remel

10B BROTH
(Liquid and Lyophilized)

INTENDED USE
Remel 10B Broth is a liquid transport and growth medium recommended for use in qualitative procedures for specimen transport, cultivation, and presumptive identification of *Ureaplasma urealyticum.*

SUMMARY AND EXPLANATION
U. urealyticum has been implicated in nonspecific urethritis, reproductive failure, and infertility.[1,2] Although urine is a good source of this organism, unfrozen specimens quickly become heavily contaminated if delayed in transit and freezing urine has not been effective for preserving *U. urealyticum*.[3] Shepard et al. developed the urease color test fluid (U-9B) to transport and presumptively identify *U. urealyticum*.[4] Shepard further modified the U-9 test broth with the addition of GHL tripeptide as a growth supplement and designated this broth U9C. This broth has since been modified to develop a standard fluid medium (10B) used for the general cultivation of *U. urealyticum* from clinical material.[5]

PRINCIPLE
U. urealyticum possesses an enzyme capable of hydrolyzing urea with the consequent production of ammonia. 10B Broth contains heart infusion, peptone, yeast extract and horse serum to supply nutrients required for the growth of *Ureaplasma*. GCHI Enrichment, a defined supplement, is added to promote growth. Penicillin is incorporated in the medium to inhibit contaminating bacteria. *U. urealyticum* hydrolyzes urea with the subsequent release of ammonia into the broth medium. This process causes an increase in pH, which changes the color of the test fluid to pink.

REAGENTS (CLASSICAL FORMULA)*

Beef Heart, Infusion from	50.0	g
Peptone	10.0	g
Sodium Chloride	5.0	g
Horse Serum	200.0	ml
Yeast Extract 25%	100.0	ml
Penicillin (100,000 U/ml)	10.0	ml
•GCHI Enrichment	5.0	ml
Urea Solution 10%	4.0	ml
L-Cysteine Hydrochloride 4%	2.5	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
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 in 10B Broth may 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. Swab specimens should be transported to the laboratory within one (1) hour of collection or transported in 10B Broth. Use only swabs with calcium-alginate or polyester tips on plastic or wire shafts. *U. urealyticum* is 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. If not, 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.[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) Sterile demineralized water, (7) Stereomicroscope, (8) Shrink seals (REF R522600), gas permeable strips.

PROCEDURE
Reagent Preparation:
- 10B Broth, lyophilized, (REF R20302) is reconstituted by adding 1.8 ml of sterile demineralized water to each vial and mixing to dissolve.
- Liquid 10B Broth (REF R20304 and R20128) is supplied ready for use and does not require reconstitution.

Specimen Preparation[6]
1. Body Fluids:
   a. Concentrate fluids by centrifugation at 1500 rpm for 15 minutes.
   b. Inoculate 0.1-0.2 ml of sample concentrate into 10B 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 10B 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 10B 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[6]
1. For optimal recovery, serially dilute the sample in 10B 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 or A8 Agar.
3. If possible, freeze remainder of original sample at -70°C for future confirmation.

Incubation[7]

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 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 daily, through the bottom, using a stereomicroscope at 20-60 X.
   c. Incubate for up to 72 hours before discarding as negative.

QUALITY CONTROL

All lot numbers of 10B 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
Ureaplasma urealyticum ATCC® 27618	CO_2, up to 72 h @ 35-37°C	Pink color, good recovery on subculture
Staphylococcus aureus ATCC® 25923	CO_2, up to 72 h @ 35-37°C	Inhibition, marked to complete

LIMITATIONS

1. This broth is not totally selective for *Ureaplasma.*
2. On rare occasion, *U. urealyticum* will produce delayed positive urease reactions, taking up to 3-5 days.[7]
3. *U. urealyticum* is susceptible to a rapid, steep death phase in culture primarily due to urea depletion and elevated pH. To maintain viability of potential isolates, subculture to appropriate solid media or fresh 10B Broth just as pH indicator begins to change color.[4]
4. Blood culture medium, if used, should not contain sodium polyanethol sulphonate (SPS).[1]
5. 10B Broth should not be incubated in CO_2 as it contains phenol red indicator.
6. False positive reactions may occur due to alkaline by-products of the medium and are generally produced by filamentous fungi and *Candida* spp. Subcultures to plated media are required to confirm growth.[8]

BIBLIOGRAPHY

1. 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.
2. Weissfeld, A.S. 1983. Clin. Microbiol. Newsl. 5:35-37.
3. Kundsin, R.B. and S.G. Driscoll. 1970. Ann. N.Y. Acad. Sci. 174:794-797.
4. Shepard, M.C. and C.D. Lunceford. 1970. Appl. Microbiol. 20:539-543.
5. Shepard, M.C. and C.D. Lunceford. 1978. J. Clin. Microbiol. 8:566-574.
6. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington DC.
7. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams and Wilkins, Baltimore, MD.
8. Razin, S. and J.G. Tully. 1983. Methods in Mycoplasmology. Vol. 1. Academic Press, Inc., New York, NY.

PACKAGING

REF R20302 10B Broth (lyophilized), 1.8 ml/Vial.....6/Pk
REF R20304 10B Broth (liquid), 1.8 ml/Vial 100/Cs
REF R20128 10B Broth (liquid), 250 ml/Bottle ..... 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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