
TODD HEWITT BROTH w/ COLISTIN and NALIDIXIC ACID (LIM BROTH)

INTENDED USE

Remel Todd Hewitt Broth w/ CNA (Lim Broth) is a liquid medium recommended for use in qualitative procedures for the isolation of Group B *Streptococcus* (GBS) from clinical specimens containing mixed bacterial flora.

SUMMARY AND EXPLANATION

Since its emergence in the 1970s, GBS disease has become a significant cause of morbidity and mortality among newborns.¹ Early-onset disease (< 7 days old) accounts for roughly 80% of GBS invasive episodes and results from vertical transmission from a mother who is a carrier. Determinants of late-onset (≥7 days old) disease are not well documented, though vertical and nosocomial transmission as well as community acquisition is possible. Studies have shown that treatment of GBS disease is more costly than prevention. This knowledge has led to development of more rapid methods to identify GBS carriers. Jones et al. reported that addition of a selective enrichment broth, such as Lim Broth, to vaginal/rectal cultures decreased the time required to identify culture-positive women.² Lim et al. reported that early diagnosis using a selective enrichment broth combined with chemoprophylaxis is effective in interrupting vertical transmission of GBS and in reducing morbidity and mortality in newborn infants.^{3,4} Successful implementation of prevention programs relies on optimal identification of GBS carriers.⁵ In 2002, the Centers for Disease Control and Prevention published guidelines for the prevention of perinatal GBS disease which recommend screening all pregnant women at 35-37 weeks of gestation. These guidelines, supported by the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, include the use of a selective broth medium, such as Lim Broth, to maximize the recovery of GBS and identification of carriers.

PRINCIPLE

Peptones, heart infusion, and yeast extract supply nitrogenous compounds and vitamins essential for bacterial growth. Dextrose is a source of carbon and energy. Sodium chloride provides essential electrolytes and maintains osmotic equilibrium. Disodium phosphate and sodium carbonate are buffers which counteract the acidity produced during the fermentation of dextrose and protect the hemolysin from inactivation by the acid. Colistin and nalidixic acid are selective agents which inhibit the growth of gram-negative bacteria.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone.....	10.0 g	Sodium Carbonate	2.5 g
Meat Peptone.....	10.0 g	Sodium Chloride	2.0 g
Yeast Extract.....	10.0 g	Disodium Phosphate	0.4 g
Heart Infusion.....	3.1 g	Nalidixic Acid	15.0 mg
Dextrose	2.0 g	Colistin.....	10.0 mg
		Demineralized Water	1000.0 ml

pH 7.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Obtain a single swab or two separate swabs of the distal vagina and anorectum between 35-37 weeks gestation.
2. Inoculate Lim Broth with the swab or both swabs together.
3. Incubate tube aerobically or in 5% CO₂ at 33-37°C for 18-24 hours.
4. After incubation, subculture Lim Broth to a sheep blood agar plate (nonselective) and incubate aerobically or in 5% CO₂ at 33-37°C for 18-24 hours.
5. Examine the blood agar plate at 24 and 48 hours for large, gray, translucent colonies with a small zone of beta-hemolysis or no hemolysis. Perform a catalase test and Gram stain to verify that colonies are catalase-negative, gram-positive cocci. Definitive identification of Group B *Streptococcus* requires additional biochemical and/or serological testing. Consult appropriate references for further instructions.⁵⁻⁷

Note: In addition to subculture, Lim Broth may be tested with a streptococcal grouping method that includes an extraction step.² Follow the instructions for use provided by the manufacturer of the streptococcal grouping method in use.

QUALITY CONTROL

All lot numbers of Todd Hewitt Broth w/ CNA (Lim Broth) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Streptococcus agalactiae ATCC® 12386
Escherichia coli ATCC® 25922

INCUBATION

Aerobic, 18-24 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C

RESULTS

Good growth
Inhibition (partial to complete)

LIMITATIONS

1. Culture following enrichment remains the most sensitive method of GBS detection.⁵⁻⁷
2. A positive culture indicates colonization with Group B *Streptococcus*, which may or may not indicate infection.⁵⁻⁷

BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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