Candi*Select*™ **4** **63746**

Chromogenic agar medium for selective isolation of yeasts, direct identification of *Candida albicans* and presumptive identification of *Candida tropicalis*, *Candida glabrata* and *Candida krusei*.

IVD

1- INTENDED USE

Candi*Select*™ **4** is a chromogenic agar medium used for:

- Selective isolation of yeasts.
- Direct identification of *Candida albicans* by detecting a specific enzymatic activity, resulting in pink to purple colored colonies.
- Presumptive identification of *Candida tropicalis*, *Candida glabrata* and *Candida krusei* according to the turquoise color and the morphological appearance of the colonies.
- Detection of mixed yeast cultures.

2- SUMMARY AND EXPLANATION

The incidence of invasive fungal infections has increased steadily over the past two decades. With recent advances in medical and surgical intervention and the increasing population of immunocompromised patients, the diversity and list of human fungal pathogens continue to grow. It is widely accepted that *Candida* species are a common cause of nosocomial infection with significant associated morbidity, mortality and increased health-care costs.[1]

Candida albicans is the most common *Candida* species implicated in these infections, but non-*albicans* species such as *Candida glabrata* and *Candida krusei* are increasing in frequency. Furthermore, infections caused by these latter species often vary in their antifungal susceptibility. According to most published guidelines for the treatment of candidiasis, rapid and accurate diagnosis of infection is required before appropriate treatment can be instigated.[2]

Candi*Select*™ **4** is a selective chromogenic medium for the identification of *C. albicans* and the presumptive identification of the major pathogenic yeast species: *C. glabrata*, *C. tropicalis* and *C. krusei*.

Colonies of *C. albicans* appear pink to purple. *C. tropicalis* colonies appear as intense turquoise, spherical colonies with smooth morphotype. *C. glabrata* appear as pale turquoise colonies that are flat and shiny with smooth morphotype, and they most commonly present a characteristic so-called “fish-eye” color pattern (turquoise colonies with darker center). *C. krusei* colonies appear turquoise-blue, with a characteristically rough morpho-type, a dry appearance and an irregular outline.

3- PRINCIPLE OF THE PROCEDURE

Candi*Select*™ **4** contains chromogenic substrates that react with enzymes secreted by yeast cells, resulting in colonies with various pigmentations.

These enzymes are species-specific, allowing organisms to be identified to species level by their color and colony characteristics.[1] Identification of *Candida albicans* is based on the detection of hexosaminidase enzymatic activity, specific to this species, using a chromogenic substrate contained in the medium, inducing pink to purple-colored colonies.
Detection of phosphatase enzymatic activity, using a second substrate present in the medium, enables the presumptive identification of *Candida tropicalis*, *Candida glabrata* and *Candida krusei* based on turquoise coloring of colonies, associated with a typical morphological appearance for each of these species. The growth of most bacterial contaminants is prevented by chloramphenicol and gentamicin.

4- REAGENTS

Package of 20 Petri dishes (90 mm) (**CANDI 4**) –
Catalog No. 63746
Candi*Select*™ **4** is a ready-to-use selective medium.

Approximate media formulation (g/L)

Peptone mix	8
Glucose	5
Chromogenic substrates	0.2
Chloramphenicol + Gentamicin	0.5
Agar	13

5- WARNINGS AND PRECAUTIONS

For *in vitro* Diagnostic Use.
Observe aseptic technique and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other potentially contaminated materials must be sterilized or disposed of in accordance with defined laboratory procedures.

All human specimens should be handled as if capable of transmitting infectious disease, including hepatitis viruses and Human Immunodeficiency Virus, by using universal precautions and appropriate institutional guidelines.[3-7]

The Material Safety Data Sheet (MSDS) is available upon request (or on bio-rad.com).

Prolonged exposure of **Candi***Select*™ **4** media to light may result in reduced recovery and/or coloration of the QC organisms or patient isolates.

BIO-RAD

6- STORAGE INSTRUCTIONS

Store plates at 2-8°C protected from light.

Plates should be kept in the original sleeve wrapping and cardboard until ready to use. Close plate packaging each time after any plates are removed. Plates must be used before the expiration date indicated on the label and printed on the plate.

Minimize exposure to light before and during incubation, as light may destroy the chromogens in the media.

7- PRODUCT DETERIORATION

The medium should be slightly opalescent and very light amber in color. **Candi***Select*™ **4** agar plates that have a bluish appearance do not perform optimally. Plates should not be used if there is any visible sign of contamination, drying, cracking or any other sign of deterioration.

8- SPECIMEN COLLECTION AND HANDLING

Specimens should be collected and handled according to standard procedures for Clinical Microbiology. Use appropriate transport media if specimens will be delayed in processing.

9- PROCEDURE

Material provided:

- Bio-Rad **Candi***Select*™ **4** agar plates.

Materials required but not provided

- Incubator, 35-37°C
- Laboratory supplies required for this procedure

Optional materials not provided

- Supplementary culture media
- Quality control organisms

Test procedure

Bring the **Candi***Select*™ **4** medium to room temperature, protected from light, before use. Do not use if there is excessive moisture on the agar surface.

Inoculation:

- Inoculate the clinical samples directly onto **Candi***Select*™ **4**, using conventional isolation techniques. For samples collected on a swab, first roll the swab over one quarter of the plate, and then use a loop for isolation. Refer to current recommendations for correct storage of biological samples.[7]
- **Candi***Select*™ **4** may also be inoculated with yeast colonies that have previously been isolated on another culture medium (subculturing).
  As for any chromogenic medium, it is very important to streak at closely spaced intervals to obtain well-isolated colonies, for more distinct colony morphology and coloration.

Incubation:

- Incubate the plate between 35-37°C for 24 to 48 hours in an inverted position (agar-side up). (If there is no growth, extend the incubation period up to 72 hours). Do not incubate in a CO_2 incubator.
- Species belonging to the genii *Cryptococcus* and *Geotrichum* grow faster at 30°C than 37°C. If an infection with *Cryptococcus neoformans* is suspected, it is recommended to inoculate a second agar plate in parallel, which will be incubated at 30°C.

10- RESULTS

Reading / Interpretation:

Read the plates and identify the yeast based on the color and morphological appearance of the colonies. The optimal growth and the most specific morphology occur around 48 hours.

1) Direct identification of *Candida albicans*

Pink to purple colored colonies (positive hexosaminidase activity) indicate the presence of ***Candida albicans.***

Colonies that have a white surface and are pink to purple on the underside of the colonies (seen by turning the plate over) should be identified as *Candida albicans*.

After an incubation period of 48 hr, it is possible to observe a diffusion of color around the colony, and a mauve halo may be observed.

2) Presumptive identification

Turquoise colored colonies are positive for phosphatase activity.

- Intense turquoise, uniformly colored colonies with a spherical, smooth morphotype (S): presumptive identification of ***Candida tropicalis***.
  After an incubation period of 48 hr, the turquoise color is intense and a diffuse turquoise halo may be observed around the colony.
- Pale turquoise colonies that are flat and shiny with a smooth morphotype (S), and most commonly present a characteristic, “fish-eye” color pattern (turquoise colonies with darker center): presumptive identification of ***Candida glabrata***.
- Large turquoise-blue colonies, with a characteristically rough morphotype (R), a dry appearance and an irregular outline: presumptive identification of ***Candida krusei***.

3) Additional testing

- Following an incubation period of 48 hr, those colonies that are white or pale blue in color (and do not show any typical morphology), should be identified using conventional methods of identification.
- Conventional identification tests, such as latex agglutination tests or analysis of the biochemical, morphological and metabolic characteristics (i.e., Bio-Rad **Auxa***Color*™ **2**) and antifungal drug susceptibility testing (i.e., Bio-Rad Fungitest™) can be performed directly using colonies isolated on **Candi***Select*™ **4**. Note: Bio-Rad **Auxa***Color*™ **2** and Bio-Rad Fungitest™ are not available in the US.

11- USER QUALITY CONTROL

Before use, observe the plates for contamination or deterioration (see Section 7 above). Performance can be evaluated with organisms that are known to demonstrate specific reactions.

Recommended organisms are as follows:

Test Organism	Results expected on Candi*Select*™ 4 at 48 hr for isolated colonies
C. albicans ATCC 24433	Pink to purple colonies
C. glabrata ATCC 66032	Smooth, shiny turquoise colonies, often with a darker center
C. *tropicalis* ATCC 750	Smooth, spherical, turquoise colonies
C. *krusei* ATCC 14243	Rough, turquoise colonies, often with an irregular outline
C. parapsilosis ATCC 90018	White to slightly bluish colonies
S. aureus ATCC 25923	No growth
E. coli ATCC 25922	No growth

Quality control testing should be performed in compliance with guidelines or requirements of local, state and/or federal regulations or accreditation organizations, as well as internal laboratory Quality Control procedures. Refer to relevant CLSI guidance documents and CLIA regulations for applicable Quality Control practices.

12- LIMITATIONS OF THE PROCEDURE

- Strains of *Candida dubliniensis* have hexosaminidase activity and show a pink to purple color on this medium, but this species is only isolated in very rare cases.
- Rare strains of *Candida albicans* show white colonies after an incubation period of 48 hr.
- Some complex samples (expectorations, tracheo-bronchial samples, stools) may color the medium blue where they are inoculated directly onto the plate. Therefore, it is recommended that these samples be streaked at closely spaced intervals to obtain well-isolated colonies, dissociated from the sample.
- Some strains of *Candida parapsilosis*, *Candida kefyr* and *Candida lusitaniae* with a more intense phosphatase activity may show pale turquoise colonies after an incubation period of 48 hr.
- Certain fastidious strains of yeast have special metabolic requirements (growth factors, incubation temperature, etc.) and cannot grow on this medium.
- **Candi***Select*™ **4** enables the presumptive identification of *Candida tropicalis*, *Candida glabrata* and *Candida krusei*. For a definitive diagnosis, confirmation of identification using conventional identification tests is recommended.

13- EXPECTED VALUES

Species of the genus *Candida* are the agents most frequently implicated in invasive fungal infections.

One form of invasive candidiasis, candidemia, is the fourth most common bloodstream infection among hospitalized patients in the United States. A survey conducted at CDC found that candidemia occurs in 8 of every 100,000 persons per year.
Persons at high risk for candidemia include low birth-weight babies, surgical patients, and those whose immune systems are deficient.[8]

14- PERFORMANCE CHARACTERISTICS

Three methods were used to evaluate the performance of the medium. In these studies, identification was confirmed by reference methods that included germ tube production, micro-fermentation, microscopic morphology, chlamydo-sporulation on rice-agar-Tween medium, rapid identification tests, and assimilation tests.

1) In a prospective study, 1034 clinical samples of respiratory, urogenital, ENT or other origin were tested, and 347 isolates of *Candida* were identified; 46 of them came from 22 polymicrobial samples.

Monomicrobial samples:

- Direct identification of *Candida albicans* (out of 235 isolates identified).
  Candi*Select*™ **4** medium has a sensitivity of 29.4% and 77.0% at 24 hr and 48 hr, respectively.
  The specificity of the medium was 100%.
- Presumptive identification of *Candida glabrata* (out of 21 isolates identified), *Candida tropicalis* (out of 15 isolates identified) and *Candida krusei* (out of 3 isolates identified):
  - 85.7% of *Candida glabrata* were identified within 48 hr.
  - 53.3% of *Candida tropicalis* isolates were identified within 48 hr.
  - 66.7% of *Candida krusei* isolates (2 out of 3 isolates) were identified within 48 hr.

Polymicrobial samples:

- Direct identification:

In a study of 16 samples that were positive for *Candida albicans*, 100% were identified within 72 hours.

Species	Number of strains	Sensitivity		
		24 hr	48 hr	72 hr
Candida albicans	16	37.5% (6/16)	93.8% (15/16)	100% (16/16)

- Presumptive identification:

In polymicrobial samples, 73.3% of *Candida glabrata* isolates (15 positive samples) and 75% of *Candida tropicalis* (4 positive samples) were correctly identified within 48 hr.

Species	Number of strains	Sensitivity		
		24 hr	48 hr	72 hr
Candida glabrata	15	ND	73.3% (11/15)	86.7% (13/15)
Candida tropicalis	4	ND	75% (3/4)	75% (3/4)

2) In a second study, 78 organisms isolated on Sabouraud agar were subcultured onto **Candi***Select*™ **4** medium. The sensitivity of **Candi***Select*™ **4** for the direct identification of *Candida albicans* and the presumptive identification of *Candida glabrata*, *Candida tropicalis* and *Candida krusei* from this study is indicated in the table below:

Species	Number of isolates	Sensitivity	
		24 hr	48 hr
Candida albicans	21	95.2% (20/21)	100% (21/21)
Candida glabrata	23	ND	91% (21/23)
Candida tropicalis	14	ND	100% (14/14)
Candida krusei	5	60% (3/5)	100% (5/5)

Of the remaining 15 isolates, 12 were other species of *Candida* and 3 were *Candida* that could not be identified to the species level with conventional identification methods.

3) The third study evaluated the growth of rarely isolated yeasts and filamentous fungi using strains from the laboratory's collection. Thus **Candi***Select*™ **4** medium enabled the growth of *Trichosporon sp., Geotrichum sp., Cryptococcus neoformans, Scedosporium apiospermum, Rhizopus sp., Fusarium sp., Aspergillus fumigatus, Aspergillus niger and Aspergillus flavus.*

15- REFERENCES

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Symbol

Stored plates must be protected from light.

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