Clinical Laboratory Department POLICY AND PROCEDURE

POLICY NUMBER: 1211
VERSION: 3

SUBJECT: Use of Routine Media for the Recovery and Isolation of Bacteria

Media Routinely Used:

Enrichment and Growth Media

BAP (Blood Agar Plate) with 5% sterile defibrinated sheep's blood. 5%Blood agar is an all-purpose substrate for the isolation and cultivation of many types of microorganisms, including streptococci, pneumococci, staphylococci and other pathogenic microorganisms from clinical specimens. Following inoculation and incubation of the plates, bacterial colonies are examined for colonial morphology and hemolysis of sheep red blood cells.

CHOC (Chocolate agar plate and slant). Chocolatized blood agar (enriched) media is used for the isolation and cultivation of pathogenic and nonpathogenic fastidious microorganisms from clinical specimens. Chocolate agar is growth medium agar base to which has been added hemoglobin and chemically defined enrichment supplement. This enriched media will support the growth of *Neisseria*, *Hemophilus* and other fastidious bacteria.

DIAMOND'S Media is a transport used to sustain the viability of *Trichomonas* and yeast.

LIM Broth. A Todd-Hewitt broth with Colistin and Nalidixic Acid added to inhibit Gram negative microorganisms, and Peptone and yeast extract to enhance the growth of Beta-Streptococcus Group B.

Selective Media

ML (Martin Lewis Agar with Lincomycin or Modified Thayer Martin). This is an enriched chocolate agar recommended for the isolation and cultivation of gonococci and meningococci from specimens with mixed flora. Antibiotics in the agar reduce the growth of saprophytic organisms, including *Candida* sp., which may be present in clinical specimens. Trimethoprim lactate is included to reduce the growth of *Proteus* sp., infamous for its tendency to swarm over growth on non-selective chocolate and blood agar plates.

MAC (MacConkey Agar Plate) is a differential plating medium for detection of enterobacteriaceae and other pathogenic and non-pathogenic Gram negative bacilli. Lactose fermenting organisms produce colonies that appear pink to deep red, while non-lactose-fermenting colonies are transparent and colorless. Selective inhibition of Gram positive bacilli is due to the presence of bile salts and crystal violet in the media.

GRANADA medium is used for the primary isolation and detection of group B Streptococcus. Production of an orange carotenoid pigment is unique to beta hemolytic group B Streptococcus.

Miscellaneous Media

TSB (Tryptic Soy Broth) with 15% Glycerol is used in freezing stock cultures. The glycerol acts as a preservative and prevents ice crystals from forming which can cause autolysis.

SAB DEX agar (Sabaroud Dextrose) plates and bottles are used for the recovery of fungi and yeast.

INHIBITORY MOLD Agar is used for the recovery of fungi and yeast. Antibiotics are added to discourage the growth of contaminants and saprophytes.

CHROMAGAR CANDIDA is used for the isolation and identification of yeasts. Contains chloramphenicol to inhibit bacteria. *C. albicans* colonies are green, *C. krusei* turn pink, *C. tropicalis* are blue. Ideal for detecting mixed cultures of yeasts.

Routine Incubation of Media

CO_2 enriched atmosphere, 35°C. CO_2 incubator, with a backup CO2 generating system as a backup if the percentage of CO_2 falls below 3%. All media intended to grow CO_2 dependent organisms must be incubated in a CO_2 enriched atmosphere. This includes most enriched agar plates (those with obvious blood enrichment such as BAP and CHOC) and those selective media designed to recover fastidious species such as *Neisseria* and *Hemophilus*. Biochemical media and susceptibility testing must not be incubated in a CO_2 enriched atmosphere.

Plates generally incubated in the CO_2 incubator are:

- BAP
- CHOC
- ML

Room air, 35°C. Gram negative selective media and most tubed media are routinely incubated in room air at 35°C.

- MAC
- LIM Broth
- DIAMOND'S media

Anaerobic Media, 35°C using the anaerobic atmosphere generating jar system placed in the room air incubator, or using anaerobic biobags.

- Granada plates

30°C Incubator, room air

ChromAgar Candida
SabDex Agar Plates

Room Temperature, Room air (25°C)

Inhibitory Mold Agar Bottles
Sabdex Bottles

References:

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Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., *Manual of Clinical Microbiology, 6th Edition*. American Society of Microbiology, Washington, D.C., 1995.

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Sutter, V.; Citron,D.; Finegold,S. Anaerobic Bacteriology Manual, 3rd Editon. C.V. Mosby Co. 1980

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Date: 05/04/2017	**Original Date:** 07/22/1988
Reviewed: 05/04/2017	**Next Review Date:** 05/04/2018
Revised:	8/21/95cw, 12/27/00cw,5/3/03 removed TSA, XV, incubation error,RW, 9/3/03 changed HDH to HDHS DB, 5/7/04 add MRSA agar, update anaerobe condition list, 30°C, room air list JH, 6/24/04 remove Strep B broth, added LIM broth, remove biphasic BC bottle ,jh, rw, 12/2/05 Add urea for yeast @ 30 & rm air jh, 4/8/08 reformat, rem CDCana, add brucella, rem Todd-Hewitt, added CO2 biobag LG , 5/17/10 jh-removed unused media, 5/3/13 jh removed unused media, 3/30/17jh removed BHI Broth & BHIA slants & changed approver to Dr.Lee
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