INSTRUCTIONS FOR USE

TODD HEWITT BROTH

Ready-to-use tubes

Todd Hewitt Broth
from left: uninoculated tube, *S.pyogenes*

1 - INTENDED USE

In vitro diagnostic device. General purpose liquid medium primarily used for the cultivation of β-haemolytic streptococci especially for serological studies.

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 carbonate	2.0 g
Disodium hydrogen phosphate	0.4 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

Todd Hewitt Broth is a medium developed by Todd and Hewitt[1] in 1932 for the production of streptococcal haemolysin and later modified by Updyke and Nickle[2] in 1954 for the growth of β-haemolytic streptococci for use in fluorescent antibody test procedures.
Todd Hewitt Broth enhances the growth of β-haemolytic streptococci and the production of antigenic streptococcal haemolysin; it favours the production of type-specific M protein and it is used in the procedures for extracting group antigens.[3]
Todd Hewitt Broth is also used as general all-purpose medium for the cultivation of most pathogenic microorganisms.[3]
Todd Hewitt 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 protecting produced haemolysin from destruction.

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 Broth	Ready-to-use tubes	552134	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 Broth may be inoculated with any type of clinical specimens from which it is intended to isolate streptococci (e.g. throat swabs). 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 in the Todd Hewitt Broth, break off the swab stick and replace the cap. Caps should be kept loose during incubation.
Incubate the inoculated tubes in ambient air or 5% CO_2 for 2-5 hours prior to use in fluorescent antibody procedures for the identification of group A streptococci.
Continue incubation for 18-24 hours for antigen extraction procedures prior to serotyping and subculture on isolation plates of suitable selective or non-selective blood agar medium.
Todd Hewitt Broth may be inoculated with pure culture of streptococci for the preparation of extracts for serological typing.
Consult appropriate references for further instructions.[4]

9 - READING AND INTERPRETATION

After incubation, growth of organisms is indicated by turbidity of inoculated tubes.
Perform serotyping according to the IFU of manufacturer.

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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, 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.pyogenes ATCC 12834	35-37°/ 18-24H / A	good growth
S.pneumoniae ATCC 6303	35-37°/ 18-24H / A	good growth

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 Broth and of the raw material used for the production of prepared tubes (dehydrated Todd Hewitt Broth REF 402134) is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes, incubating at 35-37°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *S.pyogenes* ATCC 12834, *S.pyogenes* ATCC 19615, *S.pneumoniae* ATCC 6303, *E.faecalis* ATCC 19433, *S.salivarius* ATCC 7073, *S.bovis* ATCC 9809. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

12 - LIMITATIONS OF THE METHOD

- Todd Hewitt Broth is an enrichment broth: sub-culture on selective or non-selective media is necessary for pathogen isolation and identification.
- Todd Hewitt Broth cannot be used for bile solubility testing.[3]
- After the enrichment in Todd Hewitt 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 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.
- 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* diagnostics.
- 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).

15 – REFERENCES

1. Todd EW, Hewitt LF. A new culture medium for the production of antigenic streptococcal hemolysin. J Pathol Bacteriol 1932; 35:973
2. Updyke EL, Nickle MI. A dehydrated medium for the preparation of type specific extracts of group A streptococci. Appl Microbiol 1954; 2:117
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
4. 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** *In vitro* 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 1	Updated layout and content in compliance with IVDR 2017/74	2021/05
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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