INSTRUCTIONS FOR USE

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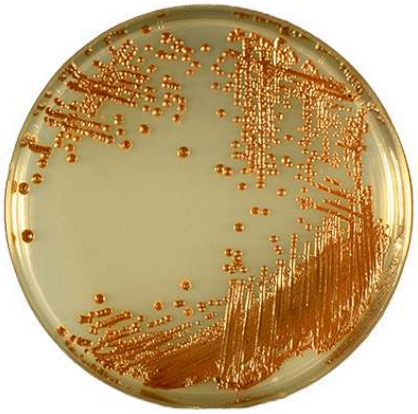
CANDIDA AGAR (NICKERSON)

Dehydrated culture medium



Candida Agar Nickerson : *Candida albicans*

1 - INTENDED USE

In vitro diagnostic. Selective medium for the isolation and differentiation of *Candida* spp. from clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Glycine	10.00 g
Bismuth ammonium citrate	5.00 g
Sodium sulphite	3.00 g
Glucose	10.00 g
Yeast extract	1.00 g
Agar	15.00 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

Candida Agar (Nickerson), also known as BiGGY (Bismuth Glucose Glycine Yeast) Agar is prepared on the basis of the formulation described in 1953 by Nickerson.[1]

Candida Agar (Nickerson) is used for the isolation and presumptive differentiation of species of the genus *Candida* on the basis of colony morphology and colour from clinical specimens.[2-4]

Bismuthyl hydroxy sulphite complex produced into the medium by heat, is extracellularly reduced by *Candida* spp. to sulphide in a neutral or acidic environment and this reduction, depending on the intensity, results in brown to black pigmentation of the yeast colonies.[1]

Bismuth sulphite, bismuth ammonium citrate, glycine at high concentrations, act as selective compounds and the medium is not favourable for the development of schizomycetes; yeast extract and glucose are the nutritive bases.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 44 g in 1000 ml of cold purified water and mix thoroughly. Heat with frequent agitation until boiling and continue to boil for 30 to 60 seconds to dissolve the agar and obtain a uniform suspension. Cool to approximately 50°C, mix gently to disperse the precipitate evenly and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	white with flocculent precipitate
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Candida Agar (Nickerson)	Dehydrated medium	4012802	500 g (11,4 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Candida Agar (Nickerson) 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. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[5]

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.

Incubate in aerobic condition at 28-30°C for up to 5 days and examine daily for evidence of sulphite reduction.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record each specific morphological and chromatic characteristic of the colonies. The development of brown to black colonies allows a presumptive diagnosis of the genus. This diagnosis must be confirmed at least by microscopic examination (fresh preparation, clarified with methyl blue lactophenol: branched pseudomycelium, budding yeast cells, presence of chlamydospores, absence of ascospores). Presumptive species identification can be made by taking into account the colony characteristics of the main *Candida* species, summarised below.[2]

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Candida albicans: smooth, circular or hemispherical brown-black colonies, slight mycelial fringe; no colour diffusion into surrounding medium; no sheen.
Candida tropicalis: smooth, discrete dark brown colonies with black coloured centres; slight mycelial fringe; diffuse blackening of medium after 72 hours; sheen.
Candida krusei: large, flat, wrinkled silvery brown-black colonies with brown peripheries; yellow halo diffused into medium.
Candida pseudotropicalis: medium size, flat, dark reddish-brown glistening colonies; slight mycelial fringe; no diffusion.
Candida parakrusei: medium size, flat, wrinkled, glistening dark reddish-brown colonies with light peripheries; extensive yellow mycelial fringe.
Candida stellatoidea: medium size, flat, dark brown colonies; very light mycelial fringe.

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 60193	30°C / 72 H / A	growth with brown-black colonies
C.tropicalis NCPF 8841	30°C / 72 H / A	growth with dark brown colonies and metallic sheen
E.coli ATCC 25922	30°C / 72 H / A	growth inhibited
S.aureus ATCC 25923	30°C / 72 H / A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Candida Agar (Nickerson) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique with the target strains *C.albicans* ATCC 10231, *C.albicans* ATCC 18804, *C.tropicalis* NCPF 8841, *C.krusei* ATCC 6258, *C.intermedia*, clinical isolate.
After incubation at 30°C for up to 72 hours, the amount of growth on the plates and colonies' characteristics are evaluated and recorded: they shall be comparable in both batches and according to specifications.
The 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 *E.coli* ATCC 25922, *S.aureus* ATCC 25923 *A.brasiliensis* ATCC 9642, *P.chrysogenum* ATCC 10106. *A.brasiliensis* is partially inhibited while the growth of the other non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- Pigmented bacteria and yeast-like fungi are usually inhibited on the medium. If they grow brown colonies, they are easily distinguished by microscopic observation; dermatophytes and moulds rarely grow and are distinguishable by the formation of aerial mycelia.[2]
- The medium should be prepared fresh, just prior to use.[2]
- Results in test tubes are not satisfactory.[1]
- 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.
- Apply Good Manufacturing Practice in the preparation process of plated or tubed 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 +10°C /+30°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 medium should be prepared fresh, just prior to use. The user is responsible for the manufacturing and quality control processes.

16 - REFERENCES

1. Nickerson, W. J. 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of Candida. J. Infect. Dis. 93:43.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
4. Lindsley M. Reagents Stains and Media: Mycology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. Berkow EL, McGowan KL. Specimen Collection, Transport and Processing: Mycology . In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/04
Revision 2	Removal of obsolete classification	2023/04

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

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