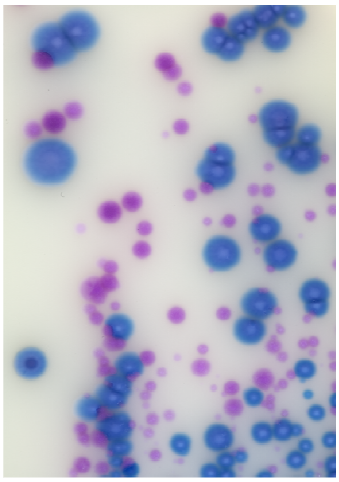
INSTRUCTIONS FOR USE

ChromArt

CHROMOGENIC STREPTO B AGAR BASE

CHROMOGENIC STREPTO B SUPPLEMENT

Dehydrated culture medium and supplements



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

1 - INTENDED USE

In vitro diagnostics. Chromogenic basal medium and selective supplement for the presumptive detection of Lancefield group B streptococci (*Streptococcus agalactiae*; GBS) carriage in clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Chromogenic Strepto B Agar Base (REF 408010)

Peptones	28.000 g
Buffer salts	5.250 g
Growth factors	6.700 g
Inorganic salts	8.500 g
Antimicrobial mix	0.027 g
Chromogenic mix	0.300 g
Opacifying compounds	6.500 g
Agar	15.000 g

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

VIAL CONTENTS (FOR 500 mL OF MEDIUM)

Chromogenic Strepto B Supplement (REF 4240053)

Antimicrobial mix	0.04 g
Chromogenic mix	0.05 g

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 Base completed with Chromogenic Strepto B Supplement, 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- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.14 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and, under aseptic conditions, add the contents of one vial of Chromogenic Strepto B Supplement (4240053), reconstituted with 5 mL of sterile purified water, under aseptic conditions. Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Chromogenic Strepto B Agar Base

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	white, opaque
Final pH at 20-25 °C	7.2 ± 0.2

Chromogenic Strepto B Supplement

Appearance of the lyophilized	high, homogeneous, yellow pastille
Appearance of the solution	opalescent yellowish

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Strepto B Agar Base	Dehydrated medium	4080102 4080104	500 g (7,1 L) 5 kg (71 L)
Chromogenic Strepto B Supplement	Freeze-dried supplement	4240053	10 vials, each for 500 mL of medium base

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7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, 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

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.

9- 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.

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.

10 - 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.

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
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

12- 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.
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 dehydrated Chromogenic Strepto B Agar Base REF 408010, supplemented with Chromogenic Strepto B 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, 3 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.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.

13 - 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 full 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.
- The basal medium and the supplement are 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.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement must be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Material Safety Data Sheets.
- The 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, tubed or bottled media.
- Chromogenic Strepto B Supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring of the vials to avoid injury.
- 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, supplements and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the products 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

Chromogenic Strepto B Agar Base

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).

Chromogenic Strepto B Supplement

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles), and the storage method applied (temperature and packaging).

16 - 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	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 3	Updated layout and content in compliance with IVDR 2017/746	2022/01
Revision 4	Removal of obsolete classification	2023/04
Revision 5	Addition of the 5kg packaging	2025/02

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

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