HealthLink®
800-638-2625

Product No. 1405
Revision No. 01

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

MacCONKEY / MANNITOL SALT / CHOCOLATE / TSA 5% AGAR QUAD PLATE

INTENDED USE

The MacConkey / Mannitol Salt / Chocolate / TSA 5% Agar Quad Plate is a four-chambered plate containing media designed to assist in the isolation and presumptive identification of bacterial upper respiratory tract (throat/pharyngeal) pathogens.

SUMMARY AND EXPLANATION

MacConkey Agar: Since this bile salt, neutral red, and lactose agar was described by MacConkey in 1905, its formulation has been modified many times. The current formulation was designed to exhibit better differentiation of lactose fermenting versus non lactose fermenting organisms, enhance the growth of enteric pathogens, and improve the inhibition of swarming *Proteus* species.

Mannitol Salt Agar: In 1942, Koch reported that staphylococci were not inhibited by a 7.5% solution of sodium chloride. Chapman confirmed this finding and determined that the addition of 7.5% sodium chloride to phenol red mannitol agar produced a medium on which staphylococci that coagulated rabbit plasma produced colonies with yellow zones.

Chocolate Agar: Bacto GC Medium Base was designed in 1947. Christensen and Schoenlein demonstrated that this base, enriched with hemoglobin and supplements yielded accelerated early growth of *Neisseria gonorrhea*.[1] This medium was improved upon by substituting a chemical supplement for the yeast concentrate.[2] In addition to aiding the growth of gonococci, other fastidious organisms, e.g. *Haemophilus* species was also improved.

Trypticase Soy Agar: The nutritional composition of Trypticase Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. Trypticase Soy Agar with 5% or 10% sheep blood is used extensively for the cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiation characteristics for bacteria, especially *Streptococcus* species.

PRINCIPLES OF THE PROCEDURE

MacConkey Agar: The selective property of this medium is due to the addition of bile salts and crystal violet which inhibit the growth of gram positive organisms. Differentiation of lactose fermenting versus non lactose fermenting is achieved with the indicator neutral red. Colonies will appear either colorless or pink to red based on the organisms ability to ferment this carbohydrate.

Mannitol Salt Agar derives its nutritive qualities from the peptone and beef extract content in the formula. These components supply essential growth factors such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride either completely or partially inhibits the growth of organisms other than staphylococci. The presence of mannitol as a fermentable carbohydrate serves to differentiate *Staphylococci* species. Staphylococci that have the ability to ferment mannitol will produce colonies with yellow zones as indicated by the change in the phenol red indicator.

Chocolate agar contains a GC agar base, bovine hemoglobin, and a chemically defined enrichment. Casein and meat peptones contained in the GC agar base provide the nitrogenous elements, phosphate buffers maintain the pH and corn starch neutralizes any toxic fatty acids that may be present in the agar. Hemin (X factor) is provided by the bovine hemoglobin, necessary for growth of *Haemophilus* species. The chemically defined enrichment solution provides nicotinamide adenine dinucleotide/NAD (V factor), vitamins, amino acids, co-enzymes, dextrose, ferric ions and other factors for improved growth of *Neisseria* species.

The presence of casein and soy peptones in the Trypticase Soy Agar base makes this medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger chained peptides. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood, added to enrich the base medium, provides excellent growth and beta hemolysis of *Streptococcus pyogenes* in addition to appropriate hemolytic reactions of other organisms. It is a suitable medium for use with low concentration bacitracin discs (0.04 unit, Taxo® A) for presumptive identification of group A Streptococci, and for performing the CAMP test for presumptive identification of group B Streptococci (*S. agalactiae*).

TYPICAL FORMULA AND APPEARANCE

MacConkey Agar

Appearance =pinkish-purple, slightly opalescent
(Approximate formula* per liter of processed water)

Pancreatic Digest of Gelatin	17.0g
Pancreatic Digest of Casein	1.5
Peptic Digest of Animal Tissue	1.5
Lactose	10.0
Bile Salts	1.5
Sodium Chloride	5.0
Neutral Red	0.03
Crystal Violet	0.001
Agar	13.5

*adjusted and/or supplemented to meet performance criteria.

Mannitol Salt Agar

Appearance = pinkish red, slightly opalescent
(Approximate formula* per liter of processed water)

Beef Extract	1.0g
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Sodium Chloride	75.0
D-Mannitol	10.0
Phenol Red	0.025
Agar	15.0

*adjusted and/or supplemented to meet performance criteria.

Chocolate Agar

Appearance = opaque, chocolate brown
(Approximate formula* per liter of processed water)

Pancreatic Digest of Casein	7.5g
Selected Meat Peptone	5.0
Corn Starch	1.0
Dipotassium phosphate	4.0
Monopotassium phosphate	1.0
Sodium Chloride	5.0
Agar	12.0
Hemoglobin	10.0
Chocolate enrichment solution	10 ml

*adjusted and/or supplemented to meet performance criteria.

Trypticase Soy Agar
Appearance = opaque, cherry red
(Approximate formula* per liter of processed water)

Pancreatic Digest of Casein	14.5g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Agar	14.0
Defibrinated Sheep Blood	5%

*adjusted and/or supplemented to meet performance criteria.

PRECAUTIONS
This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria.

STORAGE/SHELF LIFE
Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used. Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

SPECIMEN COLLECTION
The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results. Sterile swabs and collection containers should be used. Specimens should be collected prior to the initiation of antimicrobial therapy.

A properly collected throat swab is essential. Under adequate lighting, depress the tongue and rub a sterile swab vigorously over each tonsillar area and posterior pharynx. Any exudate should be touched, and care should be taken to avoid areas containing large amounts of normal respiratory flora (i.e., tongue, uvula, cheeks, lips, etc.). Nasal or nasopharyngeal specimens are often submitted for culture but show poor correlation to sinus aspirates for the diagnosis of sinusitis. These types of specimens should not be submitted for diagnosis. Flushing infected sinuses with saline to promote proper drainage is sometimes performed. Any purulent material collected in this manner may be cultured but interpretation is difficult because this type of specimen is often contaminated with *S. aureus* from the anterior nares and normal flora of the nasopharynx.[2]

Plates should be inoculated promptly after specimen collection. If a delay in inoculation of more than 1-2 hours is unavoidable, transport medium such as Stuart's or Amies should be used. *Haemophilus influenzae* and other respiratory pathogens do not tolerate refrigerated conditions therefore storage of respiratory specimens at refrigerated temperatures is NOT recommended. Every effort should be made at prompt specimen inoculation. Extended delays in processing permit overgrowth of potential pathogens by normal respiratory flora.

Detailed information on proper specimen collection may be obtained from microbiology reference materials.

MATERIALS PROVIDED
Respiratory Quad Plates

MATERIALS REQUIRED BUT NOT PROVIDED
Mueller Hinton Agar Plates
1 CO_2 kit with 10 generators and bags
Taxo® A discs
Taxo® P discs
Incubator maintaining 33-37°C.
Antibiotic sensitivity disc cartridges
Disc dispenser (6 or 8 place)
Measuring device in millimeters(Zone Overlay)
Ancillary culture media, reagents and laboratory equipment as required.

PROCEDURE
DAY ONE
1. Remove the Quad Plate from refrigerated storage and allow to warm to room temperature. Agar surfaces should be free of excessive moisture which could cause confluent organism growth. Label plate with specimen identification number and inoculation date.

2. Roll the specimen swab, or swab immersed in purulent material, over the initial one third of each agar sector to ensure contact with all swab surfaces. Begin with Sector 4 followed by Sectors 3, 2, and 1 respectively.

Note: If additional test procedures are to be performed (i.e., Gram stain etc.), it is recommended that multiple specimen swabs be collected.

3. Using a plastic sterile disposable inoculating loop, streak in a zig-zag fashion over swabbed areas and continue through the full length of each sector for isolation of colonies. Avoid applying excess pressure to the agar surface during inoculation to prevent gouging and splitting of the agar medium. (See Diagram 1)

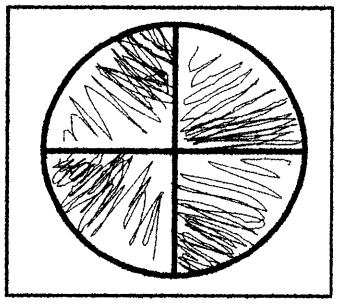
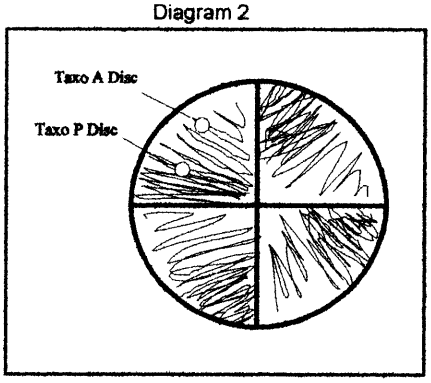


Diagram 1

4. If testing with differentiation discs (i.e., Taxo A® and Taxo P®) is desired on primary inoculum, aseptically place one of each disc on Sector 4 according to Diagram 2. Note: It is recommended that discs should be spaced no closer than 24mm apart from center to center.[3] (See also Section titled LIMITATIONS)

Diagram 2



5. Insert the inoculated plate and a CO_2 generator into a zip-lock bag. Seal the bag, crush the generator in an upright position and allow the liquid contained in the vial to contact the pellets. When effervescing has stopped, place the unopened zip-lock bag into the incubator media side up.

6. Incubate the plates at 33-37°C for 18 - 48 hours. Pathogens may be detected as early as 18 hours however, it is recommended that inoculated plates be incubated for a total of 48 hours before reporting a culture as "normal flora".

DAY TWO

1. Remove the plate from the incubator. (Note: The CO_2 atmosphere within the zip lock bag will dissipate upon opening the bag. Examination of culture results prior to detection of organism growth or 48 hours of incubation may be accomplished by; 1) examining the plate through the unopened bag or 2) using an additional CO_2 generator upon reincubating the opened and examined culture plate. (HealthLink® Catalog #3425)

2. Presumptively identify the suspected pathogenic organism(s) present by comparing the growth reactions of each chamber to the those listed in this product information and quality control sheet (see Expected Results section).

Interpretation of Taxo A® and Taxo P® Zones of Inhibition

3.1 Taxo A®: Observe Sector 4 for growth of beta hemolytic streptococci. Any zone of inhibition of beta hemolytic streptococci around the disc should be interpreted as positive.[4] Interpretation is as follows:

Zone present = "beta hemolytic *Streptococci*, presumptive group A by bacitracin"

Zone absent = "beta hemolytic *Streptococci* species, presumptively not group A by bacitracin.

3.2 Taxo P®: Observe Sector 4 for growth of alpha hemolytic streptococci. A zone of inhibition of alpha hemolytic streptococci around the disc of ≤ 14mm is interpreted as positive. A zone of 6-13mm is considered questionable and requires additional testing with supplemental materials.

Zone = ≤14mm: *S. pneumoniae* presumptive by optochin

Zone absent = alpha hemolytic streptococci, not pneumococci by optochin

ANTIBIOTIC SUSCEPTIBILITY PROCEDURE / INDIRECT DISC METHOD

Refer to the NCCLS document M2-A6, "*Performance Standards for Antimicrobial Disk Susceptibility Tests*" for detailed procedural instructions.[3]

Note$_1$: This procedure is applicable to common rapidly growing bacterial pathogens (example = *S. aureus, Enterobacteriaceae*, etc.). Fastidious organisms such as *S. pneumoniae* and *H. influenzae* require modifications of this procedure using supplemental media and reagents.

Note$_2$: This procedure is not routinely recommended for organisms such as *S. pyogenes* (Group A beta hemolytic Strep) which generally exhibit susceptibility to a highly effective drug (i.e., penicillin).

Note$_3$: Direct susceptibility testing is NOT recommended for respiratory specimens.

1. Select at least 4 - 5 well isolated colonies of the same morphological type from an agar Sector. Touch the top of each colony with an inoculation loop or needle and transfer to a tube containing approximately 5 ml of broth medium (example: trypticase soy broth.)

2. Incubate the broth culture at 35 - 37° C until it meets or just exceeds the turbidity of a 0.5 McFarland barium sulfate standard (approx. 2 - 8 hours).

3. Adjust the turbidity of the growing broth culture with sterile saline or broth to obtain a turbidity visually comparable to that of the 0.5 McFarland Standard. Tubes should be visually compared under adequate light against a white background with contrasting black lines.

4. Within 15 minutes after turbidity adjustment, dip a sterile swab into the suspension and rotate it firmly against the tube wall to express excess fluid.

5. Inoculate the surface of a Mueller Hinton (HealthLink® cat. no. 1103) plate by streaking the swab over the entire agar surface. Repeat this procedure two more times, rotating the plate approximately 60° each time to ensure even inoculum distribution. Note: Mueller Hinton plates should be free of excessive moisture prior to inoculation.

6. Place the impregnated antimicrobial discs on the surface of the agar plate using forceps or a disc dispenser. Gently press down each disc to ensure complete contact with the agar surface. Note: Discs should not be moved once in contact with the agar surface due to the instantaneous diffusion of some of the drug.

7. Invert the plates and place them in a 33-37°C incubator within 15 minutes after the discs are applied. Note: Plates should not be incubated in an atmosphere of increased carbon dioxide.

8. Examine plates after 16-18 hours of incubation by holding against a non-reflective black background illuminated from above. Measure the zones of complete inhibition, including the diameter of the disc, to the nearest whole millimeter. The endpoint should be taken as the area showing no obvious visible growth with the unaided eye. Do not include the area of faint growth of tiny colonies at the edge of the obvious zone of inhibition.

Note: A confluent "lawn" of growth should be achieved. If only

isolated colonies are present, the inoculum was too light and the test should be repeated.

9. Refer to the "Zone Diameter Interpretive Standards Table" contained within NCCLS document M2-A6 for expected values for testing common, rapidly growing pathogens.

EXPECTED RESULTS
NCCLS CONTROL ORGANISMS (ATCC STRAINS)*

Sector #1 - MacConkey Agar
***Escherichia coli* (25922)** - Growth, pink colonies
***Proteus mirabilis* (12453)** - Growth, colorless colonies, inhibition of swarming
***Pseudomonas aeruginosa* (27853)** - Growth, colorless colonies
***Enterococcus faecalis* (29212)** - Inhibition (partial)

Sector #2 - Mannitol Salt Agar
***Staphylococcus aureus* (25923)** - Growth, colonies have yellow zones at 48 hours
***Staphylococcus epidermidis* (12228)** - Growth, colonies are white, may have slight pink zones
***Proteus mirabilis* (12453)** - Inhibition (partial)

Sector #3 - Chocolate Agar
***Haemophilus influenzae* (10211)** - Growth, gray and moist with "mousey" odor
***Neisseria meningitidis* (13090)** - Inhibition
***Staphylococcus aureus* (25923)** - Inhibition (partial to complete)
***Neisseria gonorrhea* (43069)** - Growth, small, translucent , gray colonies.
***Streptococcus Pneumoniae* (6305)** - Growth

Sector #4 - TSA with 5% sheep blood
***Streptococcus pyogenes* (19615)** - Growth, beta hemolysis
***Streptococcus pneumoniae* (6305)** - Growth, alpha hemolysis
***Escherichia coli* (25922)** - Growth
***Staphylococcus aureus* (25923)** - Growth

HealthLink® conforms to all NCCLS recommendations for quality control of commercially prepared microbiological media where applicable.

LIMITATIONS
The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation, initiation of antiinfective therapy prior to specimen collection, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

Definitive identification of certain organisms requires additional testing which may include; Gram stain, oxidase, catalase, and other biochemical tests. Additional information on organism identification can be found in microbiology reference materials.[4,5,6]

Identification of *Haemophilus influenzae* from other species of *Haemophilus* requires additional testing with supplemental materials for determination of X and V factor requirements.

Interpretation of Taxo A® and Taxo P® tests for presumptive identification of *S. pyogenes* and *S. pneumoniae* are based on tests involving pure culture. Taxo A® discs placed on nonselective media inoculated directly with throat swabs presumptively identify between 50 - 97% of group A *Streptococci.*[4] Furthermore, many alpha hemolytic *Streptococci*, including *S. pneumoniae*, are susceptible to bacitracin.

Isolation of pathogenic or potentially pathogenic organisms does not necessarily indicate involvement in the disease process. These organisms may also be detected in the "carrier" state. Various respiratory pathogens (i.e. *S. pneumoniae, H. influenzae,* members of the *Enterobacteriaceae,* and *S. aureus*) may be transient or minor components of the oropharyngeal flora in healthy individuals without consequent development of infection. Because of this, the physician must often make diagnostic decisions on etiological suspicion in addition to culture results.[2]

REFERENCES

1. Chapin, K. C. , G.V. Doern. 1983. Selective Media for Recovery of *Haemophilus influenzae* from Specimens Contaminated with Upper Respiratory Tract Microbial Flora. J. of Clin. Microbiology . Vol 17/No. 6:1163-1165.

2. Bannatyne, R.M., C. Clausen, L.R. McCarthy. 1979. Cumitech 10. Laboratory Diagnosis of Upper Respiratory Tract Infections. Coordinating ed., I.B.R. Duncan. American Society for Microbiology, Washington, D.C.

3. National Committee for Clinical Laboratory Standards. 1997. Approved Standard: M2-A6. Performance Standards for Antimicrobial Disk Susceptibility Tests, 6th ed. National Committee for Clinical Laboratory Standards, Villanova, PA.

4. Lennette, E.H., ed. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

5. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby Company, St, Louis.

6. Koneman, E.S., S.D.Allen, V.R. Dowell, Jr. and H. M. Sommers. 1983. Color Atlas and Textbook of Microbiology, 2nd ed. J.B. Lippincot Company, Philadelphia.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLink® recommends that the following quality assurance and quality control procedures be performed on each batch of product.

QUALITY ASSURANCE
The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within the recommended range: 2-8°C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 22-35°C.

QUALITY CONTROL
The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory :

1. Inspect plates according to instructions contained on the "Quality Control Log Sheet." (See also STORAGE/SHELF LIFE)
2. Peel off the lower portion of a product bag label (Quality Control Certificate) for the lot being accepted into the laboratory and affix it to the Quality Control Log Sheet.

3. Initial and date the Quality Control Log Sheet.

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

TECHNICAL SERVICE

HealthLink® provides a toll free technical service line (1-800-638-2625) to assist with product usage. To receive additional product information, procedural instructions, QA/QC log sheets, Material Safety Data Sheets, or to have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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