Regan-Lowe Charcoal Agar
Regan-Lowe Charcoal Agar without Cephalexin

Intended Use

Regan-Lowe Charcoal Agar is a selective medium used for isolation of *Bordetella pertussis* from clinical specimens. Regan-Lowe Charcoal Agar without Cephalexin is used for the cultivation of *B. pertussis* from clinical specimens and for subcultures of the bacterium.

Summary and Explanation

Regan-Lowe Charcoal Agar plates are used in clinical laboratories for the isolation of *Bordetella pertussis*, the etiologic agent of whooping cough, from nasopharyngeal swabs and other sources of pharyngeal exudate. This medium was developed by Regan and Lowe as a transport medium for whooping cough specimens, but proved useful as an enrichment medium for the selective isolation of *B. pertussis* and *B. parapertussis*. It consists of charcoal agar as a basal medium supplemented with cephalexin to inhibit bacteria indigenous to the nasopharynx and defibrinated horse blood to support the growth of *Bordetella* species.[1-3]

Use of the medium without cephalexin in parallel with Regan-Lowe Charcoal Agar is recommended, since a few strains (<10%) of *B. pertussis* will not grow on selective plates; also the nonselective medium is used for subcultures to obtain a larger amount of growth for additional testing, such as agglutination or immunofluorescence testing.[3,4]

The medium in 10 mL prepared tubes (deeps) with screw-caps offers a longer shelf-life than the pre-poured plated medium.

To prepare the medium from the agar base, 10% horse blood is added and cephalexin can be added to achieve selectivity.

Principles of the Procedure

Beef extract and enzymatic digest of gelatin provide the amino acids and other complex nitrogenous substances necessary

User Quality Control

Identity Specifications

BBL™ Regan-Lowe Charcoal Agar Base

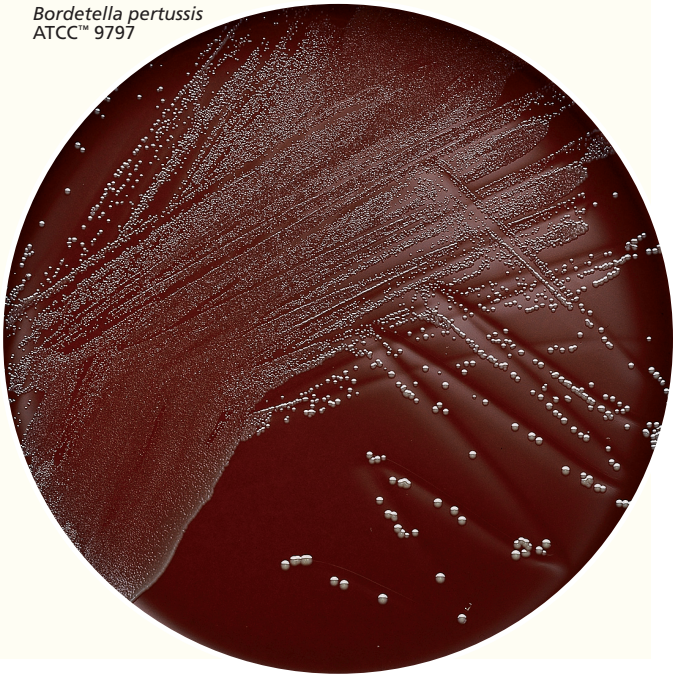
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is charcoal black, homogeneous, opaque.
Prepared Appearance:	Charcoal black, homogeneous, opaque.
Reaction of 5.1% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Regan-Lowe Charcoal Agar Base

Prepare the medium per label directions. Inoculate with fresh broth cultures diluted 1:10 and incubate at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY
Bordetella pertussis	9797	Good
Bordetella parapertussis	15311	Good



Bordetella pertussis
ATCC™ 9797

to support bacterial growth. Sodium chloride maintains the osmotic equilibrium. Defibrinated horse blood supplies nutrients required for the cultivation of *Bordetella* species. Nicotinic acid is a vitamin that promotes growth. Charcoal and starch neutralize substances toxic to *Bordetella* species, such as fatty acids and peroxides. Cephalexin is a cephalosporin antibiotic that inhibits most normal flora of the nasopharynx.

Formula

BBL™ Regan-Lowe Charcoal Agar Base

Approximate Formula* Per Liter		
Beef Extract	10.0	g
Pancreatic Digest of Casein	10.0	g
Soluble Starch	10.0	g
Sodium Chloride	5.0	g
Charcoal	4.0	g
Niacin	0.01	g
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 51 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.
4. For preparation of blood plates, add 10% sterile, defibrinated horse blood to sterile agar which has been previously melted and cooled to 45-50°C.
5. For selective isolation of *B. pertussis* and *B. parapertussis*, add 40 µg of cephalexin per mL.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) in a moist chamber at 35°C for 7 days. Colonies of *B. pertussis* may not be visible without the aid of a microscope for 2-4 days. Plates may be discarded as negative after 7 days of incubation.

Expected Results

Examine the plates daily with and without a dissecting microscope (oblique illumination) to detect the presence of *B. pertussis*. *B. pertussis* produces small, domed, glistening, white to gray colonies. To prevent overgrowth by spreading colonies or molds, use a sterile scalpel or needle to remove the portions of the agar that contain these contaminants.

References

1. Regan and Lowe. 1977. J. Clin. Microbiol. 6:303.
2. Sneed. 1992. *In* Isenberg (ed.), Clinical microbiology procedure handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Marcon. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Regan-Lowe Charcoal Agar Base

Cat. No. 298123 Dehydrated – 500 g

BBL™ Regan-Lowe Charcoal Agar

BS12 CMPH2 MCM9

Cat. No. 297883 Prepared Plates – Pkg. of 10*
297855 Prepared Tubes (Deeps), 10 mL – Pkg. of 10*

BBL™ Regan-Lowe Charcoal Agar without Cephalexin

Cat. No. 298326 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*