



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Regan-Lowe Charcoal Agar is a selective medium used for the isolation of *Bordetella pertussis* from clinical specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
  - a. For *S. aureus* and *E. coli*, streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **BD BBL Trypticase™** Soy Broth diluted to yield 10<sup>6</sup> – 10<sup>7</sup> CFU/mL; incubate at 35 ± 2 °C under appropriate atmospheric conditions.
  - b. For *Bordetella*, inoculate with 1 µL (0.001 mL) from appropriate working dilution which contains 10<sup>6</sup> – 10<sup>7</sup> CFU/mL; incubate at 35 ± 2 °C under appropriate atmospheric conditions.
  - c. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for inhibited strains.
2. Examine plates up to 7 days for growth and selectivity.
3. Expected Results

Organisms	ATCC®	Recovery
* <i>Bordetella pertussis</i>	9797	Fair to heavy growth
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)
* <i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)

\*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Note the firmness of the agar beds during the inoculation procedure.
4. Incubate uninoculated representative plates at 33 – 37 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Regan-Lowe Charcoal Agar is a selective medium used for the isolation of *Bordetella pertussis* from clinical specimens.

V 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 to be 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 cephalixin to inhibit bacteria indigenous to the nasopharynx and defibrinated horse blood to support the growth of *Bordetella* species.<sup>1-4</sup>

Use of the medium without cephalixin in parallel with Regan-Lowe Charcoal Agar is recommended, since a few stains (<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.<sup>3,4</sup>

VI PRINCIPLES OF THE PROCEDURE

Beef extract and enzymatic digest of gelatin provide the amino acids and other complex nitrogenous substances necessary 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. Cephalixin is a cephalosporin antibiotic that inhibits most normal flora of the nasopharynx.

VII REAGENTS

Regan-Lowe Charcoal Agar

Approximate Formula\* Per Liter Purified Water

Beef Extract.....	10.0 g	Niacin .....	0.01 g
Pancreatic Digest of Gelatin .....	10.0 g	Agar.....	12.0 g
Starch.....	10.0 g	Cephalixin .....	40.0 mg
Sodium Chloride.....	5.0 g	Horse Blood, defibrinated .....	10%
Charcoal.....	4.0 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>5-8</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared plates, specimen containers and other contaminated materials by autoclaving.

**Storage Instructions:** On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration.

## VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.<sup>2-4,9-11</sup>

Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Regan-Lowe Charcoal Agar.

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the plate to obtain isolated colonies.

Material not being cultured from swabs should be streaked onto the medium with a sterile inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

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.

**User Quality Control:** See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

## X RESULTS

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine the plates daily with and without a dissecting microscope (oblique illumination) to detect the presence of *Bordetella 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.

## XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.<sup>2-4,11,12</sup>

## XII AVAILABILITY

Cat. No.	Description
297883	BD BBL™ Regan-Lowe Charcoal Agar

## XIII REFERENCES

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