remel

10B ARGININE BROTH

INTENDED USE

Remel 10B Arginine Broth is a liquid transport and growth medium recommended for use in qualitative procedures for the cultivation of *Ureaplasma urealyticum* and *Mycoplasma hominis.*

SUMMARY AND EXPLANATION

Mycoplasmas, members of the class *Mollicutes*, are much smaller than most bacteria and are distinguished by the lack of a cell wall. Though most are considered normal commensal flora, a few have become well-established pathogens. *U. urealyticum* and *M. hominis* are primarily associated with genital tract colonization and disease in adults and respiratory tract colonization and disease in newborns.[1,2] Though controversial, these organisms have been associated with endometritis, chorioamnionitis, premature rupture of membranes, stillbirth, premature birth, low birth weight, post-partum infections, and infertility. Of particular concern is the causal relationship between central nervous system (CNS) infections in the premature newborn and *U. urealyticum*. The serious complications of disease associated with *Ureaplasma* and *Mycoplasma* infection have led to an increased demand for laboratory identification of these organisms. The extremely small amount of genetic material possessed by these organisms makes them demanding in their nutritional requirements for cultivation. Shepard and Lunceford developed a standard broth medium for the general cultivation of *U. urealyticum* which became known as 10B.[3] This formula has been further modified with the addition of arginine, cefoperazone, and deoxyribonucleic acid (DNA) to become 10B Arginine Broth for the simultaneous detection of *U. urealyticum* and *M. hominis.*[4]

PRINCIPLE

This medium contains beef heart infusion and peptone, which supply nitrogenous substances and amino acids essential for growth. Sodium chloride maintains osmotic equilibrium. Horse serum is added as a source of protein. Yeast extract supplies B-complex vitamins and serves with DNA as a growth enhancer. GCHI Enrichment is a defined supplement, which provides vitamins, amino acids, coenzymes, dextrose, and ferric ions, which promote growth. L-cysteine hydrochloride is a reducing agent to minimize inhibitory substances. Cefoperazone is a third generation cephalosporin, which is generally active against most gram-negative bacilli and streptococci. *U. urealyticum* hydrolyzes urea with the subsequent release of ammonia causing the phenol red indicator to change from yellow to pink as a result of an alkaline shift. *M. hominis* hydrolyzes arginine by deamination to citrulline and then to ornithine and carbamoyl phosphate, which is hydrolyzed to carbon dioxide and ammonia with the production of ATP. This results in an alkaline shift from yellow to pink in arginine positive mycoplasma cultures.

REAGENTS (CLASSICAL FORMULAE)*

Beef Heart Infusion	50.0 g
Peptone	10.0 g
Sodium Chloride	5.0 g
L-Arginine	2.0 g
Deoxyribonucleic Acid (DNA)	0.2 g
Horse Serum	200.0 ml
Yeast Extract 25%	100.0 ml
•GCHI Enrichment	5.0 ml
Urea Solution 10%	4.0 ml
L-Cysteine Hydrochloride 4%	2.5 ml
Cefoperazone 2%	1.0 ml
Phenol Red 1%	1.0 ml
Demineralized Water	700.0 ml

pH 6.0 ± 0.2 @ 25°C

•GCHI Enrichment

Dextrose	100.0 g
Cysteine Hydrochloride	25.9 g
L-Glutamine	10.0 g
L-Cystine	1.1 g
Adenine	1.0 g
NAD	0.25 g
Cocarboxylase	0.1 g
Guanine Hydrochloride	30.0 mg
Ferric Nitrate	20.0 mg
ρ-Aminobenzoic Acid	13.0 mg
Vitamin B12	10.0 mg
Thiamine Hydrochloride	3.0 mg
Demineralized Water	1000.0 ml

*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

This product is ready for use and no further preparation is necessary. 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

Specimens for transport and culture in 10B Arginine Broth may include sterile body fluids, urine, blood, wounds, tissue (lung, lymph node, placenta, endometrial, autopsy), respiratory (sputum, bronchial washing, tracheobronchial secretions, bronchoalveolar lavage, nasopharyngeal or throat swabs), and urogenital (vaginal, cervical, urethral swabs or secretions). Ureaplasmas and mycoplasmas are extremely sensitive to adverse environmental conditions, particularly drying, osmotic changes, toxic metabolites, and temperature fluctuations. Specimens should be transported to the laboratory within 6-12 hours of collection or refrigerated at 4°C. For longer storage, freeze specimens at -70°C and place on dry ice for transport. Do not freeze blood. Swab specimens should only be taken with calcium-alginate, or polyester-tipped swabs on plastic or wire shafts. Cotton swabs or wooden shafts should not be used.[6]

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), A-8 Agar (REF R20205), or alternative supplemental media, (5) Quality control organisms, (6) Centrifuge, (7) Sterile scalpel, pipettes, (8) Sterile demineralized water, (9) Stereomicroscope, (10) Shrink seals (REF R522600), gas permeable strips.

PROCEDURE

Specimen Preparation

1. Body Fluids:
   a. Concentrate body fluids by centrifugation at 1500 rpm for 15 minutes.
   b. Inoculate 0.1-0.2 ml of sample concentrate into 10B 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 the 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 10B Arginine Broth.
3. Blood:
   a. Collect blood free of anticoagulants.
   b. Immediately inoculate into broth in a 1:5 to 1:10 ratio (preferably 5-10 ml for adults).
4. Swab Specimens:
   a. Place swab in broth and swirl.
   b. Express excess liquid by pressing swab against the inside of the tube.
   c. Discard the swab.
   d. Vortex prior to processing.

Inoculation

1. For optimal recovery, serially dilute the sample in 10B Arginine Broth to at least 10^{-3} (i.e., 0.2 ml of sample in 1.8 ml of broth), preferably 10^{-5}, 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 A8 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 or A8 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 10B 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
Ureaplasma urealyticum ATCC® 27618	Ambient, up to 24 h @ 35-37°C	Pink color; growth on subculture

LIMITATIONS

1. *U. urealyticum* is susceptible to a rapid, steep death phase in culture primarily due to urea depletion and elevated pH. Broth cultures showing color changes should be subcultured to fresh broth or agar immediately.[2]
2. Blood should be collected without an anticoagulant. If collected and placed into a blood culture medium, it is imperative that the medium does not contain sodium polyanethol sulphonate (SPS).[5]
3. 10B Arginine Broth should not be incubated in CO_2 as it contains phenol red indicator.
4. Other *Mycoplasma* spp., though infrequently isolated and with questionable pathogenicity, may hydrolyze arginine (*M. buccale, M. faucium, M. lipophilum, M. orale, M. primatum, M. fermentans*, *M. salivarium,* and *M. penetrans*) making further identification necessary.[6]
5. False-positive reactions may occur due to alkaline byproducts of the medium. They are generally produced by filamentous fungi and *Candida* spp. Subcultures to plated media are recommended to confirm growth.[7]

EXPECTED VALUES

ORGANISM	ARGININE	UREA
Mycoplasma hominis	+	-
Mycoplasma orale	+	-
Mycoplasma salivarium	+	-
Ureaplasma urealyticum	-	+

PERFORMANCE CHARACTERISTICS

An in house study was conducted using 23 strains derived from the American Type Culture Collection (ATCC) (8 strains of *M. hominis* and 15 strains of *U. urealyticum*). All serotypes were represented in the study. All organisms were recovered from 10B Arginine after 12 hours at room temperature and after 24 and 72 hours at -70°C.

BIBLIOGRAPHY

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PACKAGING

REF R20305, 10B Arginine Broth, 1.8 ml/Vial 100/Cs

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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IFU 20305-PI, Revised January 13, 2011 Printed in U.S.A.

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