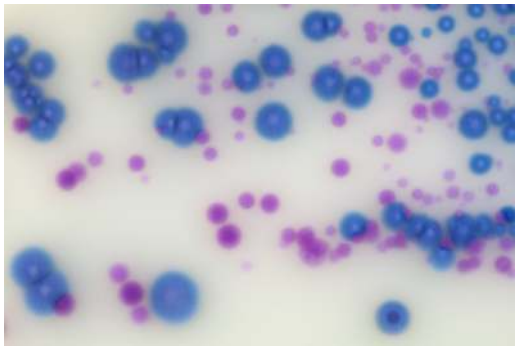
INSTRUCTIONS FOR USE

ChromArt

CHROMOGENIC STREPTO B AGAR

Ready-to-use plates



Mixed culture of *Streptococcus agalactiae* (pink-magenta colonies) and *Enterococcus* sp. (blue colonies)

1 - INTENDED USE

In vitro diagnostic device. Selective chromogenic medium for the presumptive detection of Lancefield group B streptococci (*Streptococcus agalactiae*; GBS) carriage in clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Peptones	28.000 g
Buffer salts	5.250 g
Growth factors	6.700 g
Inorganic salts	8.500 g
Antimicrobial mix	0.067 g
Chromogenic mix	0.800 g
Opacifying compounds	6.500 g
Agar	15.000 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

Lancefield group B streptococci (GBS), or *Streptococcus agalactiae*, are facultatively anaerobic, oxidase-negative, catalase-negative, Gram-positive cocci occurring in chains, that cause invasive disease primarily in infants, pregnant or postpartum women, and older adults, with the highest incidence among young infants.[1,2]

Despite substantial progress in prevention of perinatal group B streptococcal disease since the 1990s, GBS remains the leading cause of early-onset neonatal sepsis. Universal screening at 35–37 weeks gestation for maternal GBS colonization and use of intrapartum antibiotic prophylaxis has resulted in substantial reductions in the burden of early-onset GBS disease among newborns.[2]

Optimum yield will be achieved by selective enrichment procedures and subculture to selective and non-selective media, applied to swabs obtained from the vagina and the anorectum which increase the likelihood of GBS isolation compared with vaginal or cervical culture alone.[1-3]

Chromogenic Strepto B Agar is a selective and chromogenic medium for the isolation of Group B Streptococci (*S.agalactiae*) from clinical specimens and for the differentiation of the colonies based on a typical colour.

The medium consists in a buffered nutritive base containing antibiotics and chromogenic compounds. Gram-negative bacteria are strongly inhibited while the growth of Gram-positive organisms other than GBS is inhibited with different extent depending of genus and species of the organisms. The differential characteristics are based on specific enzymatic reactions, which allow the differentiation of *S.agalactiae* colonies (pink-magenta) from other bacteria not inhibited by selective agents (e.g. enterococci) which grow with green-blue, blue, without or with a pink halo or colourless colonies. The opaque white background helps in recognizing the colours of the colonies.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	white, opaque
Final pH at 20-25 °C	7.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Strepto B Agar CND: W0104010402, EDMA: 14.01.04.02; RDM: 1403814/R	Ready-to-use plates	548010	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 identification of the colonies.

7 - SPECIMENS

Specimens consist of maternal low vaginal and anorectal swabs collected and placed in appropriate transport medium (Amies or Stuart with or without charcoal).[1,4] While the culture counts decline to some extent, viability of *S.agalactiae* is preserved in transport medium kept at room temperature or 4°C for up to 4 days.[4] Maternal high vaginal swabs should not be collected as these have a lower sensitivity.[1] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; collect specimens before antimicrobial therapy where possible.

8- TEST PROCEDURE

Chromogenic Strepto B Agar can be used according to two protocols:

- Inoculation of the plate after pre-enrichment in Todd Hewitt Broth supplemented with colistin and nalidixic acid (recommended because it is validated in the clinical study reported below and because it increases the sensitivity and specificity of the method).
- Direct inoculation of the specimen onto the agar surface.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

Remove the cap aseptically from the specimen container and place the swab(s) in Todd Hewitt CNA Broth, break off (or cut) the swab stick(s) and replace the cap. Caps should be kept loose during incubation. Incubate at 35-37°C, 5% CO_2, for 18-24 hours.
Allow plates to come to room temperature in the dark. Subculture from the selective broth with a sterile loop and spread inoculum onto the agar surface.
For the direct inoculation, roll the swab(s) over a small area of the surface at the edge; then streak from this inoculated area.
Incubate the inoculated plates at 35 to 37°C, in air, for 24-48 hours.
Reading at 24 hours is possible in cases of urgency but increases the rate of false positivity. In any case, the final reading of the results must be made after incubation for full 48 hours.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
- Typical *S.agalactiae* colonies: round colonies of varying size, pink or pink-magenta or magenta. Most strains develop good size (3-4 mm) round magenta colonies after 48 hours of incubation. At 24 hours some *Enterococcus* strains develop small pink or pink colonies with grey shades or have two types of small colonies: pink and grey. Colonies of these strains usually show a strong blue, grey-blue or purple colour at 48 hours.
- The presence of colourless, blue, green-blue, grey-blue, purple colonies with or without magenta halo should be interpreted as belonging to species other than *S.agalactiae* and the sample should be considered as negative.

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
S.agalactiae	ATCC 13813	35-37°C / 44-48H / A	growth, pink-magenta colonies
E.faecalis	ATCC 19433	35-37°C / 44-48H / A	growth, blue colonies
P.aeruginosa	ATCC 27853	35-37°C / 44-48H / A	inhibited

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

11- PERFORMANCES CHARACTERISTICS

Chromogenic Strepto B Agar was evaluated by an independent Clinical Microbiological Laboratory in Italy on 225 anovaginal specimens. The medium was inoculated after enrichment of the specimen in Todd Hewitt CNA Broth. Reading was performed after 24 and 48 hours of incubation at 37°C. Chromogenic Strepto B Agar has been compared to a chromogenic medium of the market.

The table below shows a comparison of Chromogenic Strep B Agar with a Chromogenic Medium used as reference medium.

		Chromogenic Strepto B Agar			
		True negatives	False negatives	True positives	False positives
Chromogenic Medium used as reference	True negatives	168			4**
	False negatives			3*	
	True positive			44	
	False positive	5**			1**

*Strains identified as Group B Streptococci by latex agglutination
**Strains identified as Non-Group B Streptococci by latex agglutination

168 samples have been found “negative” with both chromogenic media; 44 samples have been found “positive” with both chromogenic media. 3 strains have been found “positive” with Chromogenic Strepto B Agar, “negative” with the reference medium and confirmed as Group B Streptococci by latex agglutination.
4 samples on Chromogenic Strep B Agar and 5 samples on the reference medium originated small pink colonies identified as Enterococci (false positive in the above table).
1 sample originated doubtful colonies on both media confirmed as non-Group B *Streptococcus* and considered in the above table as a “false positive”.
Chromogenic Strepto B Agar didn’t give any false negative result: sensitivity 100%
Chromogenic Strepto B Agar gave 5 false positive results: specificity: 97,2%
After 24 hours of incubation, 5 samples have been found “negative” on the Chromogenic Medium used as reference and originated typical colonies on Chromogenic Strepto B Agar; after 48 hours of incubation typical colonies were observed on the reference medium too.
The performance characteristics have been evaluated with 20 clinical collection *S.agalactiae* strains: all strains developed typical colonies on both media after 24 hours of incubation.

Prior to release for sale a representative sample of all lots of ready-to-use plates of Chromogenic Strepto B Agar and of the raw materials used for the production of prepared plates (dehydrated Chromogenic Strepto B Agar Base REF 408010, supplemented with Strepto B Chromogenic Supplement REF 4240053) are 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 following target strains: *S.agalactiae* ATCC 13813, *S.agalactiae* ATCC 12386, 5 clinical isolates identified as Group B streptococci. After incubation at 35-37°C for full 48 hours all target strains show a good growth with typical chromatic characteristics (pink-magenta colonies).
Selectivity is evaluated by semi-quantitative ecometric technique by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target organisms: *E.gallinarum* ATCC 49573, *E.faecium* ATCC 700221, *E.faecalis* ATCC 19433, *S.pyogenes* ATCC 19615, *S.pneumoniae* ATCC 6301, *S.saprophyticus* ATCC 15305, *S.xylosus* ATCC 35033, *C.albicans* ATCC

10231, *P.aeruginosa* ATCC 27853. After incubation at 35-37°C for full 48 hours, the growth of *P.aeruginosa* and *C.albicans* is totally inhibited, the growth of *E.gallinarum, S.saprophyticus, S.xylosus*, is partially inhibited with the development of light blue colonies, the growth of *S.pyogenes, S.pneumoniae* is partially inhibited with the development of small pink colonies, while *E.faecalis* and *E.faecium* are not inhibited and grow with blue or blue-grey colonies.

12 - LIMITATIONS OF THE METHOD

- It is possible that few strains of *S.agalactiae* with specific growth requirements, may not grow on this medium. Optimum detection of GBS may require the use of more than one culture medium (e.g. selective medium and blood agar).[1]
- Some species (e.g. *Enterococcus* spp.) which are resistant to antibiotics may develop and produce colonies with an atypical colour. However, during the validation tests, 5 strains of enterococci produced small pink colonies.
- Group A streptococci and pneumococci may produce small pink colonies.
- The final reading and colonies interpretation shall be done after a complete 48 hours incubation time.
- 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. On the isolates, 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 the microscopic and/or 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 the product doesn't 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 sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product 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 in the dark. 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. Public Health England. UK Standards for Microbiology Investigations (SMI) Bacteriology, B58, Issue no:3, Issue date: 26.06.18 Detection of Carriage of Group B Streptococci (*Streptococcus agalactiae*).
2. Verani JR, McGee L, Schrag SJ. Prevention of Perinatal Group B Streptococcal Disease. MMWR Recomm. Rep. 2010 Nov 19; 59 (RR-10):1-36
3. Aila NA, Tency I, Claeys G, Saerens B, Cools P, Verstraelen H et al. Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect Dis 2010;10:285.
4. Spellerberg B, Brandt C, Sendi P. *Streptococcus*. *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	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 2	Updated layout and content in compliance with IVDR 2017/746	2020/12

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it