INSTRUCTIONS FOR USE

ChromArt

CHROMOGENIC CANDIDA AGAR

Dehydrated culture medium

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

1-INTENDED USE

In vitro diagnostic. 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 (AFTER RECONSTITUTION WITH 1 L OF WATER) *

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

*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-grey colonies while other species of the genus *Candida* hydrolyse only the second chromogenic compound and grow with colonies with different shades of pink.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 39.5 g in 1000 mL of cold purified water; heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50ºC, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25°C	6.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Candida Agar	Dehydrated medium	4080052	500 g (12.65 L)
Chromogenic Candida Agar	Dehydrated medium	4080054	5 kg (126 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - 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.

9- 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.

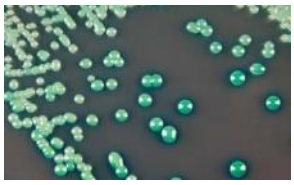
Biolife Italiana S.r.l., Viale Monza 272, 20128 Milano, Italia
Tel. +39 02 25209.1 E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it

Incubate inoculated plates in aerobic conditions at 35-37°C for 18-24 and 48 hours.

10 - READING AND INTERPRETATION

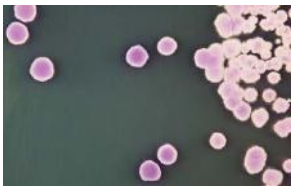
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Here below a short interpretation guide is reported.

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



Grey-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.

11 - 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, the end user can perform its own Quality Control 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-grey 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.

12 - 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 dehydrated Chromogenic Candida Agar REF 408005 is 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 2091, *C.dubliniensis* NCPF 3949, *C.intermedia* clinical isolate, *C.krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, *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 specific chromatic characteristics (after 48 hours of incubation):

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.

13 - 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/grey/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 grow or may not produce colour.
- Some rare bacterial strains which may be resistant to chloramphenicol may grow 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 diseases; 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.

14 - 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.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium 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.
- Apply Good Manufacturing Practice in the preparation process of plated or bottled media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (plates/bottles), and the storage method applied (temperature and packaging).

16 - 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-chromogenic 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.

408005 CHROMOGENIC CANDIDA AGAR
SDS rev 3
Regulation (EU) 2020/878

Contains: CHLORAMPHENICOL

Classification:

Carcinogenicity, category 2	H351 Suspected of causing cancer.
Eye irritation, category 2	H319 Causes serious eye irritation.
Hazardous to the aquatic environment, Chronic toxicity, category 3	H412 Harmful to aquatic life with long lasting effects.

Hazard pictograms:

Labelling
Signal words: Warning

Hazard statements:

H351	Suspected of causing cancer.
H319	Causes serious eye irritation.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statements:

P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P201	Obtain special instructions before use.
P308+P313	IF exposed or concerned: Get medical advice / attention.
P337+P313	If eye irritation persists: Get medical advice / attention.
P273	Avoid release to the environment.

TABLE OF APPLICABLE SYMBOLS

REF o 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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content	2022/01
Revision 2	Removal of obsolete classification, addition of SDS chapter	2023/04

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milano, Italia
Tel. +39 02 25209.1 E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it