

## Puritan<sup>®</sup> Lim Broth with Colistin and Nalidixic Acid

### Intended use

The Puritan<sup>®</sup> Lim Broth Medium is a selective enrichment broth medium for use in selective qualitative procedures for the isolation of Group B Streptococcus (GBS) from clinical specimens.

### Summary and explanation

Group B Streptococcus (GBS) is the most common cause of infections such as sepsis, meningitis and pneumonia among newborns. The disease is transmitted to newborns through the mother who carries GBS in her rectum or genital tract during birth. Approximately 7-20% of pregnant women are colonized with GBS in the vagina or rectum.<sup>1,2</sup> To reduce the risk of infection, the Centers for Disease Control and Prevention (CDC) and other organizations have published guidelines for screening and prevention of neonatal GBS disease. The CDC suggests using vaginal and rectal swabs with selective enrichment broths to detect GBS colonization from the suspected pregnant women for culture-based screening between 35 and 37 week's gestation.<sup>3-6</sup>

Puritan Lim Broth Medium consists of a polypropylene screw-cap vial containing 2 mL of modified Lim Broth enrichment medium. Modified Lim Broth medium is a selective enrichment broth. The peptones, dextrose and yeast extract provide nutritional base for growth of GBS. Nalidixic acid and colistin suppress growth of gram-negative bacteria.<sup>7</sup>

### Principles of the procedure

Once a specimen is collected with a swab, it should be placed into the vial containing Lim Broth enrichment medium and incubated aerobically at 35-37°C for 18 to 24 hours prior to being subcultured onto a blood agar plate.

### Reagents

Approximate modified Lim Broth enrichment medium formulation per liter

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

Optimum pH 7.8 ± 0.2 @ 25°C

### Precautions

For *in vitro* Diagnostic Use

- Clinical specimens are considered biohazard and must be handled in manner to protect laboratory personnel
- To be used by trained and qualified personnel using aseptic technique
- Clinical samples may contain human pathogens including hepatitis virus and Human Immunodeficiency Virus. Institutional and universally recognized guidelines should be followed when handling items contaminated with blood and other body fluids.<sup>8</sup>
- Specimen vials and other contaminated materials must be sterilized by autoclave before discarding.
- Do not use if the vial is damaged or detecting evidence of contamination, discoloration or leakage.

### Storage

For optimum performance, store at 2-25°C. Avoid freezing and overheating.<sup>7,9</sup>

### Directions for use

[1] Obtain swab samples from distal vagina and anorectum between 35-37 weeks gestation.

[2] Inoculate Lim Broth Medium with swabs.

[3] Incubate tube aerobically or in 5% CO<sub>2</sub> at 35-37°C for 18-24 hours.

[4] After incubation, subculture Lim Broth enrichment medium to a nonselective blood agar plate and incubate aerobically or in 5% CO<sub>2</sub> at 35-37°C for 18-24 hours.

[5] Check blood agar plate at 24-48 hours for large, gray, translucent colonies with a small zone of beta-hemolysis or no hemolysis.

- If plating with a microbiology automation system, refer to the automation manual. Make sure to remove swab from the tube and discard prior to processing.

## Specimen Collection and Handling

Specimens suitable for culture may be handled using various techniques. For detailed guidance, refer to appropriate references.<sup>10, 11</sup> Specimens should be obtained before antimicrobial agents have been administered.

## Quality Control

All raw materials used in the manufacture of Puritan Lim Broth Medium are tested and qualified before use. Each batch of medium is tested prior to release for bacterial or fungal contamination, medium pH, and ability to support growth of GBS and suppress gram-negative bacteria over predefined time periods. All bacterial test isolates and testing procedures were established using the criteria outlined in the Clinical and Laboratory Standards Institute's M22-A3 document and dehydrated media manufacturer recommendations where applicable.<sup>12, 13</sup>

### Control

*Streptococcus agalactiae* ATCC 12386  
*Escherichia coli* ATCC 25922

### Incubation

Aerobic, 18-24 hr @ 35-37°C  
Aerobic, 18-24 hr @ 35-37°C

### Results

Good growth  
Inhibition (partial to complete)

## Limitations

Definitive identification of GBS requires additional biochemical and/or serological tests. Refer to appropriate reference standards for further instructions.<sup>10, 11</sup>

## References

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207-876-3311 • [puritanmedproducts.com](http://puritanmedproducts.com)  
[sales@puritanmedproducts.com](mailto:sales@puritanmedproducts.com)  
Puritan Medical Products Co. LLC  
31 School Street, Guilford, Maine 04443-0149 USA  
ISO 9001:2008 ISO 13485:2003 CE

