IFU

Instructions for Use

REGAN-LOWE AGAR (CHARCOAL BLOOD AGAR)

Cat. no. A65	Regan-Lowe Agar, 15x100mm Plate, 22ml	10 plates/bag
Cat. no. Q32	Regan-Lowe Semi-Solid, 13x100mm Tube, 4ml Deep	20 tubes/box
Cat. no. A63	Regan-Lowe Agar without Cephalexin, 15x100mm Plate, 24ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Regan-Lowe Agar is a selective medium recommended for the isolation and cultivation of *Bordetella* spp. Regan-Lowe Agar without Cephalexin does not contain selective agents and is recommended for the cultivation of *Bordetella*.

SUMMARY

Several types of media have been developed over the years for the isolation of *Bordetella pertussis*. Regan and Lowe, in developing a medium for use in the transport of whooping cough specimens, discovered an enrichment medium useful for the selective isolation of *B. pertussis* and *B. parapertussis*.(6)

The basal medium of Regan-Lowe Agar consists of charcoal agar supplemented with defibrinated horse blood. Charcoal, along with starch, neutralizes fatty acids and peroxides, which are toxic to *Bordetella*. Horse blood is an added enrichment which supports the growth of *Bordetella* spp. Cephalexin inhibits the growth of normal flora of the nasopharynx. Yeasts and fungi are inhibited by the inclusion of amphotericin B (Cat. no. Q32). Beef extract and enzymatic digest are incorporated in the medium to supply amino acids and other nitrogenous substances that are necessary for bacterial growth. Osmotic equilibrium is maintained by the addition of sodium chloride. Niacin (nicotinic acid) is a vitamin which is added for growth promotion.

Katzko, et al., recently reported enhanced recovery of *Bordetella* spp. from nasopharyngeal swabs by extending the incubation of plated primary cultures beyond the usual seven days to a total of twelve days.(10)

FORMULA

Ingredients per liter of deionized water:*

Regan-Lowe Agar:	
Charcoal Agar	66.5gm
Cephalexin	0.04gm
Horse Blood	100.0ml

In addition, Regan-Lowe Agar without Cephalexin (Cat. no. A63) is the same formulation as above, without

cephalexin.

Regan-Lowe Deep (Cat. no. Q32) is a semi-solid medium. It contains half the amount of basal medium as the isolation medium (Cat. no. A65) and, in addition, contains amphotericin B. The half-strength formula allows *Bordetella* to be exposed to air and moisture, two parameters necessary for survival.[7,8]

Final pH 7.4 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If possible, it is recommended to inoculate the plate at the patient's bedside. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use for Regan-Lowe Deep (Cat. no. Q32): The medium should be brought to room temperature prior to inoculation. Providing the physical nature of the specimen is suitable for a stab inoculation, submerge the specimen into the medium. If using a swab, the tip must be submerged well into the medium. Break or cut any portion of the swab that is protruding from the tube. Tighten the cap and deliver immediately to the laboratory. Once received by the lab, the specimen should be immediately plated onto Regan-Lowe Agar and a non-selective medium. Return the swab to the Regan-Lowe Deep medium and incubate with CO_2 at 35ºC. for 48 hours, at which time a duplicate set of plates should be inoculated and incubated.

Method of Use for Regan-Lowe Agar (Cat. no. A65) and Regan-Lowe Agar without Cephalexin (Cat. no. A63): The medium should be brought to room temperature and the agar surface should be dry, prior to inoculation. Using aseptic techniques, inoculate the agar surface and streak for isolation. Incubate the plates in an aerobic, humidified atmosphere with CO_2, at 35ºC. for up to 12 days.[10] It is recommended that the plates be incubated in a zip-lock bag with a moist cotton ball. Plates should be examined daily for typical colonial growth and morphology.

INTERPRETATION OF RESULTS

Plates should be examined daily with and without a dissecting microscope. When colonies of *Bordetella* spp. are visible to the unaided eye, they should appear small, gray, shiny, convex, smooth, and raised with a pearl-like luster; somewhat like a mercury droplet.

As incubation is lengthened, the colonies become whiter, resembling a bisected pearl.[9]

LIMITATIONS

This medium is intended for the primary isolation of *Bordetella* spp.

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Often, selective media will inhibit specific strains of organisms for which they are designed to isolate. At the same time, contaminating organisms may prove resistant to the added antimicrobics, and thus result in growth. It is recommended, therefore, that Regan-Lowe Agar and a non-selective medium be inoculated in parallel, in order to ensure recovery of potential pathogens.[9]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Regan-Lowe Agar without Cephalexin:					
Bordetella pertussis ATCC® 9797	B	48-96hr	35°C	CO_2**	Growth; small, pearly-white colonies
Additionally, the following organisms are tested on Regan-Lowe Agar and Semi-Solid:					
Staphylococcus aureus ATCC®25923	B	24hr	35°C	Aerobic	Partial to complete inhibition
Escherichia coli ATCC®25922	B	24hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Atmosphere of incubation is enriched with 5-10% CO_2.

Refer to the above Procedure Section for a description of the recommended inoculation procedures for Cat. no. Q32 Regan-Lowe Semi Solid.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

- Regan-Lowe Media should appear opaque, and black in color.
    - Regan-Lowe Deeps are semi-solid.

Bordetella pertussis (ATCC® 9797) growing in Regan-Lowe Agar (Cat. no. A65). Incubated in CO_2 for 72 hours at 35ºC.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Regan and Lowe. 1997. *J. Clin. Microbiol.*; 6:303.

7. Brumfitt, W. 1959. *J. Pathol. Bacteriol.*; 77:95-100.

8. Preston, N.W. 1970. *Lab. Pract.*, 19:482-486.

9. Gilchrist, Mary J.R., Ph.D. 1970. *Clin. Micro. Newsletter*; Vol. 12, No. 7.

10. Katzko, Gary, et al. 1996. *J. Clin. Microbiol.*; 34:1563-1564.

IFU-10725[B]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]