

INSTRUCTIONS FOR USE

TODD HEWITT CNA BROTH

Ready-to-use tubes

 Todd Hewitt CNA Broth – from left: uninoculated tube, *S. agalactiae*
1 - INTENDED USE

In vitro diagnostic device. Liquid medium for the selective enrichment of Group B streptococci (*S. agalactiae*) from clinical specimens.

2 - COMPOSITION -TYPICAL FORMULA *

Beef heart infusion from	500.0 g
Peptones	20.0 g
Glucose	2.0 g
Sodium chloride	2.0 g
Sodium bicarbonate	2.0 g
Disodium hydrogen phosphate	0.4 g
Yeast extract	10 g
Colistin	10 mg
Nalidixic acid	15 mg
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

Group B Streptococci (GBS) or *Streptococcus agalactiae* are facultatively anaerobic, oxidase-negative, catalase-negative, Gram-positive cocci that cause postpartum infections (neonatal sepsis, pneumonia, respiratory diseases) within seven days from birth, but also within the first 24 hours of life.¹ Approximately 50% of women who are colonized with GBS will transmit the bacteria to their new-borns. Vertical transmission usually occurs during labour or after rupture of membranes. In European countries and the United States, the incidence of the disease is around 0.5-1 per thousand live births.^{1,2}

Todd Hewitt CNA Broth, also known as Lim Broth, is based on a modification of the formulation of Lim *et al.*^{3,4} who proposed a selective enrichment broth and the slide co-agglutination test for the rapid screening of these obstetric patients.

At least 50% of women with GBS give false negatives results if the culture is performed by inoculating the specimens directly into primary isolation medium rather than by performing a preliminary step in the selective enrichment broth.⁵ The use of Todd Hewitt broth with colistin and nalidixic acid, followed by plate isolation, is recommended to maximize the recovery of GBS.⁵⁻⁷

Todd Hewitt CNA Broth has a high concentration of peptones which promotes excellent microbial growth and prevents the formation of proteases; glucose stimulates haemolysin production; sodium chloride maintains the osmotic balance; sodium carbonate and disodium hydrogen phosphate neutralize the acidity that is formed during microbial growth and glucose fermentation. Colistin and nalidixic acid inhibit Gram negative bacteria, promoting the growth of streptococci.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	yellow, limpid
Final pH at 20-25°C	7.8 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Todd Hewitt CNA Broth W0104010206, EDMA: 14.01.02.01, RDM:1514925/R	Ready-to-use tubes	552134B	20 x 9 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, ancillary culture media and reagents for the isolation and the identification of the colonies.

7 - SPECIMENS

Todd Hewitt CNA Broth may be inoculated with maternal low vaginal and anorectal swabs; maternal high vaginal swabs should not be collected as these have a lower sensitivity.⁶ A lower vaginal and rectal swab should be obtained with either one or two different swabs.⁷ Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. GBS isolates can remain viable in transport media (Amies or Stuart with or without charcoal) for several days at room temperature. However, the recovery of isolates declines over 1-4 days, especially at elevated temperatures, which can lead to false-negative results.^{6,7}

Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.





8 - TEST PROCEDURE

Remove the cap aseptically from the container and place the swab(s) in the Todd Hewitt CNA Broth, break off the swab stick(s) and replace the cap. Caps should be kept loose during incubation.

Incubate the inoculated tubes in ambient air or 5% CO₂ for 18-24 hours.⁵⁻⁷

Sub-culture with a sterile loop and inoculate appropriate plating media (Blood Agar and/or Columbia CNA Blood Agar and/or Chromogenic Strep B Agar). Optimum detection of GBS may require the use of more than one culture medium.⁶

9 - READING AND INTERPRETATION

After incubation, growth of organisms is indicated by turbidity of inoculated tubes.

After an overnight incubation of the isolation media, observe plates for suggestive GBS colonies and identify them. If negative after overnight incubation, re-incubate an additional 24 hours before reporting a negative result. On blood agar, suggestive colonies of GBS are grey, translucent, with a surrounding zone of beta-haemolysis (or no haemolysis: very rare).⁶

Perform serological identification on the colonies by latex agglutination test with a group B antiserum.

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
<i>S.agalactiae</i> ATCC 12386	35-37 °C / 16-24h / A or CO ₂	good growth
<i>E.coli</i> ATCC 25922	35-37 °C / 16-24h / A or CO ₂	inhibited

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to-use tubes of Todd Hewitt CNA Broth is tested for productivity and selectivity. Productivity is tested by inoculating less than 1000 CFU of target strains *S.agalactiae* ATCC 12386 and *S.agalactiae* ATCC 13813 in test tubes and incubating at 35-37°C for 16-24 hours. Good turbidity shall be observed.

Selectivity is tested by inoculating more than 1000 CFU of non-target strain *E.coli* ATCC 25922 in test tubes and incubating at 35-37°C for 16-24 hours. No turbidity shall be observed.

12 - LIMITATIONS OF THE METHOD

- The isolation rate of GBS from clinical specimens depends on several factors; studies have shown that detection of GBS colonisation can be improved by attention to the timing of cultures, the sites swabbed and the microbiological method used for culture of microorganisms.⁶
- Todd Hewitt CNA Broth is an enrichment broth: sub-culture on selective or non-selective media is necessary for pathogen isolation and identification.
- After the enrichment in Todd Hewitt CNA Broth, even if the microbial colonies on the isolation 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.

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 tubes 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- Ready-to-use tubes of Todd Hewitt CNA Broth are subject to sterilization by autoclaving.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes 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 tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour, precipitates).

15 – REFERENCES

1. Società Italiana di Neonatologia Prevenzione della malattia perinatale da streptococco di gruppo B [2010]
2. Istituto Superiore di Sanità. Infezioni neonatali precoci e tardive da streptococco di gruppo B in Italia [2011]
3. Lim DV, Kanarek KS, Peterson ME. Magnitude of colonization and sepsis by group B streptococci in newborn infants. *Curr Microbiol* 1982;7:99-101.
4. Jones DE, Friedl EM, Kanarek KS, Williams JK, Lim DV. Rapid identification of pregnant women heavily colonized with group B streptococci. *J Clin Microbiol.* 1983;18(3):558-60.
5. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. *Recommendations and Reports*, November 19, 2010 / 59(RR10);1-32.
6. Public Health England- UK Standards for microbiology investigations (UK SMI): Detection of Carriage of Group B Streptococci (Streptococcus agalactiae) B58, 2018
7. Spellenberg B, Brandt B, 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 <i>In vitro</i> Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 0	Updated layout and content in compliance with IVDR 2017/74	2020/05
Revision 1	Updates and corrections in the chapters 2, 3, 4, 7, 8, 12, 13, 14	2021/01

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

