REGAN-LOWE AGAR

- For in vitro use only - Catalogue No. PR35 & PR36

Our Regan-Lowe Agar can be used for the primary isolation of *Bordetella pertussis* from clinical specimens. We offer two formulations, a non-selective medium (PR36) and a selective medium containing cephalexin (PR35).

Bordetella was first cultured on a charcoal-based medium by Pollack in 1947. Our current formulation is based on the innovative research of Mishulow, Sharpe, and Cohen who devised a non-selective beef-heart charcoal agar for the preparation of pertussis vaccines. The high nutrition value of this medium made isolation of *Bordetella* difficult since the lengthy incubation time required by *Bordetella* allowed contaminating nasopharyngeal flora to overgrow it. Numerous researchers tried incorporating different antibiotics with varying degrees of success. Regan and Lowe as well as Sutcliffe and Abbott when using the first generation cephalosporin, cephalexin, reported the best suppression of nasopharyngeal flora. Cephalexin was superior when compared to other antimicrobials being used at that time including penicillin and methicillin. Regan and Lowe were the first researchers to use both a charcoal-based enrichment transport medium (TR35 Regan-Lowe Transport Medium) and a selective charcoal agar to selectively isolate *Bordetella* and to their credit Regan-Lowe Agar has become synonymous with charcoal agar.

Numerous peptones and extracts and the addition of defibrinated horse blood makes Regan-Lowe Agar especially nutritious. Nicotinic acid is an essential growth factor, and its addition is a necessity, as shown by Proom, to cultivate *Bordetella* species. Charcoal and starch are the two detoxifying agents that help neutralize toxic fatty acids and metabolites contained in the medium.

Formula per Litre of Medium

Beef Extract	10.0 g
Meat Peptone	10.0 g
Soluble Starch	10.0 g
Sodium Chloride	5.0 g
Charcoal	4.0 g
Nicotinic Acid	0.001 g
Agar	12.0 g
Horse Blood (Defibrinated)	100.0 mL

Additional Ingredients per Liter:

PR35 Regan-Lowe Agar

Cephalexin	0.04 g

pH 7.4 ± 0.2 at 25°C.

Recommended Procedure

General Procedure for PR35 & PR36

1. Allow medium to adjust to room temperature prior to inoculation.
2. Inoculate sample and perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate plates at 35°C in a moist aerobic atmosphere (60-70% humidity). [To obtain a moist environment, place inoculated plates into a **partially** sealed container along with some damp paper towels]
4. Check plates daily and incubate plates for a minimum of 7 days before reporting plates as negative.

Recommended Clinical Diagnosis Procedure

1. Collect specimen from the posterior nasopharynx (not the throat) using a Dacron™ or calcium alginate swab (not cotton).
2. Place specimen swab into Regan-Lowe Transport Media (Dalynn TR35) for enrichment and transport of potential *B. pertussis* isolates. (Swabs can remain in the transport medium for up to 48 hours as it serves as an enrichment step)
3. Once in the laboratory, streak the specimen swab onto a selective Regan-Lowe Agar plate with antibiotics (PR35).
4. Incubate plates at 35°C in a moist aerobic atmosphere (60-70% humidity). [To obtain a moist environment, place inoculated plates into a **partially** sealed container along with some damp paper towels]
5. Check plates daily for growth. Incubate plates a minimum of seven days before reporting specimen plates as negative. (A 12 day incubation period may be warranted as a 1996 study reported a 18% increase in recovery of isolates)
6. If characteristic *Bordetella* colonies are observed, select one or two well-isolated colonies, and subculture them onto a non-selective Regan-Lowe Agar plate (Dalynn PR36) so further testing can be performed from pure culture.

Interpretation of Results

Bordetella pertussis colonies appear as small, greyish-white, glistening colonies on Regan-Lowe Agar usually within 72 hours. Selective plates containing Cephalexin require prolonged incubation and typically good growth is observable after 5 to 6 days of incubation. *B. parapertussis* grows quicker and mature colonies usually become visible after 48 hours of incubation.

Some clinical laboratory use a direct fluorescent antibody (DFA) test to detect *Bordetella*, however DFA cannot replace culturing methods due to its low sensitivity and low specificity. Sensitivity is directly correlated with the number of *B. pertussis* cells contained in the sample therefore specimens lacking sufficient numbers will give false negatives. The potential for cross reactivity with resident respiratory flora can also be quite high resulting in the poor specificity of DFA. DFA can provide rapid results and therefore it can be a valuable companion test when used in conjunction with classical culturing methods. Rapid detection and confirmation can be done by PCR, which shows both a high specificity and sensitivity.

- *Patients whose disease has progressed beyond the catarrhal or paroxysmal stage, or have undergone antimicrobial treatment make detection and isolation of Bordetella detection more difficult and may result in false negatives*
- *Some organisms such as Pseudomonas species may grow on Regan-Lowe Agar*
- *The presence of cephalexin in the selective medium retards the growth of Bordetella and a minimum incubation period of 4 or 5 days is required for visible growth*

Quality Control

Organism	Expected Result	
	PR35 (with AB)	**PR36 (w/o AB)**
Bordetella pertussis ATCC 8467	Growth	Growth
Staphylococcus aureus ATCC 25923	Inhibition	Growth
Escherichia coli ATCC 25922	Inhibition	Growth

AB = Antibiotic (Cephalexin)

Storage and Shelf Life

Our Regan-Lowe Agar should be stored at 4°C to 8°C with medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions it has a shelf life of 8 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PR35	Regan-Lowe Agar [Standard 15x100-mm plate]	10/pkg
PR36	Regan-Lowe Agar w/o AB [Standard 15x100-mm plate]	10/pkg

References

1. Mishulow L, Sharpe LS, Cohen LL. Beef-heart charcoal agar for the preparation of pertussis vaccines. Am J Public Health 1953; 43:1466.
2. Proom H. The minimum nutritional requirements of organisms of the genus *Bordetella lopez*. J Gen Microbiol 1955; 12:63-75.
3. Regan J, Lowe F. Enrichment medium for the isolation of *Bordetella*. J Clin Microbiol 1977; 6:303-309.
4. Sutcliffe EM, Abbott JD. Brit Med J 1972; 6:732-3.
5. Regan J. The laboratory diagnosis of whooping cough. Clin Micro Newsletter 1980; 18:Sept 15.
6. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
7. Katzko G, Hofmeister M, Church D. Extended incubation of culture plates improves recovery of *Bordetella spp*. J Clin Micro 1996; 34: 1563-4.
8. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington, DC: ASM, 1999.
9. Recommendations from CDC web-site.

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