INSTRUCTIONS FOR USE

ChromArt

CHROMOGENIC CANDIDA AGAR

Ready-to-use plates

Chromogenic Candida Agar:
C.albicans (green-blue colonies), *C.tropicalis* (blue-gray colonies) and *C.krusei* (large pink-violet colonies)

1-INTENDED USE

In vitro diagnostic device. Selective and chromogenic medium for the isolation of *Candida* spp. from clinical specimens and for the differentiation of *Candida albicans/Candida dubliniensis* group from *Candida tropicalis* and other species of the genus *Candida*.

2 - COMPOSITION -TYPICAL FORMULA *

Peptones	10,30 g
Growth factors	11,70 g
Inorganic salts	4,60 g
Chloramphenicol	0,50 g
Chromogenic mix	0,36 g
Agar	12,00 g
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Yeast infections require prompt diagnosis to allow the early initiation of appropriate antifungal therapies. Since the 90s, advances have been made in laboratory methods for diagnosis of *Candida* species, especially *Candida albicans*, resulting in more rapid and reliable identification.[1-3] One of these methods was the incorporation of chromogenic substrates directly into the isolation media. A common principle of these media is the use of a chromogenic substrate for β-hexosaminidase, to differentiate *C.albicans/C.dubliniensis* group from other yeasts and a second chromogenic substrate (usually to detect phosphatase or β-glucosidase) to provide further discrimination between species.[4] The main advantage of such chromogenic media is their ability to detect mixed yeast cultures, because different species often form colonies with different colours.
Chromogenic Candida Agar is a "second generation" chromogenic and selective medium for the isolation of *Candida* spp. from clinical specimens and for the differentiation of clinically important *Candida* spp.: *C.albicans-C.dubliniensis* group from *Candida tropicalis, Candida krusei* and other *Candida* spp. The selectivity of the medium is due to the presence of chloramphenicol which suppresses the growth of bacteria. Differentiation is obtained by the presence of two chromogenic compounds. The hydrolysis of the substrate for the detection of β-hexosaminidase enzyme of *C.albicans* and *C.dubliniensis* results in the release of an insoluble chromophore that remains inside the colonies giving them a typical green-blue colour. The hydrolysis of the second chromogenic substrate results in the release of an insoluble pink chromophore and orients in the identification of other species: *Candida tropicalis* splits both the compounds with the formation of blue-gray colonies while other species of the genus *Candida* hydrolyse only the second chromogenic compound and grow with colonies with different shades of pink.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, limpid
Final pH at 20-25 °C	6.0 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Candida Agar CND:W0104030202; EDMA:14.03.02.02; RDM: 1443953/R	Ready-to-use plates	548005	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the complete identification of the colonies.

7 - SPECIMENS

Chromogenic Candida Agar is intended for the bacteriological processing of non-sterile clinical specimens such as mouth, throat, pharyngeal, vaginal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

8 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate by rolling the swab over a small area of the surface at the edge; then streak from this inoculated area to obtain well isolated colonies.
Incubate inoculated plates in aerobic conditions at 35-37°C for 18-24 and 48 hours.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Here below a short interpretation guide is reported.

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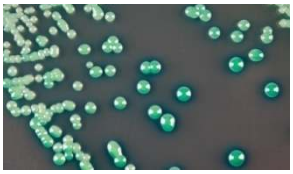
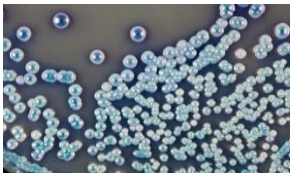
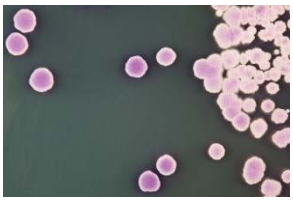
Brillant green-blue colonies: characteristic of *C.albicans /C.dubliniensis. (C.albicans* here below)



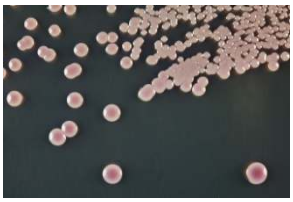
Gray-blue colonies with purple tinges and/or a violet halo: characteristic of *C.tropicalis.*



Enlarged, flat pink-red or violaceous colonies, with a rough fine texture: characteristics of *C.krusei*.



White or pink or pink-purple colonies: characteristics of other *Candida* species (*C.glabrata* here below)



Candida kefir produces violet-red colonies.
Candida parapsilosis complex produces pink, pink-violet colonies.
Gram positive and Gram negative bacteria are almost inhibited.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C.albicans	ATCC 10231	35-37°C / 44-48 h / A	good growth, green-blue colonies
C..tropicalis	NCPF 8841	35-37°C / 44-48 h / A	good growth, blue-gray colonies
E.coli	ATCC 25922	35-37°C / 44-48 h / A	inhibited
S.aureus	ATCC 25923	35-37°C / 44-48 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCPF: Public Health England, National Collection of Pathogenic Fungi.

11 - PERFORMANCES CHARACTERISTICS

The performances characteristics of Chromogenic Candida Agar were evaluated by Andreoni *et al.*[5] with 82 yeast strains isolated from human specimens, identified with a phenotypic system and confirmed with a spectrometric method and stored at -80°C and with 80 clinical specimens isolated from respiratory material, vaginal exudates, urine and positive blood cultures. Chromogenic Candida Agar was compared with a chromogenic medium of the market. The conclusions have been the following: for yeast strains isolated from human specimens, the comparison between the two media, in general showed a better growth, in terms of colony dimension at 24 and 48 hours, on Chromogenic Candida Agar; the colony colour as well, in terms of tonality and intensity, resulted more evident on Chromogenic Candida, allowing a better differentiation between species with similar colours. The findings confirmed that the Chromogenic Candida Agar can substantially ensure the presumptive identification of frequent clinical isolation species, allowing an orientation for presumptively identifying yeasts species of lower isolation frequency. The rapid growth and the colour intensity moreover guarantee a morphological and colour evaluation in a shorter time.

Prior to release for sale a representative sample of all lots of ready-to-use plates of Chromogenic Candida Agar and of the raw material used for the production of prepared plates (dehydrated Chromogenic Candida Agar Agar REF 408005) are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity and specificity are evaluated by semi-quantitative ecometric technique with the following strains: *C.albicans* ATCC 10231, *C.albicans* ATCC 20912, *C.dubliniensis* NCPF 3949, *C.intermedia* clinical isolate, *C.krusei* ATCC 6258, *C. parapsilosis* ATCC 6258, *C.stellatoidea* ATCC 11006, *C.tropicalis* NCPF 8841, *C.glabrata* clinical isolate. After incubation at 35-37°C for 8-24 and 48 hours, the amount of growth and the chromatic characteristics of the colonies are evaluated and recorded. All *Candida* species develop a good growth with the following chromatic characteristics (after 48 hours of incubation):

C. albicans	green-blue colonies
C. dubliniensis	green-blue colonies
C. tropicalis	blu-grey colonies
C. intermedia	pale-pink colonies
C. krusei	pink-violet colonies
C. parapsilosis	pale pink colonies
C. stellatoidea	green-blue colonies
C. glabrata	pale-pink colonies

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *P.aeruginosa* ATCC 27853 and *E.faecalis* ATCC 19433. The growth of non-target strains is totally inhibited.

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12 - LIMITATIONS OF THE METHOD

- *C.dubliniensis* is ß-hexosaminidase positive and grows with green-blue colonies and therefore it is not differentiable from *C.albicans*.[5]
- Chromogenic Candida agar does not differentiate between *C.parapsilosis*, *C.orthopsilosis* and *C.metapsilosis.*[5]
- The best colour differentiation of *Candida* spp. is obtained after 48 hours of incubation.[5]
- *Candida* spp. other than *C.albicans/C.dubliniensis* and *C.tropicalis* appear as a variety of pink/gray/violet colours, due to the mixture of natural pigmentation and the released chromophores. The experience of the microbiologist can help to differentiate these species by colour and morphology of the colonies.
- Growth depends on the requirements of each individual microorganisms. It is possible that yeasts with specific metabolic requirements may not growth or may not produce colour.
- Some rare bacterial strains which may be resistant to chloramphenicol may growth on the medium with coloured colonies.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Polacheck I, Melamed M, Bercovier H, Salkin IF. Beta-Glucosidase in Candida albicans and its application in yeast identification. J Clin Microbiol 1987;25:907-10.
2. Perry JL, Miller GR. Umbelliferyl-labeled galactosaminide as an aid in identification of Candida albicans J Clin Microbiol 1987;25:2424-5.
3. Willinger BW, Manafi M, Rotter ML. Comparison of rapid methods using fluorogenic-chormogenic assays for detecting Candida albicans. Letters App Microbiol 1994; 18:47-49
4. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017 Apr;30(2):449-479.
5. Andreoni S., Molinari G.L., Ruzza P., Dellera A. Evaluation of Chromogenic Candida Agar for isolation and presumptive identification of yeasts. XLI AMCLI Italian Clinical Microbiologists Association Congress Rimini, November 13-16, 2012.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/10

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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