IFU

Instructions for Use

ROSE AGAR

Cat. no. A66	Rose Agar, 15x100mm Plate, 17ml	10 plates/bag
Cat. no. J85	Rose / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Rose Agar is recommended for use as a selective growth medium for the cultivation and isolation of gram-positive cocci from clinical and non-clinical specimens which contain mixed flora.

SUMMARY

Rose Agar consists of half Columbia CNA Agar and Half Phenylethyl Alcohol Agar Base. The combination of the two basal mediums provides a more selective growth medium for gram-positive cocci than the individual mediums solely provide. Atypical hemolytic reactions may occur with *Streptococcus* spp., therefore, determination of hemolysis is not recommended.

Columbia Blood Agar was first described in 1966 by Ellner, Stoessel, Drakeford, and Vasi who incorporated animal derived peptone, enzymatic digests of casein, and defibrinated sheep blood into one medium.[3] It was found to be an improved form of blood agar, promoting both luxuriant and rapid growth, improved pigment production, typical colony morphology, and sharply defined hemolytic reactions. Ellner, et al. also described the use of nalidixic acid and colistin in Columbia Blood Agar.[3] Columbia CNA Agar was designed to suppress the growth of most gram-negative bacteria, including *Klebsiella*, *Proteus*, and *Pseudomonas* species from mixed flora specimens, thus isolating for gram-positive staphylococci and streptococci.[3]

Phenylethyl Alcohol Agar was developed by Brewer and Lilley in 1949 for the selective isolation of gram-positive organisms, particularly gram-positive cocci.[8,9] Phenylethyl alcohol permits the growth of gram-positive organisms while inhibiting most gram-negative organisms, especially swarming *Proteus* spp. Nitrogen, carbon, sulfur and trace nutrients are made available by the presence of peptones. Osmotic equilibrium is maintained by the addition of sodium chloride. The addition of 5% sheep blood to the basal medium provides many growth factors, however, atypical hemolytic reactions may occur. Therefore, determination of hemolytic reactions should not be made on PEA with 5% sheep blood.

The combination of the two formulas provides a rich, stable media with improved performance over the individual formulations. The antimicrobics of Columbia CNA combined with phenylethanol provide inhibition of swarming *Proteus* and *Pseudomonas* spp.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic and Enzymatic Digests of Casein	25.0gm

Casein Yeast Peptone	10.0gm
Sodium Chloride	10.0gm
Papaic Digest of Soybean Meal	5.0gm
Tryptic Digest of Beef Heart	3.0gm
Phenylethanol	2.5gm
Corn Starch	1.0gm
Colistin Sulfate	8.25mg
Nalidixic Acid	4.0mg
Sheep Blood	50.0ml
Agar	30.0gm

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: Consult listed references for information on specimen collection.[1,2,4,6] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to

be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Method of Use: Allow the plates to warm to room temperature, and the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates at 35-37ºC for 24-48 hours in an aerobic atmosphere supplemented with 5-10% CO_2. Examine for typical colonial morphology and characteristics.

INTERPRETATION OF RESULTS

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1,2,4,6]

LIMITATIONS

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.

Atypical hemolytic reactions may occur with *Streptococcus* spp., therefore, determination of hemolysis is not recommended. It is recommended that the organism be subcultured to a Blood Agar Plate (Cat. no. A10) to confirm hemolysis.

Some gram-positive organisms may be inhibited by phenylethyl alcohol; additional incubation time may be warranted.

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, swabs, applicator sticks, other culture media, 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	
Streptococcus pyogenes ATCC® 19615	A	24hr	35°C	CO_2**	Growth; beta-hemolysis may appear atypical
Streptococcus pneumoniae ATCC® 6305	A	24hr	35°C	CO_2**	Growth; alpha-hemolysis may appear atypical
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	CO_2**	Growth
Proteus mirabilis ATCC® 12453	B	24hr	35°C	CO_2**	Partial to complete inhibition
Pseudomonas aeruginosa ATCC® 27853	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.

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:

Rose Agar should appear opaque, and cherry red in color.

Streptococcus pyogenes (ATCC® 19615) colonies growing on Rose Agar (Cat. no. A66). Showing beta-hemolysis. Incubated in CO_2 for 24 hours at 35ºC.

Streptococcus pneumoniae (ATCC® 6305) colonies growing on Rose Agar (Cat. no. A66). Showing alpha-hemolysis. Incubated in CO_2 for 24 hours at 35ºC.

Staphylococcus aureus (ATCC® 25923) colonies growing on Rose Agar (Cat. no. A66). Incubated in CO_2 for 24 hours at 35ºC.

Proteus mirabilis (ATCC® 12453) growth inhibited on Rose Agar (Cat. no. A66). Incubated in CO_2 for 24 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. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. Ellner, et al. *Am. J. Clin. Path.*; 45:502.

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

5. MacFaddin, J.F. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria,* Vol. I. Williams & Wilkins, Baltimore, MD.

6. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

8. Brewer, J.H., and B.D. Lilley. Presented before a meeting of the Maryland Association of Medical and Public Health Laboratories, Dec. 2, 1949.

9. Lilley and Brewer. 1953. *J. Am. Pharm. Assoc.*; 42:6.

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

HDQA 2207F [D]