remel

ARGININE BROTH (Liquid and Lyophilized)

INTENDED USE

Remel Arginine Broth is a liquid medium recommended for use in qualitative procedures for the cultivation and presumptive identification of *Mycoplasma hominis.*

SUMMARY AND EXPLANATION

Dienes and Edsall first encountered *Mycoplasma* when doing a study on *Streptobacillus moniliformis*.[1] Additional studies suggested "pleuropneumonia-like organisms" (PPLO) could be recovered from human genitalia in both sexes. In 1947, Edward devised a *Mycoplasma* medium which consisted of beef heart infusion, peptone, yeast extract, and serum.[2] Velleca, Bird, and Forrester at the Centers for Disease Control and Prevention recommended the use of a mycoplasma broth with the addition of arginine for the isolation of *M. hominis.*[3]

PRINCIPLE

M. hominis metabolizes arginine with subsequent release of ammonia into the broth medium. This results in an alkaline shift of the medium, as indicated by a color change in the phenol red indicator from salmon to red.

REAGENTS (CLASSICAL FORMULA)*

Beef Heart Infusion	35.0	g
Yeast Extract	25.0	g
Peptone	7.0	g
Sodium Chloride	3.5	g
Erythromycin	100.0	mg
Phenol Red	20.0	mg
Polymixin B	6.5	mg
Amphotericin B	5.0	mg
Horse Serum	200.0	ml
L-Arginine	6.6	ml
Penicillin	500,000	U
Demineralized Water	700.0	ml

pH 7.0 ±.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of contamination, (2) the color has changed, (3) the expiration date has passed, or (4) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Acceptable specimens for testing in Arginine Broth include sterile body fluids, urine, blood, wounds, tissue (lung, lymph node, placenta, endometrial, autopsy), respiratory (throat swabs, sputum, bronchial washing, tracheobronchial secretions, bronchoalveolar lavage, nasopharyngeal), and urogenital (vaginal, cervical, urethral) swabs or secretions. Any swab specimen for *Mycoplasma* should be transported to the laboratory within one (1) hour of collection or placed in appropriate transport media. Swab specimens should only be collected with calcium alginate- or polyester-tipped swabs on plastic or wire shafts. Cotton swabs or wooden shafts should not be used. Mycoplasmas are extremely sensitive to adverse environmental conditions, particularly drying, osmotic changes, toxic metabolites, and temperature fluctuations. Specimens should be refrigerated at 4°C until transported to the laboratory, if delivery can be achieved within 6-12 hours. Otherwise, immediately freeze at -70°C and place on dry ice for transport. Do not store at -20°C for even short periods, as this will result in loss of viability. Do not freeze blood.[4,5]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) A-7 Agar (REF R20201), PPLO Agar (REF R20260), or alternative supplemental media, (5) Quality control organisms, (6) Sterile demineralized water, (7) Stereomicroscope, (8) Shrink seals (REF R522600), gas permeable strips.

PROCEDURE

Reagent Preparation:

- Arginine Broth, lyophilized, (REF R20358) is reconstituted by adding 1.8 ml of sterile demineralized water to each vial and mixing to dissolve.
- Arginine Broth, liquid, (REF R20002 and R20004) is supplied ready for use and does not require reconstitution.

Specimen Preparation

1. Body Fluids:
   a. Concentrate fluids by centrifugation at 1500 rpm for 15 minutes.
   b. Transfer 0.1-0.2 ml of sample concentrate into Arginine Broth using a sterile pipette.
   c. If centrifugation is not possible, inoculate fluid into broth in a 1:10 ratio.
   d. Sputum may be inoculated directly into broth.
2. Tissue:
   a. Mince tissue into fragments using a sterile scalpel. Avoid grinding as it tends to pulverize tissue, releasing growth inhibitors.
   b. Place minced tissue directly into Arginine Broth.
3. Blood:
   a. Collect blood free of anticoagulants.
   b. Immediately inoculate into Arginine Broth in a 1:5 to 1:10 ratio (preferably 5-10 ml for adults).
4. Swab Specimens:
   a. Place swab in Arginine Broth and swirl.
   b. Express excess liquid by pressing swab against the inside of the tube.
   c. Discard the swab.

Inoculation

1. For optimal recovery, serially dilute the sample in Arginine Broth to at least 10^{-3}, preferably 10^{-5} (for example, 0.2 ml of sample in 1.8 ml of broth), to overcome potential inhibitory substances and facilitate quantitation.
2. Inoculate an aliquot (0.2 ml) of the original sample transported and each broth dilution onto plated media, such as A7 Agar or PPLO Agar.
3. If possible, freeze remainder of original sample at -70°C for future confirmation.

Incubation

1. Broth media:
   a. Incubate all broth dilution tubes in ambient air at 35-37°C.
   b. Closely monitor the broth (2-3 times daily) for a color change to pink.
   c. Discard broth cultures not showing a color change after 8 days of incubation.
   d. Subculture 0.1-0.2 ml from positive broth cultures to plated media (A7 Agar or PPLO Agar).
   e. If possible, freeze positive broth cultures at -70°C immediately after subculture for future reference.

2. Plated media:
   a. Seal plates to prevent dehydration and incubate in 5% CO_2 at 35-37°C.
   b. Examine plates for growth through the bottom using a stereomicroscope at 20-60 X every 1-3 days.
   c. Incubate for 7-10 days before discarding as negative.

INTERPRETATION

Positive - Color change to pink with recovery on solid media
Negative - No color change

QUALITY CONTROL

All lot numbers of Arginine Broth have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Mycoplasma hominis ATCC® 23114	Ambient, up to 72 h @ 35-37°C	Pink color, growth on subculture

LIMITATIONS

1. Blood culture medium, if used, should not contain sodium polyanethol sulphonate (SPS).[6]
2. Arginine Broth should not be incubated in CO_2, as it contains phenol red indicator.
3. Arginine Broth is not totally selective for *M. hominis.*
4. Arginine Broth should be subcultured before the pH becomes too alkaline, preferably as soon as a color change is detected.[3]
5. Subculture to plated media followed by growth of typical colonies is required to confirm the isolation of *M. hominis.*[3]

BIBLIOGRAPHY

1. Tully, J.G. and R.F. Whitcomb. 1979. The Mycoplasmas. Vol. 2. Academic Press, Inc., New York, NY.
2. Razin, S. and J.G. Tully. 1983. Methods in Mycoplasmology. Vol. 1. Academic Press, Inc., New York, NY.
3. Velleca, W.M., B.R. Bird, and F.T. Forrester. 1980. Laboratory Diagnosis of Mycoplasma Infections. Centers for Disease Control and Prevention, Atlanta, GA.
4. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed. Vol. 1. ASM Press, Washington, D.C.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
6. Waites, K.B., C.M. Bebear, J.A. Robertson, D.F. Talkington, and G.E. Kenny. 2001. Cumitech 34, Laboratory Diagnosis of Mycoplasmal Infections. Coordinating ed., F.S. Nolte. ASM, Washington, D.C.

PACKAGING

REF R20358 (lyophilized), 1.8 ml/Vial 6/Pk
REF R20002 (liquid), 1.8 ml/Vial 100/Pk
REF R20004 (liquid), 250 ml ..... Ea

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128