IFU

Instructions for Use

RTF AGAR (MODIFIED CASMAN)

Cat. no. A68	RTF Agar (Modified Casman), 15x100mm Plate, 17ml	10 plates/bag

INTENDED USE

Hardy Diagnostics RTF Agar is recommended for the cultivation and differentiation of fastidious microorganisms, especially *Haemophilus* spp. by hemolytic reaction.

SUMMARY

Casman, in 1947, developed a medium which allowed for the cultivation of fastidious microorganisms but did not require the use of fresh meat infusion in the basal medium.[6]

The basal medium is composed of beef and yeast extracts and peptones. Beef extract replaces the infusion from fresh meat which enhances pathogenic cocci development.[7,8] Along with peptones, beef extract also provides amino acids and other complex nitrogenous nutrients. Yeast extract serves as a source of the B-complex vitamins. Dextrose, corn starch and purified agar are incorporated into the medium. Dextrose enhances the development of pathogenic cocci. Corn starch and purified agar allow the growth of *Neisseria gonorrhoeae* without interfering with hemolytic reactions.

RTF Casman is an excellent substitute for Rabbit and/or Horse Blood Agar. In comparison, the sheep blood in RTF has a longer shelf life and is less prone to spontaneous hemolysis and contamination.

The medium is supplemented with sheep blood in order to supply hemin (X-factor), and nicotinamide adenine dinucleotide (NAD or V-factor) which are growth factors required by *Haemophilus influenzae* . Nicotinamide (also known as niacinamide or nicotinic acid amide) is added to retard the nucleotidase of sheep blood erythrocytes that destroys the V-factor.[8]

RTF Casman Agar is also useful in identifying the various species of *Haemophilus* by pattern of hemolysis. *H. influenzae* and *H. parainfluenzae* will grow but will not exhibit beta-hemolysis. *H. hemolyticus* and *H. parahemolyticus* will also grow and will show a zone of beta-hemolysis.

FORMULA

Ingredients per liter of deionized water:*

Yeast Extract	10.0gm
Casein Peptone	5.0gm
Meat Peptone	5.0gm
Sodium Chloride	5.0gm
Beef Extract	3.0gm

Corn Starch	1.0gm
Niacinamide	0.5gm
Dextrose	0.5gm
Sheep Blood	50.0ml
Agar	14.0gm

Final pH 7.3 +/- 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 there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Using aseptic technique, inoculate medium then streak to obtain isolated colonies. Incubate the plate in 5-10% CO_2 at 35-37ºC. for 18-24 hours. Examine plates for typical colonial growth and hemolytic reactions.

INTERPRETATION OF RESULTS

Observe for typical colonial growth and morphology. Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1-4]

Colonies of *Haemophilus influenzae* appear colorless to gray, are transparent and moist, and produce a characteristic "mousy" odor. *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus* are similar in appearance to *H. influenzae* except that the colonies are surrounded by a zone of beta-hemolysis. Colonies of *Neisseria gonorrhoeae* appear colorless to grayish-white, are small, translucent, raised, and moist.

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.

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	
RTF Agar:					
Haemophilus influenzae ATCC® 10211	A	24-48hr	35°C	CO_2**	Growth with no hemolysis
Haemophilus parahaemolyticus ATCC® 10014	A	24-48hr	35°C	CO_2**	Growth with beta-hemolysis

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

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

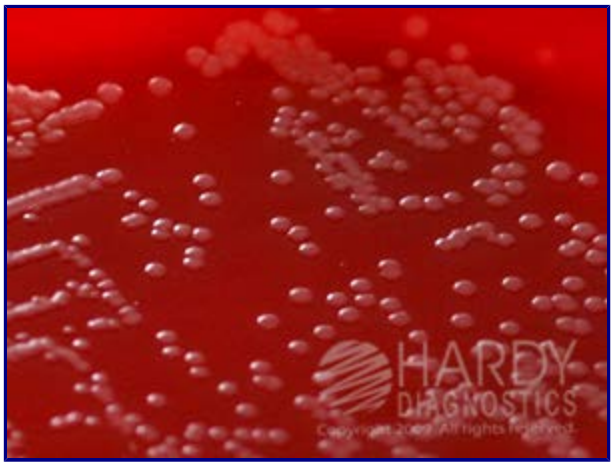
USER QUALITY CONTROL

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.

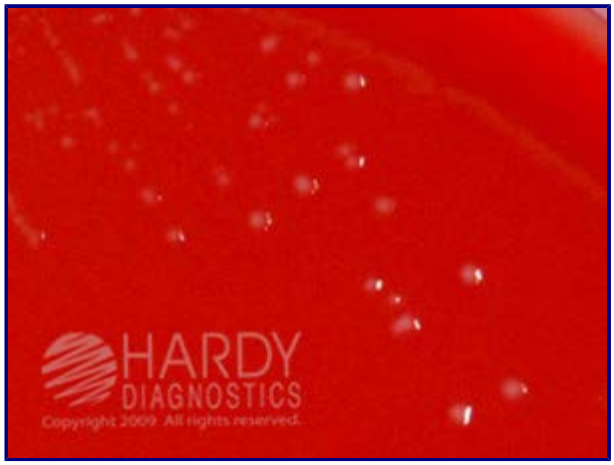
PHYSICAL APPEARANCE

RTF Casman (Modified) should appear opaque, and cherry red in color.

Haemophilus influenzae (ATCC® 10211) colonies growing on RTF Agar (Cat. no. A68). Incubated in CO_2 for 48 hours at 35ºC.



Haemophilus parahaemolyticus (ATCC® 10014) colonies growing on RTF Agar (Cat. no. A68). Incubated in CO_2 for 48 hours at 35ºC.



Haemophilus influenzae (ATCC® 10211) colonies growing on RTF Agar (Cat. no. A68). Plate shown against backlight to demonstrate lack of beta-hemolysis. Incubated in CO_2 for 48 hours at 35ºC.

Haemophilus parahaemolyticus (ATCC® 10014) colonies growing on RTF Agar (Cat. no. A68). Plate shown against backlight to demonstrate beta-hemolysis. Incubated in CO_2 for 48 hours at 35ºC.

Uninoculated plate of RTF Agar (Cat. no. A68).

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

6. Casman. 1947. *Am. J. Clin. Pathol.*; 17:281.

7. Casman. 1942. *J. Bacteriol.*; 43:33.

8. Casman. 1947. *J. Bacteriol.*; 53:561.

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

HDQA 2207F [D]