	well 2 (AB): amphotericin B (4 µg/mL)
well 3 (GAL): galactose	well 3 (NY): nystatin (200 units/mL)
well 4 (TRE): trehalose	well 4 (FCT): flucytosine (35 µg/mL)
well 5 (MAL): maltose	well 5 (ECZ): econazole (16 µg/mL)
well 6 (CEL): cellobiose	well 6 (KTZ): ketoconazole (16 µg/mL)
well 7 (RAF): raffinose	well 7 (MCZ): miconazole (16 µg/mL)
well 8 (LAC): lactose	well 8 (FCZ): fluconazole (16 µg/mL)
wells 9 et 10 are empty	wells 9 et 10 are empty

CANDIFAST ES *Twin*: 2x10-well tray, ready for use

The two rows of wells are identical to the resistance test row of the CANDIFAST tray.

Each tray allows the testing of two samples (resistance tests only).

R1: 4-mL vial of buffered agar medium for dilution and identification.

Beef extract 1.3g/L, Casein peptone 1.8g/L, Yeast extract 0.8g/L, Amino acids, vitamins, minerals 5.75g/L, Urea 20g/L, Agar 0.52g/L, Antibiotics 1.12g/L. pH: 6.05 ± 0,1.

R2: 2-mL vial of YNB (yeast nitrogen base) medium containing urea and phenol red for resistance test.

Beef extract 1.3g/L, Casein peptone 1.8g/L, Yeast extract 0.8g/L, Amino acids, vitamins, minerals 8.75g/L, Urea 20g/L, Glucose 8.5g/L, Phenol red 0.052g/L, Antibiotics 1.16g/L. pH: 7.3 ± 0,1.

TC: 4-mL vial of barium sulphate solution.

5 - PRECAUTIONS

- The reagents are intended solely for *in vitro* use and must be handled by authorized 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.
- Reagents containing raw materials of animal origin 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

The colonies used for performing the identification and the resistance test should be young (24 to 48 hours old) and perfectly isolated at room temperature or at 37 °C on an agar medium, preferably in a Petri dish. It is recommended that isolation be made on media that are specific for yeast (3).

7 - REAGENT STOCKAGE

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. One half of a CANDIFAST ES *Twin* may be stored at 2 to 8°C for 7 days, in its original packaging, resealed with the desiccant.

8 - REAGENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Paraffin oil
- Sterile pipette
- Incubator at 37°C
- Container for contaminated waste

9 – PROCEDURE

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

9.1. Preparation of the inoculum

Pick up an isolated colony with a wire loop or an occluded Pasteur pipette. Inoculate a vial of Reagent 1 with the colony. Mix well. The standardization of the inoculum can be performed in three different ways:

• With Respect to the Turbidity Control Vial

Adjust the opacity of the inoculated Reagent 1 to that of the Turbidity Control with the aid of the black lines printed on the vial labels.

If Reagent 1 is lighter (insufficient inoculum), inoculate the vial further until the opacity obtained equals that of the Turbidity Control vial.

If Reagent 1 is more turbid (inoculum too rich), dilute it with Reagent 1 from another freshly opened vial until the correct turbidity is obtained.

• With a Densitometer

Verify with a densitometer that the turbidity of inoculated Reagent 1 is equal to 1 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 yeasts in a Malassez cell. A solution of 2,500 to 3,500 yeasts per mm3 must be obtained.

9.2a Inoculation of CANDIFAST tray

Identification row

Mark the test-tray to identify the specimen being tested. Lift the adhesives tape and dispense into each of the first 8 wells as follows:

- 100 µL of inoculated standardized R1
- 2 drops of paraffin oil

Reseal the test-tray with adhesive tape.

Resistance test row

First, inoculate Reagent 2 with 100 µL of inoculated standardized Reagent 1 (see § 9.1). Then, lift the adhesive tape and dispense into each of the first 8 wells as follows:

- 100 µL of inoculated R2
- 2 drops of paraffin oil.

Reseal the test-tray with the adhesive tape.

9.2.b Inoculation of CANDIFAST ES *Twin* tray

First sample row

Inoculate reagent R2 with 100 µL of inoculated standardized reagent R1.

Label both rows of the tray.

Lift the adhesive tape and dispense into each of the first 8 wells:

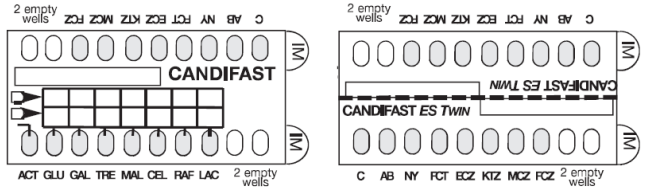
- 100 µL of inoculated Reagent 2
- 2 drops of paraffin oil.

Reseal the test-tray with adhesive tape.

Second sample row

Inoculate in the same way the second row with an additional vial of inoculated Reagent 2 as described above.

Reseal the test-tray with adhesive tape.



9.3. Incubation

Incubate the test-tray at 37°C for 24 hours; if necessary and depending on the strains, this incubation may be prolonged for up to 48 or even, 72 hours. The tray is read once the yeast have grown in the control well.

10 - READING AND INTERPRETATION

Read each test-tray in its entirety only if in the control well (C+: Well 1 of the resistance test row) there is the following colour change of the medium:

- Colour change to yellow, to orangey-yellow, or to fuchsia* (*for urease positive genera)

10.1 Identification Row

Well 1 (ACT)

The characteristic is positive when a colour change of the medium is observed:

- Colour change to yellow, to orangey-yellow, or to fuchsia

A characteristic is negative when there is no colour change to yellow, to orangey-yellow or to fuchsia.

Wells 2 to 8 (GLU to RAF)

A characteristic is positive when a colour change of the medium is observed:

- Colour change to yellow, to orangey-yellow, or to fuchsia

A characteristic is negative when there is no colour change to yellow, to orangey-yellow, or to fuchsia.

The identification of the yeasts is performed with the CANDIFAST tray in parallel with the analysis of the morphological features of the colonies.

For interpretation, refer to the table below or to the chart provided in the kit. The markings on the test-tray adhesive tape may also be used: **read the actidione well first; then, locate the last positive well, on the right of it.** The name of the germ is printed on the adhesive tape at the intersection of the actidione-positive (lower

row) or actidione-negative (upper row) characteristic and the located sugar.

Yeast	ACT	GLU	GAL	TRE	MAL	CEL	RAF	LAC
Candida albicans	+	+	+	v	+	-	-	-
Candida albicans (var stellatoïdea)	+	+	-	v	+	-	-	-
Candida glabrata	v	+	-	+	-	-	-	-
Candida kefyr	+	+	+	-	-	-	v	+
Candida krusei	v	+	-	-	-	-	-	-
Candida lusitaniae	-	+	+	+	v	+	-	-
Candida parapsilosis	-	+	+	-	v	-	-	-
Candida tropicalis	-	+	+	+	+	-	-	-
Saccharomyces	-	+	+	v	v	v	+	-
Cryptococcus or *Trichosporon* or *Rhodotorula* genera	v	positive urease						

v : variable (+ or -)

Differential Diagnosis

Cryptococcus, *Trichosporon* and *Rhodotulora* species may be differentiated by morphological examination.

- *Rhodotulora* species are characterized by distinctive orange-red colonies when grown on Sabouraud's dextrose agar.
- Some strains of *Cryptococcus* species may also produce a reddish pigmentation. Therefore, *Cryptococcus* species should be examined for the presence of a capsule by an India ink preparation.
- *Trichosporon* species produce pseudohyphae when grown on PCB agar medium.

Note

• Among *Saccharomyces* species, *Saccharomyces cerevisiae* is the main human pathogenic species.
• Strains of *Saccharomyces boulardii* or *Saccharomyces cerevisiae* can be isolated from patients undergoing a treatment based on yeast.
• A microscopic examination can differentiate the two species:
- *S. cerevisiae* are large globular yeasts.
- *S. boulardii* are smaller in size, egg-shaped or elongated.
• With the CANDIFAST test some strains of *Cryptococcus* may grow in presence of actidione.

10.2 Resistance test row

Wells 2 to 8 (AB to FCZ)

A colour change of the medium to yellow, to orangey-yellow, or to fuchsia indicates that the strain being tested is able to grow in that well and is therefore resistant to the antifungal present.
If the well remains orangey-red, the growth of the tested strain has been inhibited by the antifungal in that well.

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 90029 and *Candida parapsilosis* ATCC 22019.

Strain	Incubation	Expected results															
		ACT	GLU	GAL	TRE	MAL	CEL	RAF	LAC	C+	AB	NY	FCT	ECZ	KTZ	MCZ	FCZ
C. albicans (ATCC 90029)	24 hours	+	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-
C. parapsilosis (ATCC 22019)	48 hours	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-

12- CAUSES OF ERROR

• Preparation of an inoculum that is either too concentrated or not concentrated enough.
• Preparation of the inoculum from a mixed culture or with isolated colonies grown for more than 48 hours.
• 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

•This *in vitro* method for the determination of the resistance to antifungal agents has an indicative value for the antifungal-yeast interaction during *in vivo* treatment (2,4).
• The CANDIFAST procedure is intended for the identification and the testing of resistance to antifungal agents of yeasts of mucocutaneous origin only. It is therefore not to be used for yeast strains from systemic mycoses.
• The CANDIFAST procedure does not allow a categorization into Susceptible-Intermediate-Resistant.
• Some thermosensitive strains isolated at 20 °C grow differently at 37 °C. In this case, either the yeasts will not grow or they will grow poorly. As a consequence it would be very difficult, if not impossible, to carry out a reliable strain identification or to assess their resistance to certain antifungal agents.
• For certain strains of *C. lusitaniae* a delay of growth in the TRE well makes identification with the enclosed table difficult to achieve. Identification can also be achieved by referring to the markings on the test-tray adhesive tape.
• For certain strains of *C. glabrata*, difficulty reading the GAL characteristic makes identification with the enclosed table difficult to achieve. Identification can also be achieved by referring to the markings on the test-tray adhesive tape.
• The interpretation of the resistance tests does not take into account clouding of the medium that can appear after incubation with azole antifungal agents.

14 - PERFORMANCE CHARACTERISTICS

Collection strains

The study was carried out using 80 collection strains (31 *Candida albicans*, 17 *C. glabrata*, 11 *C. tropicalis*, 6 *C. lusitaniae*, 3 *C. parapsilosis*, 2 C. *kefyr*, 2 *C. krusei*, 5 *Saccharomyces spp*., 2 *Cryptococcus neoformans* and 1 *Trichosporon cutaneum*). 78 strains were correctly identified with the aid of the markings on the test-tray adhesive tape and gave a concordance of 97.5% with the ELITech MICROBIO FUNGICHROM method and the Biomérieux API 32C method. The overall concordance for the resistance test compared with the Biorad FUNGITEST and the Biomérieux ATB Fungus tray, was 87.4%.

Clinical strains

The study was carried out on 100 freshly isolated strains from clinical samples taken from patients in community health practices (72 *Candida albicans*, 9 *C. glabrata*, 8 *C. parapsilosis*, 5 *C. tropicalis*, 3 *C. krusei*, 1 *C. kefyr*, 1 *C. inconspicua* and 1 *Saccharomyces spp*).
98 strains were correctly identified in comparison with the Biomérieux VITEK method.
The overall concordance for the resistance test compared with the Biorad FUNGITEST and the Biomérieux ATB Fungus tray, was 98,5%. Among the 10 discrepancies, 3 are major errors and 7 are minor errors. These discrepancies concern 4 strains (2 *C. glabrata*, 1 C. *parapsilosis* and 1 *C. tropicalis*) and 3 antifungal agents: econazole (2 R/I and 1 R/S), ketoconazole (1 R/I and 2 R/S) and miconazole (4 R/I).

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 – BIBLIOGRAPHIE

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The changes from the previous version are highlighted in grey

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