HPC060 Rev.00 Date 2016.07

LBM™
LIM Broth

Package insert and How to use guide

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ENGLISH

LIM - Product Leaflet and User Guide

INTENDED USE
LIM broth (TODD HEWITT CNA) is a prepared medium for the selective enrichment of group B streptococci (*Streptococcus agalactiae*). After appropriate incubation, the clinical samples enriched in broth can be cultivated on nutrient agar.

SUMMARY AND PRINCIPLES

The Todd Hewitt CNA Broth base is a general-purpose medium primarily used for the cultivation of streptococci, especially for serologic studies. The buffering system (sodium bicarbonate and sodium phosphate) helps to maintain the pH by counteracting the acidity produced during the fermentation of sugar. The addition of an antimicrobial supplement (nalidixic acid and colistin) suppress the growth of gram-negative bacteria, thus making it a selective medium.

LIM broth can be processed manually or with automated systems as for example WASP™ and WASPLab™.

PRODUCT DESCRIPTION
LIM broth can be supplied in the configurations presented in the table below:

code	Product description	Package dimensions	Suitable for automation
083CU.A	5 ml of LIM broth in a 16X100 polypropylene tube with screw cap and conical shape bottom	50 units in each inner box. 6x50 units in each case.	YES
476CE.A	2 ml of LIM broth in bulk	50 units in each inner box. 6x50 units in each case.	YES
476CE01.A	2 ml of LIM broth in bulk with regular flocked swab	6 boxes containing 100 minigrips	YES
4E022N.A	2 ml of LIM broth in round-bottom test tube for automation	50 units in each inner box. 6x50 units in each case.	YES

REAGENTS
Components of LIM broth:

Infusion of meat (fat-free)
Tryptone
Glucose
Sodium bicarbonate
Sodium chloride
Di-sodium phosphate
Colistin sulphate
Nalidixic acid

pH at the time of batch release 7.7 ± 0.2 at 25°C

REQUIRED MATERIALS BUT NOT INCLUDED
Suitable materials for the cultivation and isolation of bacteria; refer to the laboratory reference manuals for the recommended culture techniques and identification procedures.

STORAGE
The LIM Broth is ready for use and requires no further preparation. The packaged and unopened product can be stored at a temperature of 5-25°C until use or up to the expiration date. Do not overheat. Do not incubate or freeze prior to use. Improper storage can cause a loss of effectiveness. Do not use after the expiration date, which is clearly printed on the product packaging.

RESTRICTIONS
1. The sample collecting conditions, transport times and volume for the culture are important variables for obtaining reliable culture results. Follow the recommended guidelines for the collection and management of clinical samples.
2. An adequate collection of samples from the patient is an extremely critical factor for the successful isolation and identification of infectious organisms. Consult the published reference manuals for specific guidance on the sample collecting procedures. Samples should be collected as soon as possible after clinical signs of the disease appear. Higher bacterial concentrations may be present in the acute phase of the disease.
3. LIM Broth is a selective and enrichment broth for the isolation of GBS samples (Group B *Streptococco agalactiae*).

WARNINGS and PRECAUTIONS
1. For *in vitro* diagnostic use.
2. ② This product is intended for single use; if reused, there is the risk of obtaining misleading results.
3. Not suitable for any other application different from its intended use.
4. This product must only be used by properly trained and qualified personnel. The manufacturer cannot be held responsible for any use by unauthorized or unqualified persons.
5. Wear latex gloves and other protection commensurate with universal precautions when handling clinical specimens.
6. The use of this product in association with any diagnostic tests or any diagnostic instrumentation should be previously evaluated by the user.
7. Do not use the product if it is visibly damaged.
8. Do not ingest the product.

10. The manufacturer cannot be held responsible for any improper or unqualified use of the product.
11. All clinical samples are considered potentially infectious and must be handled with appropriate precautions.
12. This LIM Broth product is intended for *in vitro* diagnostic use only and must not be used for therapeutic or prophylactic purposes under any circumstances.
13. Observe approved biohazard precautions and aseptic techniques. The product is only to be used by adequately trained and qualified personnel.
14. Carefully read and follow the instructions.
15. For product code 476CE01.A: Do not use excessive force or pressure when collecting swab samples from patients as this may result in the accidental breakage of the swab shaft.
16. For product code 476CE01.A: Do not use sampling systems other than the one provided in the kit and do not put more than one flocked swab in the tube as this interferes with the proper closing of the tube.

PRODUCT DETERIORATION

Do not use the **LIM Broth** if: (1) the product has visible signs of damage or contamination; (2) there is evidence of leaking; (3) it has passed the expiration date; (4) there are other signs of deterioration.

INSTRUCTIONS FOR USE

Sample collection

An adequate collection of samples from the patient is an extremely critical factor for the successful isolation and identification of infectious organisms. Consult the published reference manuals for specific instructions on the sample collection procedures.
Vaginal/rectal swabs are normally used to screen for bacterial colonization of Streptococcus B in pregnant women. The Copan eSwab system or a regular size flocked swab can be used as the sampling system. Contact Copan or refer to the product IP for instructions.

Laboratory use

MANUAL PROCEDURE

1. Open the bag and remove the tube and the pouch containing the swab.
2. Remove the flocked swab from the individually wrapped package and collect the sample from the patient
3. Unscrew the cap of the LIM broth tube.
4. Inoculate the sample in the open tube following the procedure indicated below:

For samples taken with flocked swabs included in the kit:

- Break the swab off into the tube as follows (fig. 1):

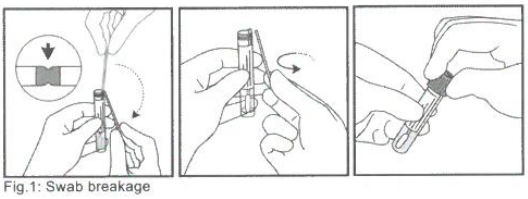

Fig.1: Swab breakage

  - Hold the tube in one hand pointing the opening of the tube away from your face
  - With the other hand grasp the swab shaft at the very end with the thumb and first finger
  - Lean the part of the shaft with the breaking point against the rim of the tube
  - Bend the swab shaft at a 180 degrees angle to break it off at the colored ink breakpoint mark. If needed, gently rotate the swab shaft to complete the breakage and take away the upper part of the swab shaft.
  - Discard the broken handle part of the swab shaft into an approved medical waste disposal container.
- Vortex for 10 seconds at 2000-2500 rpm.
- Directly incubate the LIM broth tube at 35°C ± 2°C for 18-24 hours.
- After incubation, automatically or manually seed the LIM Broth tube on a suitable culture media plate.

For samples collected using the ESwab:

- Vortex the tube for 10 seconds at 2000-2500 rpm.
- Unscrew the cap and transfer the swab from the ESwab to the LIM broth tube.
- Close the ESwab and the LIM Broth tube.
- Vortex for 10 seconds at 2000-2500 rpm.
- Directly incubate the LIM broth tube at 35°C ± 2°C for 18-24 hours.
- After incubation, check for the presence/absence of turbidity in the tube and then directly seed from the LIM Broth tube onto a suitable culture media plate either manually or using automatic systems.

Alternatively, transfer a minimum an aliquot of Amies culture medium directly into the LIM broth using a micro pipette:

- Close the ESwab and LIM Broth tubes and vortex for 5-10 seconds at 2000-2500 rpm.
- Incubate the LIM broth tubes at 35°C ± 2°C for 18-24 hours.
- After incubation, check for the presence/absence of turbidity in the tube and then directly seed from the LIM Broth tube onto a suitable culture media plate either manually or using automatic systems.

Seeding onto plates can be done directly using the swab or by seeding an aliquot of sample taken with a suitable instrument (micropipette, loop, etc.) and

then streaking the plate.
It is recommended that the plate be seeded with at least 1 µL of the original sample or 1 µL of the transport medium in which the original sample is stored.

Incubate the plates at 35°C ± 2°C for 18-24 hours, or up to 48 hours if necessary, as per the standard laboratory procedure.
Read the plates after the incubation period. Check for the growth of characteristic grey-white beta-hemolytic or non hemolytic colonies (GBS is a gram positive, catalase negative coccus). Incubation should be extended to 48 hours if there were no group B Streptococcus colonies after 18-24 hours.
Use the isolated colonies to perform further confirmations with the agglutination test or other recommended tests for the detection of Group B Streptococcus antigens by referring to the manufacturer's instructions for use and reading the results.

OPERATIONS USING AUTOMATIC SYSTEMS (WASP ™/WASPLab ™)
LIM Broth is produced in a format suitable for use by WASP™/WASPLab™ type automatic sample processing systems.

BROTH INOCULATION WITH A WASP™/WASPLab™ AUTOMATIC SYSTEM
Refer to the WASP™/WASPLab™ User Manual for further information.

SEEDING A PLATE OF ENRICHMENT BROTH WITH A WASP™/WASPLab™ AUTOMATIC SYSTEM
Refer to the WASP™/WASPLab™ User Manual for further information.

WASTE DISPOSAL
The unused reagents can be considered non-hazardous waste and be disposed of accordingly. Consult the product MSDS for disposal.
Used reagents and any other contaminated waste material must be disposed of following the procedures for infected or potentially infected products. It is the laboratory's responsibility to handle waste and the waste fluids produced according to their nature and the degree of hazard, treating and disposing of them (or having them treated and disposed of) as indicated in any applicable regulation.
The unused swabs can be considered non-hazardous waste and be disposed of accordingly.
Used swabs must be disposed of as contaminated disposable materials in accordance with the required procedures for infectious or potentially infectious products.

RESULTS
Results obtained will largely depend on proper and adequate sample collection, as well as timely transport and processing in the laboratory.

QUALITY CONTROL PROCEDURE

PERFORMANCE TEST
- Prepare a 0.5 McFarland bacterial suspension in PBS from a fresh culture of *Streptococcus agalactiae* ATCC 12386.
- Prepare a 10^{-5} serial dilution from the 0.5 McFarland bacterial suspension.
- Inoculate the LIM Broth with 200 ul of the dilution in PBS.
- Recap the tubes.
- Homogenize on a Vortex for 10 seconds at 2500 rpm.
- To check the zero-time, seed the appropriate culture medium (Blood agar 5% sheep) with 100 ul of the microbial suspension.
- Incubate the LIM broth tube at 35°C ± 2°C for 18-24 hours.
- After incubation, seed the appropriate nutrient culture medium (Blood agar 5% sheep) with 100 ul of LIM Broth.
- Incubate the plates at 35°C ± 2°C for 18-24 hours.

The zero-time plate counts should be between 30 and 300 CFU/plate; turbidity in the tube after 18 to 24 hours of incubation and the subsequent confluent growth on the plate are used as the batch release parameters.

INHIBITION TEST
- Prepare a 0.5 McFarland bacterial suspension in PBS from a fresh culture of *E.Coli* ATCC 25922.
- Prepare a 10^{-1} serial dilution from the 0.5 McFarland bacterial suspension.
- Inoculate the LIM Broth tube with 200 ul of inoculum.
- Recap the tubes.
- Homogenize on a Vortex for 10 seconds at 2500 rpm.
- To check the zero-time, seed the nutrient medium (Tryptic soy agar) with 100 ul of the microbial suspension.
- Incubate the LIM broth tube at 35°C ± 2°C for 18-24 hours.
- After incubation, seed the nutrient culture medium (Tryptic soy agar) with 100 ul of LIM Broth.
- Incubate the plates at 35°C ± 2°C for 18-24 hours.

The zero-time counts should show a semi-confluent growth and the growth on the plate must be partially or totally inhibited after 18-24 hours.

RESULTS OF THE PERFORMANCE TEST

STRAIN	ZERO-TIME: CFU/PLATE	TIME 18-24H CFU/PLATE
Streptococcus agalactiae ATCC 12386	172	CONFLUENT GROWTH
Escherichia coli ATCC 25922	SEMI-CONFLUENT GROWTH	FROM PARTIAL TO TOTAL INHIBITION

¥ The results of the tests mentioned above were obtained with ATCC strains in the laboratory.