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Plated Media

| BBL™ Product Name | Label Control Number | Revision Level | Revision Date |
|--|-----------------------------|-----------------------|----------------------|
| Bacteroides Bile Esculin Agar (BBE) | L007348 | Rev. 06 | October 2006 |
| BCYE Agar | L007349 | Rev. 07 | February 2007 |
| Bile Esculin Agar | L007350 | Rev. 10 | April 2006 |
| Brain Heart CC Agar with 10% Sheep Blood and Gentamicin (Deep Fill) | L008065 | Rev. 06 | August 2006 |
| Brain Heart Infusion Agar (Deep Fill) | L007351 | Rev. 05 | May 2006 |
| Brain Heart Infusion Agar with 10% Sheep Blood (Deep Fill) | L007352 | Rev. 06 | January 2006 |
| Brain Heart Infusion Agar with 10% Sheep Blood, Gentamicin and Chloramphenicol (Deep Fill) | L007353 | Rev. 08 | April 2006 |
| Brucella Agar with 5% Horse Blood | L007354 | Rev. 06 | March 2008 |
| Campy CVA Agar | L007355 | Rev. 05 | January 2006 |
| Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Blaser) | L007356 | Rev. 11 | September 2007 |
| CDC Anaerobe 5% Sheep Blood Agar | L007357 | Rev. 09 | April 2006 |
| CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV) | L007358 | Rev. 10 | February 2007 |
| CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA) | L007359 | Rev. 08 | December 2006 |
| CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) | L007360 | Rev. 10 | February 2007 |
| Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitaleX™) | L007361 | Rev. 08 | September 2007 |
| Chocolate II Agar with Bacitracin | L007362 | Rev. 04 | October 2006 |
| Chocolate II Agar with Pyridoxal | L007362 | Rev. 04 | October 2006 |
| Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitaleX™) and Martin-Lewis Agar - Bi-Plate | L007364 | Rev. 04 | September 2007 |
| Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitaleX™) & Modified Thayer-Martin (MTM II) Agar-Bi-Plate | L007365 | Rev. 08 | September 2007 |
| CHROMagar™ Candida | 8012620 | 01 | October 2008 |
| CHROMagar™ MRSA | 8012632 | 05 | October 2008 |

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| BBL™ Product Name | Label Control | | Revision Date |
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| | Number | Revision Level | |
| CHROMagar™ O157 | 8010757 | 01 | October 2008 |
| CHROMagar™ Orientation | 8011255 | 01 | October 2008 |
| CHROMagar™ Orientation/TSA II with 5% Sheep Blood | 8083714 | 01 | October 2008 |
| CHROMagar™ Salmonella | 8010557 | 01 | December 2008 |
| CHROMagar™ Staph aureus | 8010756 | 01 | November 2008 |
| CIN Agar | L007366 | Rev. 06 | October 2006 |
| CLED Agar | L007367 | Rev. 04 | May 2006 |
| Columbia Agar with 5% Sheep Blood | L007369 | Rev. 06 | August 2006 |
| Columbia CNA Agar with 5% Sheep Blood | L007370 | Rev. 08 | August 2006 |
| Columbia CNA Agar with 5% Sheep Blood and MacConkey II Agar- I Plate™ | L007371 | Rev. 08 | December 2006 |
| Corn Meal Agar with Polysorbate 80 (Deep Fill) | L007372 | Rev. 06 | August 2006 |
| DNase Test Agar with Toluidine Blue | L007373 | Rev. 05 | July 2006 |
| EMB Agar, Modified, Holt-Harris and Teague | L007374 | Rev. 08 | May 2006 |
| Endo Agar | L007375 | Rev. 08 | April 2006 |
| Enterococcosel™ Agar | L007376 | Rev. 08 | April 2006 |
| GC-Lect™ Agar | L007377 | Rev. 04 | September 2007 |
| GC-Lect™ Agar, JEMBEC™ | L007377 | Rev. 04 | September 2007 |
| Group A Selective Strep Agar with 5% Sheep Blood (ssA™) | L007378 | Rev. 07 | May 2006 |
| Group A Selective Strep Agar with 5% Sheep Blood (ssA™) & Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)-Bi-Plate | L007379 | Rev. 09 | December 2006 |
| Haemophilus Test Medium Agar | L007380 | Rev. 06 | February 2007 |
| HBT Bilayer Medium | L007381 | Rev. 04 | May 2006 |
| Hektoen Enteric Agar | L007382 | Rev. 08 | May 2006 |
| Hemo ID QUAD (with Growth Factors) | L007383 | Rev. 05 | December 2006 |
| Inhibitory Mold Agar (Deep Fill) | L007384 | Rev. 06 | May 2006 |
| Inhibitory Mold Agar with Chloramphenicol and Gentamicin (Deep Fill) | L007385 | Rev. 07 | September 2007 |
| Inhibitory Mold Agar with Gentamicin (Deep Fill) | L007385 | Rev. 07 | September 2007 |
| Lecithin Lactose Agar | L007386 | Rev. 06 | October 2006 |
| Levine EMB Agar | L007387 | Rev. 09 | October 2006 |
| MacConkey II Agar | L007388 | Rev. 08 | October 2006 |
| MacConkey II Agar with Sorbitol | 8807371 | Rev. 01 | October 2006 |
| Mannitol Salt Agar | L007389 | Rev. 07 | September 2007 |
| Martin-Lewis Agar | L007390 | Rev. 08 | September 2007 |
| Martin-Lewis Agar, Gono-Pak | L007390 | Rev. 08 | September 2007 |
| Martin-Lewis Agar, JEMBEC™ | L007390 | Rev. 08 | September 2007 |
| Middlebrook and Cohn 7H10 Agar (Deep Fill) | L007391 | Rev. 10 | September 2007 |
| Modified Thayer-Martin (MTM II) Agar | L007392 | Rev. 09 | September 2007 |

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| | Number | Revision Level | |
| Modified Thayer-Martin (MTM II) Agar, Gono-Pak | L007392 | Rev. 09 | September 2007 |
| Modified Thayer-Martin (MTM II) Agar, JEMBEC™ | L007392 | Rev. 09 | September 2007 |
| Mueller Hinton II Agar | L007393 | Rev. 11 | July 2006 |
| Mueller Hinton Agar with 5% Sheep Blood | L007394 | Rev. 03 | April 2006 |
| Mueller Hinton Chocolate Agar | L007396 | Rev. 03 | January 2006 |
| Mycosel™ Agar (Deep Fill) | L007397 | Rev. 06 | January 2006 |
| Neomycin Blood Agar | L007398 | Rev. 04 | July 2006 |
| Nutrient Agar (Deep Fill) | L007399 | Rev. 06 | October 2006 |
| Oxacillin Screen Agar (Mueller Hinton Agar with 6 µg/mL Oxacillin and 4% NaCl) | L007400 | Rev. 07 | July 2006 |
| PC Agar | L009798 | Rev. 00 | July 2007 |
| Phenylethyl Alcohol Agar with 5% Sheep Blood | L007401 | Rev. 07 | May 2006 |
| Sabouraud Brain Heart Infusion Agar (Deep Fill) | L007402 | Rev. 05 | April 2006 |
| Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide (Deep Fill) | L007403 | Rev. 08 | April 2006 |
| Sabouraud Dextrose Agar | L007404 | Rev. 09 | May 2006 |
| Sabouraud Dextrose Agar with Chloramphenicol (Deep Fill) | L007405 | Rev. 07 | January 2006 |
| Sabouraud Dextrose Agar, Emmons | L007406 | Rev. 09 | August 2006 |
| Salmonella Shigella Agar | L007407 | Rev. 08 | July 2006 |
| Schaedler Agar with Vitamin K ₁ and 5% Sheep Blood | L007408 | Rev. 07 | October 2006 |
| Schaedler K-V Agar with 5% Sheep Blood | L007409 | Rev. 06 | October 2006 |
| Selective 7H11 Agar (Deep Fill) | L007410 | Rev. 11 | September 2007 |
| Selective Streptococcus Agar | 8808201 | Rev. 01 | August 2006 |
| Serum Tellurite Agar | L007411 | Rev. 09 | December 2006 |
| Seven H11 Agar (Deep Fill) | L007412 | Rev. 11 | September 2007 |
| SXT Blood Agar | L007413 | Rev. 08 | July 2006 |
| TCBS Agar | L007414 | Rev. 07 | October 2006 |
| Thayer-Martin Selective Agar | L007417 | Rev. 07 | September 2007 |
| Trypticase™ Soy Agar (Soybean-Casein Digest Agar) | L007418 | Rev. 08 | September 2007 |
| Trypticase™ Soy Agar (150 mm) | L007419 | Rev. 07 | May 2006 |
| Trypticase™ Soy Agar with 5% Horse Blood | L007420 | Rev. 04 | July 2006 |
| Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) | L007421 | Rev. 09 | July 2006 |
| Trypticase™ Soy Agar with 10% Sheep Blood | L007421 | Rev. 09 | July 2006 |
| Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitalEx™) - I Plate™ | L007422 | Rev. 08 | September 2007 |
| Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and Levine EMB Agar - I Plate™ | L007423 | Rev. 08 | May 2006 |

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|---|-----------------------------|-----------------------|----------------------|
| Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and MacConkey II Agar - I Plate™ | L007424 | Rev. 08 | December 2006 |
| Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and MacConkey II Agar with MUG - I Plate™ | L009211 | Rev. 01 | December 2006 |
| V Agar | L007425 | Rev. 06 | May 2006 |
| XLD Agar | L007426 | Rev. 09 | September 2007 |

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| BBL™ Product Name | Label Control Number | Revision Level | Revision Date |
|--|-------------------------|----------------|----------------|
| Acetamide Agar Slants | L007431 | Rev. 08 | July 2006 |
| Acetate Differential Agar Slants | L007432 | Rev. 06 | May 2006 |
| ATS Medium Slants | L008067 | Rev. 08 | October 2006 |
| Bile Esculin Agar Slants | L007435 | Rev. 08 | December 2006 |
| Blood Agar Slants | L007436 | Rev. 06 | December 2006 |
| Blood Culture Media | L007437 | Rev. 04 | July 2006 |
| Brain Heart CC Agar, Mycoflask™ | L007439 | Rev. 08 | April 2006 |
| Brain Heart Infusion | L007440 | Rev. 10 | April 2009 |
| Brain Heart Infusion with 6.5% Sodium Chloride | L007440 | Rev. 10 | April 2009 |
| Brain Heart Infusion with PABA and 0.1% Agar | L007441 | Rev. 06 | August 2006 |
| Brain Heart Infusion Agar | L007442 | Rev. 08 | July 2006 |
| Brilliant Green Bile Broth, 2% with Durham Tube | L007443 | Rev. 08 | May 2006 |
| Campylobacter Thioglycollate Medium with 5 Antimicrobics | L007445 | Rev. 09 | December 2006 |
| Chocolate II Agar Slants (GC II Agar with Hemoglobin and IsoVitaleX™) | L007446 | Rev. 08 | September 2007 |
| Cooked Meat Medium | L007448 | Rev. 10 | July 2006 |
| CTA Medium™ | L007450 | Rev. 08 | July 2006 |
| CTA Medium™ with Dextrose | L007450 | Rev. 08 | July 2006 |
| CTA Medium™ with Lactose | L007450 | Rev. 08 | July 2006 |
| CTA Medium™ with Maltose | L007450 | Rev. 08 | July 2006 |
| CTA Medium™ with Mannitol | L009478 | Rev. 00 | January 2006 |
| CTA Medium™ with Salicin | L009478 | Rev. 00 | January 2006 |
| CTA Medium™ with Sorbitol | L009478 | Rev. 00 | January 2006 |
| CTA Medium™ with Sucrose | L009478 | Rev. 00 | January 2006 |
| CTA Medium™ with Xylose | L009478 | Rev. 00 | January 2006 |
| Enterococcosel™ Agar Slants | L007452 | Rev. 09 | December 2006 |
| Enterococcosel™ Broth | L007453 | Rev. 08 | July 2006 |
| Fluid Thioglycollate Medium | L007454 | Rev. 10 | April 2009 |
| GN Broth | L007455 | Rev. 10 | February 2007 |
| Indole Nitrite Medium | L007456 | Rev. 07 | May 2006 |
| Jordan's Tartrate Agar Deep | L007457 | Rev. 07 | January 2007 |
| Kligler Iron Agar Slants | L007458 | Rev. 07 | February 2007 |
| Lactose Broth with Durham Tube (Double Strength) | L007459 | Rev. 07 | May 2006 |
| Lauryl Sulfate Broth with Durham Tube | L007460 | Rev. 09 | October 2006 |
| Lauryl Sulfate Broth, Durham Tube (Double Strength) | L007460 | Rev. 09 | October 2006 |

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| | Number | Revision Level | |
| Lim Broth | L007461 | Rev. 04 | February 2007 |
| Litmus Milk | L007462 | Rev. 09 | May 2006 |
| Loeffler Medium Slants | L007463 | Rev. 07 | January 2007 |
| Lowenstein-Jensen Medium | L007464 | Rev. 08 | January 2007 |
| Lowenstein-Jensen Medium with 5% Sodium Chloride | L007464 | Rev. 08 | January 2007 |
| Lysine Iron Agar Slants | L007465 | Rev. 07 | January 2007 |
| Malonate Broth, Ewing Modified | L007466 | Rev. 08 | January 2007 |
| Middlebrook 7H9 Broth with Glycerol | L007467 | Rev. 09 | December 2006 |
| Middlebrook and Cohn 7H10 Agar | L007468 | Rev. 09 | September 2007 |
| Moeller Decarboxylase Broth Base | L009479 | Rev. 00 | January 2006 |
| Moeller Decarboxylase Broth with Arginine | L009479 | Rev. 00 | January 2006 |
| Moeller Decarboxylase Broth with Lysine | L007470 | Rev. 08 | February 2007 |
| Moeller Decarboxylase Broth with Ornithine | L007470 | Rev. 08 | February 2007 |
| Moeller KCN Broth Base | L007471 | Rev. 06 | May 2006 |
| Motility Indole Ornithine (MIO) Medium | L007472 | Rev. 08 | February 2007 |
| Motility Test Medium | L007473 | Rev. 09 | February 2007 |
| MR-VP Broth | L007474 | Rev. 06 | May 2006 |
| Mueller Hinton II Broth (Cation-Adjusted) | L007475 | Rev. 11 | July 2006 |
| Mycobactosel™ L-J Medium | L007477 | Rev. 08 | October 2006 |
| Mycose™ Agar | L007479 | Rev. 08 | January 2007 |
| Nitrate Broth with Durham Tube | L007480 | Rev. 07 | May 2006 |
| Nutrient Agar | L007481 | Rev. 08 | July 2006 |
| Nutrient Broth | L007482 | Rev. 08 | October 2006 |
| Nutrient Gelatin | L007483 | Rev. 06 | January 2006 |
| OF Basal Medium | L007484 | Rev. 08 | March 2007 |
| OF Medium with Dextrose | L007484 | Rev. 08 | March 2007 |
| OF Medium with Lactose | L009476 | Rev. 00 | January 2006 |
| OF Medium with Maltose | L009476 | Rev. 00 | January 2006 |
| OF Medium with Mannitol | L009476 | Rev. 00 | January 2006 |
| OF Medium with Sucrose | L009476 | Rev. 00 | January 2006 |
| OF Medium with Xylose | L009476 | Rev. 00 | January 2006 |
| Petragnani Medium Slants | L007485 | Rev. 08 | July 2006 |
| Phenol Red Broth Base | L007486 | Rev. 09 | July 2006 |
| Phenol Red Broth with Dextrose and Durham Tube | L007486 | Rev. 09 | July 2006 |
| Phenol Red Broth with Xylose and Durham Tube | L007486 | Rev. 09 | July 2006 |
| Phenol Red Broth with Adonitol and Durham Tube | L009481 | Rev. 00 | January 2006 |

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| BBL™ Product Name | Label Control Number | Revision Level | Revision Date |
|--|-------------------------|----------------|----------------|
| Phenol Red Broth with Arabinose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Cellobiose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Dulcitol and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Glycerol and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Inositol and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Inulin and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Lactose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Maltose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Mannitol and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Raffinose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Rhamnose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Salicin and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Sorbitol and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Sucrose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenol Red Broth with Trehalose and Durham Tube | L009481 | Rev. 00 | January 2006 |
| Phenylalanine Agar Slants | L007487 | Rev. 06 | October 2006 |
| Potato Dextrose Agar | L007488 | Rev. 08 | May 2006 |
| Pseudose™ Agar Slants | L007489 | Rev. 07 | May 2006 |
| Rapid Fermentation Medium, Base | L007491 | Rev. 08 | July 2006 |
| Rapid Fermentation Medium, Dextrose | L007491 | Rev. 08 | July 2006 |
| Rapid Fermentation Medium, Lactose | L007491 | Rev. 08 | July 2006 |
| Rapid Fermentation Medium, Maltose | L007491 | Rev. 08 | July 2006 |
| Rapid Fermentation Medium, Sucrose | L007491 | Rev. 08 | July 2006 |
| Sabouraud Dextrose Agar | L007492 | Rev. 08 | March 2007 |
| Sabouraud Dextrose Agar with Chloramphenicol | L007492 | Rev. 08 | March 2007 |
| Sabouraud Dextrose Agar, Emmons | L007493 | Rev. 07 | August 2006 |
| Sabouraud Liquid Broth, Modified (Antibiotic Medium 13) | L007494 | Rev. 07 | May 2006 |
| Salt Broth, Modified | L007495 | Rev. 08 | May 2006 |
| Schaedler Broth with Vitamin K ₁ | L007496 | Rev. 08 | February 2007 |
| Selenite-F Broth | L007497 | Rev. 09 | February 2007 |
| Serum Tellurite Agar Slants | L007499 | Rev. 08 | October 2006 |
| Seven H11 Agar Slants | L007500 | Rev. 09 | September 2007 |
| Seven H11 Agar Slants with Aspartic Acid and Sodium Pyruvate | L007501 | Rev. 07 | October 2006 |
| SF Broth | L007502 | Rev. 11 | September 2007 |
| SIM Medium | L007503 | Rev. 08 | April 2008 |
| Simmons Citrate Agar Slants | L007504 | Rev. 06 | February 2007 |

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| BBL™ Product Name | Label Control | | Revision Date |
|---|---------------|----------------|----------------|
| | Number | Revision Level | |
| Sodium Hippurate Broth | L007505 | Rev. 05 | May 2006 |
| Standard Methods Agar (APHA) | L007506 | Rev. 08 | October 2006 |
| Standard Methods Agar with Lecithin and Polysorbate 80 Deeps | L007507 | Rev. 06 | May 2006 |
| Thioglycollate Medium, Enriched with Vitamin K ₁ and Hemin | L007509 | Rev. 10 | April 2009 |
| Thioglycollate Medium, Enriched (with Vitamin K ₁ and Hemin), with Calcium Carbonate | L007510 | Rev. 04 | April 2009 |
| Thioglycollate Medium, Fluid without Dextrose or Eh Indicator | L007511 | Rev. 06 | May 2006 |
| Thioglycollate Medium without Indicator-135C | L007512 | Rev. 11 | April 2009 |
| Todd Hewitt Broth | L007513 | Rev. 08 | March 2007 |
| Todd Hewitt Broth with Gentamicin and Nalidixic Acid | L007514 | Rev. 04 | October 2006 |
| Trypticase™ Soy Agar | L007516 | Rev. 09 | October 2006 |
| Trypticase™ Soy Broth | L007517 | Rev. 12 | April 2009 |
| Trypticase™ Soy Broth with 6.5% Sodium Chloride | L007517 | Rev. 12 | April 2009 |
| Trypticase™ Soy Broth with 5% Fildes Enrichment | L009477 | Rev. 00 | January 2006 |
| TSI Agar Slants | L007520 | Rev. 07 | February 2007 |
| Urea Agar Base Concentrate 10X | L007521 | Rev. 08 | September 2007 |
| Urea Agar Slants, Complete | L007521 | Rev. 08 | September 2007 |
| Urease Broth Concentrate 10X | L007522 | Rev. 07 | July 2006 |
| Urease Test Broth | L007522 | Rev. 07 | July 2006 |

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BBL™ Brucella Agar with 5% Horse Blood

L007354 • Rev. 06 • March 2008

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Brucella Agar with 5% Horse Blood is a culture medium which is used for the isolation and growth of both fastidious and nonfastidious bacterial species.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures diluted to contain 50–100 CFU per 0.1 mL.
 - a. Add 0.1 mL of the appropriate dilution to each plate and spread-inoculate using a sterile glass spreader.
 - b. Incubate at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
 - c. Include a Chocolate II Agar plate as a control for the *Haemophilus* strain only.
2. Examine plates after 18–24 and 48–72 h for amount of growth, colony size and hemolytic reactions.
3. Expected Results

| Organisms | ATCC™ | Recovery |
|-----------------------------------|-------|---|
| * <i>Streptococcus pyogenes</i> | 19615 | Growth, beta hemolysis |
| * <i>Streptococcus pneumoniae</i> | 6305 | Growth, alpha hemolysis |
| * <i>Staphylococcus aureus</i> | 25923 | Growth |
| * <i>Escherichia coli</i> | 25922 | Growth |
| <i>Shigella flexneri</i> | 12022 | Moderate to heavy growth. Colonies large, shiny and gray and may or may not be hemolytic. |
| <i>Haemophilus influenzae</i> | 10211 | Moderate to heavy growth. Colonies nonhemolytic, grayish, small and translucent with a distinct "mousy" odor. |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Brucella Agar with 5% Horse Blood is used in qualitative procedures for the isolation and cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens.

V SUMMARY AND EXPLANATION

Brucella Agar was developed for the cultivation of *Brucella* species from diagnostic specimens such as blood, and from foods and other potentially contaminated material. Brucella Agar with 5% Horse Blood plates are particularly useful for the cultivation of the more fastidious aerobic and anaerobic microorganisms including streptococci, pneumococci, *Listeria*, *Neisseria meningitidis* and *Haemophilus influenzae*.

VI PRINCIPLES OF THE PROCEDURE

This medium supports the growth of fastidious microorganisms due to its content of peptones, dextrose, yeast extract and blood. The peptones supply organic nitrogen. The yeast extract is a potent source of the B vitamins. Dextrose is utilized as an energy source. Horse blood supplies both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*. Sheep blood is not suitable for this purpose in that it contains enzymes which inactivate the nicotinamide adenine dinucleotide (NAD) which is the V factor.¹

Defibrinated horse blood may give hemolytic reactions different from sheep blood.¹ Some enterococci give beta-hemolytic reactions on horse blood but non-beta-hemolytic reactions on sheep blood.² This may result in the isolate being mistakenly reported as group A. If a hemolytic reaction is obtained, the organism should be tested with a **Taxo™** A disc and it also should be grouped serologically.¹ Beta-hemolytic streptococci and *Haemophilus hemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.

VII REAGENTS

Brucella Agar with 5% Horse Blood

Approximate Formula* Per Liter Purified Water

| | | | |
|--------------------------------------|--------|---------------------------------|--------|
| Pancreatic Digest of Casein | 10.0 g | Sodium Chloride | 5.0 g |
| Peptic Digest of Animal Tissue | 10.0 g | Sodium Bisulfite | 0.1 g |
| Dextrose | 1.0 g | Agar | 15.0 g |
| Yeast Extract | 2.0 g | Horse Blood, defibrinated | 5% |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"³⁻⁶ and institutional guidelines should be followed in handling all items contaminated with

blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.⁵

Storage Instructions: On receipt, store plates in the dark at 2-8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2-8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{1,7} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brucella Agar with 5% Horse Blood

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5% CO₂.

Incubate plates at 35± 2°C for 18–24 h in an aerobic atmosphere supplemented with carbon dioxide.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

XI LIMITATION OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{1,7,8}

XII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 221547 | BBL™ Brucella Agar with 5% Horse Blood, Pkg. of 20 plates |
| 221548 | BBL™ Brucella Agar with 5% Horse Blood, Ctn. of 100 plates |

XIII REFERENCES

1. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Teixeira, L.M. and R.R. Facklam. 2003. *Enterococcus*, p. 422-433. In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
3. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, PA.
4. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
5. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
6. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
8. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

BBL™ CHROMagar™ Candida is a selective medium for the isolation and presumptive identification of yeast and filamentous fungi and differentiation of *Candida albicans*, *C. tropicalis* and *C. krusei*.¹

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak inoculate with 10³-10⁴ CFUs of the organisms listed below.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)** plates as nonselective controls for *P. aeruginosa* and Sabouraud Dextrose Agar for all *Candida* species.
2. Examine plates after 36–48 h for amount of growth and color formation.
3. Expected Results

| Organisms | ATCC™ | Recovery | Colony Color |
|---------------------------------|-------|----------------------------------|---|
| * <i>Candida albicans</i> | 60193 | Fair to heavy growth | Light to medium green |
| <i>Candida albicans</i> | 10231 | Fair to heavy growth | Light to medium green |
| * <i>Candida krusei</i> | 34135 | Fair to heavy growth | Mauve to rose pink, flat, may have whitish border |
| * <i>Candida tropicalis</i> | 1369 | Fair to heavy growth | Dark blue to metallic blue, with or without halos |
| <i>Candida tropicalis</i> | 9968 | Fair to heavy growth | Grey blue, with or without halos |
| * <i>Pseudomonas aeruginosa</i> | 27853 | Inhibition (partial to complete) | |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 5.9 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ Candida is a selective medium for the isolation and presumptive identification of yeast and filamentous fungi and differentiation of *Candida albicans*, *C. tropicalis* and *C. krusei*.¹ Due to the differences in morphology and colors of the yeast colonies, this medium facilitates the detection of mixed yeast cultures in specimens.^{2,3} It may also be used as a selective isolation medium for other yeasts and for filamentous fungi instead of Sabouraud Dextrose Agar or similar media. Patent Pending

V SUMMARY AND EXPLANATION

The usefulness of a selective and differential medium for the primary isolation of *Candida* species has long been noted. In 1953 Nickerson developed a medium following a study of sulfite reduction by *Candida* species.⁴ In 1958 Pagano et al. added triphenyltetrazolium chloride to Sabouraud Dextrose medium to differentiate *C. albicans* from other yeasts.⁵

CHROMagar Candida is a selective and differential medium developed by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France. With the inclusion of chromogenic substrates in the medium, the colonies of *C. albicans*, *C. tropicalis* and *C. krusei* produce different colors, thus allowing the direct detection of these yeast species on the isolation plate.¹⁻³ Colonies of *C. albicans* appear light to medium green, *C. tropicalis* colonies appear dark blue to metallic-blue and *C. krusei* colonies appear light mauve to mauve, flat colonies with a whitish border. Other yeasts may appear light to dark mauve (e.g., *C. glabrata* and other species).

VI PRINCIPLES OF THE PROCEDURE

Specially selected peptones supply the nutrients in **BBL CHROMagar Candida**. The chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific enzymes. This permits the differentiation of certain species, or the detection of certain groups of organisms, with only a minimum of confirmatory tests. Chloramphenicol inhibits most bacterial contaminants.

VII REAGENTS

BBL CHROMagar Candida

Approximate Formula* Per Liter Purified Water

| | |
|-----------------------|--------|
| Chromopeptone | 10.0 g |
| Glucose | 20.0 g |
| Chromogen Mix | 2.0 g |
| Chloramphenicol | 0.5 g |
| Agar | 15.0 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over the offset lid and allow to air dry in order to prevent formation of a seal between the top and the bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and original cardboard box until time of inoculation. Plates may be inoculated up to the expiration date.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying or cracking.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁶⁻⁹ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

IX PROCEDURE

Material Provided: BBL CHROMagar Candida

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. Allow the medium to warm to room temperature before inoculation.

As soon as possible after receipt in the laboratory, inoculate the specimen onto a **BBL CHROMagar Candida** plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 36–48 h in an inverted position (agar-side up). Occasional isolates, such as *Cryptococcus neoformans* and filamentous fungi, will require a longer incubation time and possibly a lower incubation temperature.

Do not incubate in an atmosphere supplemented with carbon dioxide. Minimize exposure to light both before and during incubation.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After proper incubation, read plates against a white background. Plates from specimens containing yeasts will show growth. Depending on the yeast species, colonies will appear light to medium green (*C. albicans*), light mauve to mauve flat colonies with a whitish border (*C. krusei*), or dark blue to metallic blue (*C. tropicalis*). Colonies that appear light to dark mauve or appear in their natural cream color should be identified using standard methods.¹⁰ Identification is presumptive for these three species, confirmatory tests are recommended.

XI LIMITATIONS OF THE PROCEDURE

Consult appropriate references for detailed information and recommended procedures for the identification of isolates.^{1,3,10}

Since molds and other filamentous fungi metabolize the chromogenic substrates, the colors exhibited by these organisms on **CHROMagar Candida** medium may differ from those exhibited on Sabouraud Dextrose Agar. Do not use the appearance of growth on this medium for traditional descriptive identification from Sabouraud Dextrose Agar.

C. glabrata and *C. parapsilosis* cannot be differentiated using this product. These identifications should be confirmed using other standard laboratory methods.

It has been reported that *C. dubliniensis* produces a distinctive dark green color on primary isolation with **CHROMagar Candida** Medium.¹¹⁻¹³ However, this property may not be retained in subculture. Additional phenotypic and genotypic assays may be necessary. The clinical importance of *C. dubliniensis* requires further study.

Minimize exposure to light before and during incubation, as light may destroy the chromogens. Keep plates within original sleeve wrapping and cardboard box for the entire storage period.

XII PERFORMANCE CHARACTERISTICS¹⁴

A total of 160 clinical samples were plated onto **BBL CHROMagar Candida** plates at a large metropolitan hospital. Preliminary identification was done using the chromogenic medium. Confirming identification was done using at least one of the following reference methods: microscopy, Cream of Rice Agar, sheep blood media, Vitek™ and API™ systems.

Candida albicans: A total of 106 isolates were grown and identified with **BBL CHROMagar Candida** plates. Of the 106 isolates, 105 developed the characteristic "green" colony color of *Candida albicans* on **BBL CHROMagar Candida**. The one outlying isolate did not develop green colonies. When the confirming method was used for identification, the result was *Candida albicans*. Identification of all **CHROMagar Candida albicans** results were verified using at least one of the reference methods. It should be noted that four of these isolates were initially isolated in mixed culture with other fungi.

Candida krusei: A total of 5 isolates were grown and identified with **BBL CHROMagar Candida**. All 5 isolates developed colonies that appeared as mauve with white edges and were powdery or dry in appearance, the characteristic colony color of *C. krusei*. Identification of all **CHROMagar Candida krusei** results were verified using at least one of the reference methods.

Candida tropicalis: A total of 10 isolates were grown and identified with **BBL CHROMagar Candida**. Of the 10 isolates, all developed the characteristic "blue" to "metallic blue" color of *C. tropicalis* on the test medium. Identification of all **CHROMagar C. tropicalis** results were verified using at least one of the reference methods.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 254093 | BBL™ CHROMagar™ Candida, Pkg. of 20 plates |

XIV REFERENCES

1. Odds, F.C., and R. Bernaerts. 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *J. Clin. Microbiol.* 32:1923-1929.
2. Pfaller, M.A., A. Huston, and S. Coffman. 1996. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J. Clin. Microbiol.* 34:56-61.
3. Beighton, D., R. Ludford, D.T. Clark, S.R. Brailsford, C.L. Pankhurst, G.F. Tinsley, J. Fiske, D. Lewis, B. Daly, N. Khalifa, V. Marren, and E. Lynch. 1995. Use of CHROMagar Candida medium for isolation of yeasts from dental samples. *J. Clin. Microbiol.* 32:3025-3027.
4. Nickerson, W.J. 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of *Candida*. *J. Infect. Dis.* 93:45-56.
5. Pagano, J., J.D. Levine, and W. Trejo. 1958. Diagnostic medium for differentiation of species of *Candida*. *Antibiot. Ann.* 1957-1958:137-143.
6. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
7. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
8. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
9. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
10. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. *Bailey & Scott's diagnostic microbiology*, 10th ed. Mosby, Inc., St. Louis.
11. Schoofs, A., F.C. Odds, R. Coleblunders, M. Ieven, and H. Goosens. 1997. Use of specialized isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. *Eur. J. Clin. Microbiol. Infect. Dis.* 16:296-300.
12. Kirkpatrick, W.R., S.G. Revankar, R.K. McAtee, J.L. Lopez-Ribot, A.W. Fothergill, D.I. McCarthy, S.E. Sanche, R.A. Cantu, M.G. Rinaldi, and T.F. Patterson. 1998. Detection of *Candida dubliniensis* in oropharyngeal samples from Human Immunodeficiency Virus-infected patients in North America by primary CHROMagar Candida screening and susceptibility testing of isolates. *J. Clin. Microbiol.* 36:3007-3012.
13. Odds, F.C., L. Van Nuffel, and G. Dams. 1998. Prevalence of *Candida dubliniensis* isolates in a yeast stock collection. *J. Clin. Microbiol.* 36:2869-2873.
14. Data on file, BD Diagnostics.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

BBL™ CHROMagar™ MRSA, supplemented with chromogens and inhibitory agents, is used for the qualitative direct detection of nasal colonization by methicillin-resistant *Staphylococcus aureus*.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak the plates for isolation. For *Staphylococcus aureus* ATCC™ 43300 and 33591, use an 18-24 h broth culture diluted to yield 10³-10⁴ CFU/plate. Use an 18-24 h broth culture for all other organisms diluted to yield 10⁴-10⁵ CFU/plate.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.

NOTE: Minimize exposure to light before and during incubation.
 - c. Include **Trypticase™ Soy Agar** with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18-24 h and 42-48 h for recovery, colony size and pigmentation.
3. Expected results

| Organisms | ATCC™ | Recovery | Colony Color |
|--------------------------------|-------|-------------------------------------|--------------|
| <i>Staphylococcus aureus</i> | 29213 | Inhibition (partial to complete) | N/A |
| * <i>Staphylococcus aureus</i> | 25923 | Inhibition (partial to complete) | N/A |
| * <i>Staphylococcus aureus</i> | 43300 | Growth | Mauve |
| <i>Staphylococcus aureus</i> | 33591 | Growth | Mauve |
| <i>Enterococcus faecalis</i> | 29212 | Growth | Blue |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification 6.8 ± 0.2.
4. Note the firmness of the plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ MRSA is a selective and differential medium for the qualitative direct detection of nasal colonization by methicillin-resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings. The test is performed on anterior nares swab specimens from patients and healthcare workers to screen for MRSA colonization. **BBL CHROMagar MRSA** is not intended to diagnose MRSA infection nor to guide or monitor treatment for infections.

V SUMMARY AND EXPLANATION

MRSA are a major cause of nosocomial and life threatening infections. Infections with MRSA have been associated with a significantly higher morbidity, mortality and costs than methicillin-susceptible *S. aureus* (MSSA).¹ Selection of these organisms has been greatest in the healthcare setting; however, MRSA have also become more prevalent in the community.² To control the transmission of MRSA, the Society for Healthcare Epidemiology of America (SHEA) has recommended guidelines, which include an active surveillance program to identify potential reservoirs and a rigorous infection control program to control the spread of MRSA.¹

BBL CHROMagar MRSA is a selective and differential medium, which incorporates cefoxitin, for the detection of MRSA from anterior nares specimens.

BBL CHROMagar MRSA was developed by A. Rambach and BD. This product utilizes **CHROMagar Staph aureus**, which was developed by A. Rambach and is sold by BD under a licensing agreement with **CHROMagar**, Paris, France.

VI PRINCIPLES OF THE PROCEDURE

BBL CHROMagar MRSA medium permits the direct detection and identification of MRSA through the incorporation of specific chromogenic substrates and cefoxitin. MRSA strains will grow in the presence of cefoxitin³ and produce mauve-colored colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are incorporated for the suppression of gram-negative organisms, yeast and some gram-positive cocci. Bacteria other than MRSA may utilize other chromogenic substrates in the medium resulting in blue to blue/green colored colonies or if no chromogenic substrates are utilized, the colonies appear as white or colorless.

VII REAGENTS

BBL CHROMagar MRSA

Approximate Formula Per Liter Purified Water

| | | | |
|-----------------------|--------|-------------------------|--------|
| Chromopeptone | 40.0 g | Inhibitory Agents | 0.07 g |
| Sodium Chloride | 25.0 g | Cefoxitin | 6.0 mg |
| Chromogen Mix..... | 0.5 g | Agar | 14.0 g |

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation. Protect from light during drying. See Storage Instructions. Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus may be present in clinical specimens. "Standard Precautions"⁴⁻⁷ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and original cardboard box until time of inoculation. Prolonged exposure to light (>4 h) may result in reduced recovery and/or coloration of the QC organisms or patient isolates. Plates may be used up until the expiration date.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This device has been evaluated for performance with anterior nares specimens. Use of transport devices approved for the collection of such specimens is recommended. Follow the transport device manufacturer's recommended procedures. The user may also refer to appropriate texts for details of specimen collection and handling procedures.^{8,9}

IX PROCEDURE

Material Provided: BBL CHROMagar MRSA

Materials Required But Not Provided: Ancillary culture media, coagulase test reagents, quality control organisms and other laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. Allow the medium to warm to room temperature in the dark before inoculation.

As soon as possible after receipt in the laboratory, inoculate the specimen onto a BBL CHROMagar MRSA plate and streak for isolation. Incubate plates aerobically at 35–37°C for 24 ± 4 h in an inverted position. If no mauve colonies are recovered, reincubate for an additional 24 ± 4 h. Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation (>4 h) as light may result in reduced recovery and/or coloration of isolates. Exposure to light is permissible after colony color develops.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Read plates against a white background. Colonies of MRSA will appear mauve on the BBL CHROMagar MRSA medium. Other organisms (non-MRSA) will be inhibited or produce colorless, white, blue or blue/green colonies. Refer to Table 1 for interpretation of results.

Table 1

| 24 h Incubation | | Interpretation/Recommended Action |
|--|---------------------------|--|
| Mauve colonies morphologically resembling staphylococci* | | MRSA detected, report MRSA nasal colonization |
| No mauve colonies | | No result available, reincubate 24 additional hours |
| 48 h Incubation | Recommended Action | Interpretation |
| Mauve colonies | Perform coagulase testing | If coagulase positive – MRSA detected, report MRSA nasal colonization If coagulase negative – report no MRSA detected |
| No mauve colonies | N/A | Report no MRSA detected |

*Staphylococci typically produce moderately sized smooth mauve colonies on BBL CHROMagar MRSA medium. Mauve colonies which are very small to pinpoint are most often gram-positive rods, usually corynebacteria. If morphology is unclear, confirmatory tests such as coagulase may be used to confirm identification at 48 h.

XI LIMITATIONS OF THE PROCEDURE

Minimize exposure (<4 h) of BBL CHROMagar MRSA to light both before and during incubation, as prolonged exposure may result in reduced recovery and/or coloration of isolates. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.

At 48 h occasional strains of coagulase-negative staphylococci (such as, *S. epidermidis*, *S. cohnii*, *S. intermedius*, *S. haemolyticus*, *S. capitis*, *S. hominis* and *S. schleiferi*), *Acinetobacter* sp., *Corynebacterium* and yeast may produce mauve-colored colonies requiring a confirmatory coagulase test for confirmation of MRSA. This may also occur at a much lower rate at 24 h. In clinical studies, approximately 5% (6/120) of the mauve-colored colonies detected at 24 h were coagulase-negative staphylococci and/or corynebacteria on the BBL CHROMagar MRSA medium. If desired, a coagulase test may be performed at 24 h on mauve-colored colonies to increase specificity.

Surveillance testing determines the colonization status at a given time and could vary depending on patient treatment (e.g. decolonization regime), patient status (e.g. not actively shedding MRSA) or exposure to high risk environments (e.g. contact with MRSA carrier, prolonged hospitalization). Monitoring colonization status should be done according to hospital policies.

Results from CHROMagar MRSA should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions. The test is not intended to identify patients with staphylococcal infection. Results should not be

used to guide or monitor treatment for MRSA infections. This device can be used to identify patients for isolation or removal from isolation to control nosocomial transmission of MRSA.

A **CHROMagar MRSA** negative result following a previous positive test result may indicate treatment eradication success or may occur due to intermittent shedding.

mecA-negative *S. aureus* may grow if the oxacillin or ceftaxime MICs are at or near the resistant breakpoint.

Incubation in 5% CO₂ is not recommended and may result in false negative cultures.

Use of phenylephrine hydrochloride, a component of some nasal sprays, at a concentration of ≥10% shows an inhibitory effect on organism growth that is unrelated to medium performance.

Rare strains of MRSA have demonstrated sensitivity to the **CHROMagar MRSA** base. This sensitivity is unrelated to methicillin resistance, but is due to a component in the base. As a result, these strains may appear as falsely susceptible to methicillin.

XII EXPECTED VALUES

The prevalence of MRSA infection has increased dramatically in medical institutional settings, and the carriage rate of MRSA is rising in the community. Recent publications suggest that the population at large has *S. aureus* colonization rates ranging between 25 and 30%.¹¹ Resistance rates have steadily increased in the past fifteen years, and recent NNIS (National Nosocomial Infections Surveillance) data indicates that, in the intensive care patient setting, the proportion of MRSA among *S. aureus* infections was as high as 60% in 2003.¹²

In the external clinical evaluation of **CHROMagar MRSA**, the overall prevalence of *S. aureus* colonization was 17.2% (340/1974), as detected by either the **CHROMagar MRSA** or **Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)** plates. The overall prevalence of (non-duplicate patient) MRSA-positive specimens was 6.7% (132/1974), or about 39% (132/340) of all *S. aureus*. The TSA II plate MRSA-colonization detection rate was 6.5% (117/1974), while the **CHROMagar MRSA** rate of MRSA-colonization was 7.0% (126/1974).

XIII PERFORMANCE CHARACTERISTICS

Clinical Studies

CHROMagar MRSA was evaluated at four geographically diverse hospitals with fresh prospective surveillance specimens of the anterior nares. A total of 1,974 surveillance nares specimens were evaluated, comparing the recovery of MRSA on **Trypticase Soy Agar with 5% Sheep Blood (TSA II)** reference plates to **CHROMagar MRSA** plates. *S. aureus* recovered on TSA II were tested by a microbroth dilution Oxacillin MIC method, and an Oxacillin Screen Agar method, as well as three additional susceptibility test methods (see next section). Oxacillin MIC results followed NCCLS interpretive criteria, with MSSA ≤ 2 µg/mL and MRSA ≥ 4 µg/mL. Oxacillin Screen Agar was interpreted using manufacturer's instructions which included the presence of any colony growth as representative of MRSA. **CHROMagar MRSA** was interpreted as positive for MRSA at 24 h based on detection of mauve colony color (alone), or at 48 h based on detection of mauve colonies with confirmation as *S. aureus* by a coagulase test. Overall recovery of MRSA on **CHROMagar MRSA** was higher at 95% (126), compared to a recovery of 89% (117) on TSA II. The accuracy of identification of MRSA was compared to the Oxacillin MIC microbroth dilution method and the Oxacillin Screen Agar method. At the 24 h reading, there were 6 false positives where mauve colonies were observed on **CHROMagar MRSA** (2 *S. epidermidis*, 2 *S. haemolyticus*, and 2 *Corynebacterium*). Using colony color alone at the 24 h reading for **CHROMagar MRSA**, and confirming all mauve colonies with coagulase at the 48 h reading, the overall agreement of the **CHROMagar MRSA** test to the Oxacillin MIC test was 96% (312/325). Overall category agreement of **CHROMagar MRSA** to Oxacillin Screen Agar was 96% (312/325). Positive percent MRSA agreement and negative percent MSSA agreement of **CHROMagar MRSA** compared to these reference methods is shown in the following Tables 2–5:

Performance of **CHROMagar MRSA** (24 h mauve/48 h with coagulase combined final result) versus Oxacillin MIC Reference Result

Table 2

| CHROMagar MRSA Result | MRSA Identification | TSA II Result | | | Total |
|-----------------------|---|--------------------------------|-----|-------------------------------|-------|
| | | Growth of <i>S. aureus</i> | | No growth of <i>S. aureus</i> | |
| | | Oxacillin MIC Reference Result | | | |
| MRSA | MSSA | | | | |
| Mauve | Mauve at 24 h or mauve and coag pos at 48 h | 111 | 7 | 21* | 139 |
| | Coag neg 48 h | 0 | 3 | 68** | 71 |
| Not Mauve/ No Growth | N/A | 6 | 198 | 1560 | 1764 |
| Total | | 117 | 208 | 1649 | 1974 |

*Of 21 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on **CHROMagar MRSA**: 15 were confirmed as MRSA by positive PBP 2' latex test results; 4 were coagulase-negative staphylococci; and 2 were gram-positive rods.

Of 68 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on **CHROMagar MRSA at 48 h: 45 were confirmed as coagulase-negative staphylococci; and 23 were gram-positive rods and other organisms.

Table 3

| CHROMagar MRSA vs. Oxacillin MIC | |
|-----------------------------------|-----------------------------------|
| % Agreement of MRSA (95% CI) | % Agreement of MSSA (95% CI) |
| 94.9% (111/117) (89.2%, 98.1%) | 96.6% (201/208) (93.2%, 98.6%) |

Performance of CHROMagar MRSA (24 h mauve/48 h with coagulase combined final result) versus Oxacillin Screen Agar Reference Result

Table 4

| CHROMagar MRSA Result | MRSA Identification | TSA II Result | | No growth of <i>S. aureus</i> | Total |
|-----------------------|---|--|------|-------------------------------|-------|
| | | Growth of <i>S. aureus</i> | | | |
| | | Oxacillin Screen Agar Reference Result | | | |
| | | MRSA | MSSA | | |
| Mauve | Mauve at 24 h or mauve and coag pos at 48 h | 110 | 7 | 21* | 138 |
| | Coag neg 48 h | 0 | 3 | 68** | 71 |
| Not Mauve/ No Growth | N/A | 6 | 199 | 1560 | 1765 |
| Total | | 116 | 209 | 1649 | 1974 |

*Of 21 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on CHROMagar MRSA: 15 were confirmed as MRSA by positive PBP 2' latex test results; 4 were coagulase-negative staphylococci; and 2 were gram-positive rods.

**Of 68 specimens where no *S. aureus* was recovered on TSA II, and mauve isolates were recovered on CHROMagar MRSA: 45 were confirmed as coagulase-negative staphylococci; and 23 were gram-positive rods and other organisms.

Table 5

| CHROMagar MRSA vs. Oxacillin Screen Agar | |
|--|-----------------------------------|
| % Agreement of MRSA (95% CI) | % Agreement of MSSA (95% CI) |
| 94.8% (110/116) (89.1%, 98.1%) | 96.7% (202/209) (93.2%, 98.6%) |

These studies also compared CHROMagar MRSA to other test methods for identifying MRSA and MSSA: the PBP 2' Latex Agglutination Test, a cefoxitin (30 µg) disk diffusion test, and PCR detection of the *mecA* gene. The cefoxitin disk diffusion testing followed recent NCCLS interpretive criteria (zone size of ≤ 19 mm as MRSA, or ≥ 20 mm as MSSA). PBP 2' and PCR methods followed labeling instructions for interpretation. Percent agreement compared to these additional methods is shown in Table 6 for the MRSA and MSSA isolates. Total number of isolates tested differs between methods due to differences in individual method completion or compliance/evaluability rates.

Table 6

| CHROMagar MRSA vs. Cefoxitin Disk Diffusion | | CHROMagar MRSA vs. PBP 2' Latex Agglutination | | CHROMagar MRSA vs. PCR (<i>mecA</i>) | |
|---|------------------------------------|---|--------------------------------------|--|------------------------------------|
| % Agreement of MRSA | % Agreement of MSSA | % Agreement of MRSA | % Agreement of MSSA | % Agreement of MRSA | % Agreement of MSSA |
| 94.9% (112/118) (89.3%, 98.1%) | 98% (200/204) (95.1%, 99.5%) | 93.5% (115/123) (87.6%, 97.2%) | 98.5% (198/201) (95.7%, 99.7%) | 95.7% (111/116) (90.2%, 98.6%) | 97% (196/202) (93.6%, 98.9%) |

Challenge Testing

Testing of twenty (20) challenge strains of *S. aureus* was conducted at three of the clinical sites. In this panel, 9 were heterogeneous resistant MRSA, 5 were homogeneous resistant MRSA, and 6 were MSSA. Individual site and combined site sensitivities were all 100%, and site and overall specificities were 100%.

Expression of Resistance

CHROMagar MRSA was evaluated for its ability to detect heterogeneous and homogeneous strains. MRSA can be homogeneously or heterogeneously resistant. Heterogeneous strains may have as few as 1 in 1,000,000 cells expressing resistance,¹³ making detection by conventional antimicrobial susceptibility tests difficult. Fifteen test strains, representing 10 heterogeneous and 5 homogeneous MRSA, were evaluated for recovery and colony counts on CHROMagar MRSA compared to a nonselective medium, TSA II with 5% sheep blood. Both CHROMagar MRSA and TSA II recovered all 15 strains. CHROMagar MRSA colony counts ranged from 64–99% for heterogeneous strains and 71–100% for homogeneous strains compared to the TSA II. These results support that CHROMagar MRSA is able to detect both homogeneous and heterogeneous strains.¹⁴

Interference Study

Eight commonly used medicinal substances, human blood and five types of specimen transport devices, were evaluated for potential interference of the chromogenic reaction on the CHROMagar MRSA medium. At a 10% concentration, a nasal spray containing phenylephrine hydrochloride demonstrated antibacterial activity on CHROMagar MRSA, as well as on the nonselective control, TSA II with 5% sheep blood. No other substance or device tested interfered with the performance of the CHROMagar MRSA medium.¹⁴

XIV AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 215084 | BBL™ CHROMagar™ MRSA, Pkg. of 20 plates |
| 215181 | BBL™ CHROMagar™ MRSA, Ctn. of 100 plates |

XV REFERENCES

1. Muto, C. A., J. A. Jernigan, B. E. Ostrowosky, H. M. Richet, W. R. Jarvis, J. M. Boyce, B. M. Farr. 2003. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. *Infect. Control and Hospital Epidemiol.* May 362-386.
2. Bannerman, T. L. 2003. *Staphylococcus, Micrococcus*, and other catalase-positive cocci that grow aerobically. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenover (eds.), 8th ed., *Manual of clinical microbiology*. ASM, Washington DC.
3. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; Eighteenth Informational Supplement, M100-S18. CLSI, Wayne, PA.
4. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed., CLSI, Wayne, PA.
5. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17: 53-80.
6. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, DC.
7. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
8. Ramsay-Shea, Y. 1992. Specimen collection and transport. *In* Isenberg, H.D. (ed.), *Clinical microbiology procedures handbook*. ASM, Washington DC.
9. Miller, J.M., H.T. Holmes, K. Krisher. 2003. General principles of specimen collection and handling. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenover (eds.), 8th ed., *Manual of clinical microbiology*. ASM, Washington DC.
10. Clinical and Laboratory Standards Institute. 2004. Approved Guideline M22-A3. Quality assurance for commercially prepared microbiological culture media, 3rd ed., CLSI, Wayne, PA.
11. MRSA – Methicillin Resistant *Staphylococcus aureus*: Fact Sheet. CDC website, <http://www.cdc.gov/ncidod/hip/Aresist/mrsafaq.htm>.
12. Proportion of *S. aureus* Nosocomial Infections Resistance to Oxacillin (MRSA) Among Intensive Care Unit Patients, 1989 – 2003 (graph). CDC website, http://www.cdc.gov/ncidod/hip/AREISIT/ICU_MRSA.pdf.
13. Tomasz A., Nachman S., Leah H. 1991. Stable classes of phenotypic expression in methicillin resistant clinical isolates of staphylococci. *Antimicro. Agents Chemother.* 35:124-129.
14. Data on file, BD Diagnostics.

U.S. Patent Pending

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

BBL™ CHROMagar™ O157 is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with dilutions of the cultures listed below.
 - Streak inoculate with 10^3 - 10^4 CFUs of *E. coli* ATCC 700728, 35150 and 43895 and 10^4 - 10^5 CFUs of all other organisms.
 - Incubate plates at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.
 - Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
- Examine plates after 18–24 h for amount of growth and color formation.
- Expected Results

| Organisms | ATCC™ | Recovery | Colony Color |
|-----------------------------------|--------|----------------------------------|--|
| <i>Aeromonas hydrophila</i> | 7965 | Inhibition (partial to complete) | Colorless to light yellow |
| * <i>Enterobacter cloacae</i> | 13047 | Fair to heavy growth | Blue-green to blue |
| <i>Enterococcus faecalis</i> | 29212 | Inhibition (partial to complete) | Blue |
| * <i>Escherichia coli</i> O157:H7 | 700728 | Fair to heavy growth | Light mauve to mauve |
| * <i>Escherichia coli</i> | 25922 | Inhibition (partial to complete) | Blue |
| <i>Escherichia coli</i> | 35150 | Fair to heavy growth | Light mauve to mauve |
| <i>Escherichia coli</i> | 43895 | Fair to heavy growth | Light mauve to mauve |
| <i>Klebsiella pneumoniae</i> | 33495 | Inhibition (partial to complete) | Blue |
| <i>Proteus mirabilis</i> | 12453 | Inhibition (partial to complete) | Colorless, may have orange-brown precipitate |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification 7.4 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates aerobically at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ O157 is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7 from human clinical stool specimens.

U.S. Patent No. 6,165,743

V SUMMARY AND EXPLANATION

E. coli O157:H7 is the most frequently isolated pathogen from bloody stools.¹ However, absence of bloody diarrhea does not rule out the presence of *E. coli* O157:H7.² This serotype causes a broad range of illness from mild non-bloody diarrhea to severe bloody diarrhea (hemolytic colitis), hemolytic uremic syndrome and death.¹ The isolation of *E. coli* O157:H7 exceeds that of some other common enteric pathogens, especially *Shigella* in many areas and age groups. Transmission most often occurs through ingestion of raw or undercooked beef; other foods have also been implicated.¹ In addition, transmission may occur person to person, as well as from recreational water sources.¹

CHROMagar O157 is intended for the isolation, differentiation and presumptive identification of *E. coli* O157:H7. Due to the chromogenic substrates in the medium, colonies of *E. coli* O157:H7 produce a mauve color, thus allowing presumptive identification from the primary isolation plate and differentiation from other organisms. In samples with low numbers of *E. coli* O157:H7, enrichment methods may be helpful prior to inoculating medium.

VI PRINCIPLES OF THE PROCEDURE

CHROMagar O157 was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar O157** prepared plated medium.

Specially selected **Difco™** peptones supply the nutrients. The addition of potassium tellurite, cefixime and cefsulodin reduces the number of bacteria other than *E. coli* O157:H7 that grow on this medium. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by a specific enzyme. *E. coli* O157:H7 utilizes one of the chromogenic substrates producing mauve colonies. The growth of mauve colonies is considered presumptive for *E. coli* O157:H7 on **BBL CHROMagar O157**. Non-*E. coli* O157:H7 bacteria may utilize other chromogenic substrates resulting in blue to blue-green colored colonies or, if none of the chromogenic substrates are utilized, colonies may appear as their natural color. This facilitates the detection and differentiation of *E. coli* O157:H7 from other organisms.

VII REAGENTS

BBL CHROMagar O157

Approximate Formula* Per Liter Purified Water

| | | | |
|---------------------------|--------|------------------|---------|
| Chromopeptone | 16.0 g | Cefixime | 0.05 mg |
| Sodium Chloride | 7.0 g | Cefsulodin | 4.0 mg |
| Chromogen Mix | 0.65 g | Agar | 14.0 g |
| Potassium Tellurite | 2.5 mg | | |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation. Protect from light during drying. See Storage Instructions.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"³⁻⁶ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

As with any selective prepared culture medium, not all organisms (i.e. *E. coli* O157:H7) may grow on the medium due to factors associated with inoculation, recovery or inhibition. The result of this test is not definitive and should be evaluated in coordination with the physiological symptoms of the patient.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and original cardboard box until time of inoculation. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure of **BBL CHROMagar O157** to light both before and during incubation, as light may destroy the chromogens. Prepared plates may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII PROCEDURE

Material Provided: BBL CHROMagar O157

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Specimen Collection and Handling: For human stool use, refer to lab procedures for details on specimen collection and handling procedures.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

For clinical specimens, as soon as possible after receipt in the laboratory, inoculate onto a **BBL CHROMagar O157** plate and streak for isolation. If the specimen is cultured from a swab, roll the swab over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 18–24 h in an inverted position (agar-side up). Plates are not to be incubated beyond the 24 h time period prior to reading. Interpretation of plate results must be completed within 18–24 h after inoculation of the **BBL CHROMagar O157** plate.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the clinical user refer to pertinent Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines for appropriate Quality Control practices.

IX RESULTS

After proper incubation, read plates against a white background. Interpretation of plate results must be completed within 18–24 h after inoculation of the **BBL CHROMagar O157** plate. *E. coli* O157:H7 will produce mauve-colored colonies on **BBL CHROMagar O157** medium. All mauve colonies should be confirmed biochemically and/or serologically prior to reporting as *E. coli* O157:H7.¹ Gram-positive organisms should be completely inhibited. Gram-negative organisms, other than *E. coli* O157:H7, will either be inhibited or produce colorless, blue, green, blue-green (aqua) or natural color colonies.

X LIMITATIONS OF THE PROCEDURE

BBL CHROMagar O157 does not detect enterohemorrhagic or enteropathogenic serotypes of *E. coli* other than O157:H7, since they may differ biochemically. β-glucuronidase-positive strains of *E. coli* O157:H7 will not be detected on **BBL CHROMagar O157**; however, such strains are rare.

BBL CHROMagar O157 does not differentiate between toxin-producing and non-toxin-producing strains of *E. coli* O157:H7.

Organisms other than *E. coli* O157:H7, such as *Proteus* spp. may grow on this medium; however, they generally produce a different color. If unisolated mauve colonies are observed, isolation can be achieved by subculturing to another **BBL CHROMagar O157** plate. Rare strains of *E. coli* (biochemically similar to *Shigella*) have been found that produce false positive results on **BBL CHROMagar O157**.

Internal cross reactivity testing has demonstrated that *Salmonella* serotype Heidelberg exhibited mauve colonies when plated on **BBL CHROMagar O157** medium. As recommended, all mauve colonies should be confirmed by biochemical or serological testing prior to reporting results.

Confirmatory tests are necessary for definitive identification.

Incubation at lower than recommended temperatures may delay detection of positive reactions. If the incubation temperature is below 35 ± 2°C, the plates should be incubated a full 24 h before reporting as negative.

Plates are not to be incubated beyond the 24 h time period prior to reading.

XI PERFORMANCE CHARACTERISTICS

Analytical Testing

An interference study was conducted with substances that may be present in stool or rectal specimens. Fourteen (14) substances were tested which included lubricants, water, soap, laxatives, suppositories, and various hemorrhoidal treatments. None of the substances tested interfered with the performance of the **BBL CHROMagar O157** medium.

Internal testing of other stool pathogens was conducted in order to determine the potential cross reactivity of these organisms with **BBL CHROMagar O157**. Fifty-nine (59) non-*E. coli* O157:H7 organisms were tested, including selected species from the following genera: *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Aeromonas*, *Campylobacter*, and *Plesiomonas*.

Reproducibility testing was conducted at three different geographical locations (two [2] external clinical sites and one [1] internal) to demonstrate the ability of **BBL CHROMagar O157** to provide reproducible results with known microorganisms. A blinded panel of *E. coli* O157:H7 strains and non-*E. coli* O157:H7 strains were provided to each site for testing. Each panel was tested in triplicate on three days at each site. For all sites, the results for this study showed 100% reproducible results within each site and across all sites for the entire panel.

Clinical Testing

A clinical study was conducted at an external centralized regional clinical laboratory that routinely tests for *E. coli* O157:H7 in stool specimens. Stool specimens were inoculated onto Sorbitol-MacConkey (SMAC) and **BBL CHROMagar O157** media and incubated aerobically for 18–24 h at 35°C. Each plate was read by an independent technologist and confirmatory testing (indole and serotyping) was conducted on all suspected colony samples. A total of 3,136 stool specimens were cultured, of which 2,855 specimens provided acceptable results for this study while 281 specimens were determined to be noncompliant to the required testing matrix. The following table shows the breakdown of the results from the study:

| CHROMagar Result | SMAC Result | |
|------------------|-------------|----------|
| | Positive | Negative |
| Positive | 19 | 5 |
| Negative | 3 | 2828 |
| Totals | 22 | 2833 |

Positive Percent Agreement: 86.4%

Negative Percent Agreement: 99.8%

XII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 214984 | BBL™ CHROMagar™ O157 Prepared Plates – Pkg. of 20 plates |

XIII REFERENCES

1. Bopp, C.A., F.W. Brenner, P.I. Fields, J.G. Wells, and N.A. Strockbine. 2003. *Escherichia*, *Shigella* and *Salmonella*. In P.R. Murray, E.J. Baron, M.A. Tenover, F.C. Tenover, J.H. Jorgensen and R.H. Tenover (eds.), Manual of clinical microbiology. 8th ed. American Society for Microbiology, Washington DC.
2. CDC MMWR January 26, 2001/50 (RR02):1-69. Diagnosis and management of foodborne illness.
3. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections. 3rd ed., CLSI, Wayne, Pa.
4. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
5. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
6. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.

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**QUALITY CONTROL PROCEDURES****I INTRODUCTION**

BBL™ CHROMagar™ Orientation is a nonselective medium for the isolation, differentiation and enumeration of urinary tract pathogens.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with dilutions of the cultures listed below.
 - Streak inoculate with 10^3 - 10^4 CFUs of all organisms.
 - Incubate plates at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.
 - Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
- Examine plates after 18–24 h for amount of growth and color formation.
- Expected Results

| Organisms | ATCC™ | Recovery | Colony Color |
|-------------------------------------|-------|---|--|
| * <i>Enterobacter cloacae</i> | 13047 | Fair to heavy growth | Dark blue to medium blue with or without violet halos in the surrounding medium |
| * <i>Enterococcus faecalis</i> | 29212 | Fair to heavy growth of small size colonies | Blue-green |
| * <i>Escherichia coli</i> | 25922 | Fair to heavy growth of medium to large size colonies | Transparent, dark rose to pink, with or without halos |
| <i>Klebsiella pneumoniae</i> | 33495 | Fair to heavy growth | Medium blue to dark blue, mucoid |
| * <i>Proteus mirabilis</i> | 43071 | Fair to heavy growth of medium size colonies. Swarming is partially to completely inhibited | Transparent, pale beige to brown, surrounded by a brown halo. In areas of dense growth, the medium may be completely orange-brown. |
| * <i>Staphylococcus aureus</i> | 25923 | Fair to heavy growth of small to medium size colonies | White to cream (natural pigmentation) |
| <i>Staphylococcus epidermidis</i> | 12228 | Fair to heavy growth | White to cream (natural pigmentation) |
| <i>Staphylococcus saprophyticus</i> | 15305 | Fair to heavy growth | Light pink to rose |
| * <i>Streptococcus agalactiae</i> | 12386 | Fair to heavy growth of pinpoint to small size colonies | Light blue-green to light blue with or without halos |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification 6.9 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates aerobically at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION**IV INTENDED USE**

BBL™ CHROMagar™ Orientation medium is a nonselective differentiated medium for the isolation, differentiation and enumeration of urinary tract pathogens. **BBL CHROMagar** Orientation medium allows for the differentiation and identification of *Escherichia coli* and *Enterococcus* without confirmatory testing.

U.S. Patent Nos. 5,716,799; 5,962,251

V SUMMARY AND EXPLANATION

Escherichia coli, enterococci, the *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups are frequently encountered organisms in urinary tract infections (UTI). Most UTIs are caused by *E. coli* alone, or in combination with enterococci. *Staphylococcus saprophyticus* and *Streptococcus agalactiae* may be isolated from females, although less frequently.

Due to the different antimicrobial susceptibility patterns of the microorganisms involved, identification to the species level is necessary for effective antimicrobial therapy. The most frequently isolated species or organism groups produce characteristic enzymes. Thus, it is possible to identify these organisms to the species level with a limited number of substrate fermentation or utilization tests.¹

Some of the organisms encountered in UTIs produce enzymes either for the metabolism of lactose or glucosides or both. Other organisms produce none of these enzymes. For example, *E. coli* contains enzymes for lactose metabolism but is β -glucosidase negative. Some members of the family *Enterobacteriaceae* are β -glucosidase positive but do not contain enzymes necessary for

lactose fermentation; others may contain both types of enzymes or none of them. β -glucosidases are also found in gram-positive cocci, such as *S. agalactiae* and the enterococci. Tryptophan deaminase (TDA) is an enzyme characteristically found in the *Proteus-Morganella-Providencia* group.

CHROMagar Orientation medium was developed by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

VI PRINCIPLES OF THE PROCEDURE

Specially selected peptones supply the nutrients in **BBL CHROMagar** Orientation medium. The chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests. *Proteus* swarming is partially to completely inhibited.

VII REAGENTS

BBL CHROMagar Orientation

Approximate Formula* Per Liter Purified Water

| | |
|---------------------|--------|
| Chromopeptone | 16.1 g |
| Chromogen Mix | 1.3 g |
| Agar | 15.0 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and box until time of inoculation. Plates may be used up until the expiration date.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying or cracking.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

IX PROCEDURE

Material Provided: BBL CHROMagar Orientation

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. Allow the medium to warm to room temperature before inoculation.

A dilution of the specimen on the plate (by using calibrated loops or other techniques commonly used for plating urine specimens) is required to obtain isolated colonies with typical colors and morphology. Incubate plates aerobically at 35 ± 2°C for not less than 20 to 24 h in an inverted position (agar-side up). Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation as light may destroy the chromogens. Once the colony color develops, exposure to light is permissible.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation, the plates should show isolated colonies in the areas where the inoculum was diluted appropriately. Table 1 and Scheme 1 should be used for identification or differentiation and as a guideline for additional confirmatory reactions. A Gram stain and microscopic examination can be used to confirm results.

Confirmatory Tests: BBL CHROMagar Orientation has been validated as an acceptable medium for both identification and antimicrobial susceptibility testing on the **BD Phoenix™** System.

Do not apply any detection reagents directly onto the colonies growing on the medium. Perform the tests on filter paper with growth from the respective colonies.

For *E. coli* colonies that are dark rose to pink, but are pinpoint to small in size, do not use Kovacs' indole reagent, as the colony color may interfere with the red color of a positive indole test. Use only dimethylaminocinnamaldehyde (DMACA) indole reagent.

If other confirmatory tests or biochemical identification systems are used, follow the instructions accompanying the identification systems.

Perform confirmatory testing for *Enterococcus* only if speciation beyond the genus level is required.

Table 1: Guidelines for Identification Based on Different Colony Colors

| Organism | Appearance on BBL CHROMagar Orientation Medium | Confirmatory Tests (Necessary for further differentiation) |
|--|--|---|
| <i>E. coli</i> * | Dark rose to pink, transparent colonies, medium to large size, with or without halos in the surrounding medium | |
| KES group | Medium-blue to dark blue colonies | BBL™ Crystal™ E/NF for differentiation within the genera |
| PMP group | Pale to beige colonies surrounded by brown halos** | Indole, H ₂ S, ODC, BBL Crystal E/NF for differentiation within the genera |
| <i>Enterococcus</i> | Blue-green small colonies | |
| <i>S. agalactiae</i> * | Light blue-green to light blue, pinpoint to small colonies, with or without halos | PYR |
| <i>S. saprophyticus</i> (most strains) | Light pink to rose, small opaque colonies with or without halos | 5 µg Novobiocin disc |
| Other including yeasts | Natural (cream) pigmentation | Appropriate biochemical or serological identification methods |

Scheme 1: Guidelines for the Performance of Identification Tests on Select Organisms

| Colony Appearance | | | | |
|--|------------------------|----------------------------|---|---|
| Small, rose, opaque | ⇒ Novobiocin 5 µg disc | ⇒ sensitive ⇒ resistant | ⇒ <i>S. intermedius</i> ⇒ <i>S. xylosus</i> ⇒ <i>S. saprophyticus</i> | ⇒ Identify species with biochemical tests |
| Colorless to beige colonies, orange-brown medium | ⇒ PMP group | ⇒ DMACA | ⇒ green (positive) ↓ H ₂ S positive ⇒ <i>P. vulgaris</i> H ₂ S negative ⇒ <i>Providencia</i> spp. H ₂ S negative ⇒ <i>Morganella</i> spp. ⇒ colorless to rose (negative) ↓ ODC positive ⇒ <i>P. mirabilis</i> ODC negative ⇒ <i>P. penneri</i> | |

* See "Limitations of the Procedure."

** About 50% of *P. vulgaris* strains produce blue colonies on a brownish halo.

Key: KES = *Klebsiella-Enterobacter-Serratia* group; PMP = *Proteus-Morganella-Providencia* group; ODC = Conventional ornithine decarboxylase test; H₂S = Conventional hydrogen sulfide test; DMACA = Indole test performed with DMACA (dimethylaminocinnamaldehyde) reagent.

XI LIMITATIONS OF THE PROCEDURE

As this medium is nonselective, other UTI pathogens will grow. Colonies that show their natural color and do not react with the chromogenic substrates must be further differentiated with appropriate biochemical or serological tests to confirm identification.

E. coli colonies that are dark rose to pink but are pinpoint to small in size, require additional confirmatory tests such as spot indole (DMACA indole reagent).

Gram-negative organisms other than those belonging to the KES group may produce large blue colonies and thus require other biochemical tests for identification.

In very rare cases, *Listeria monocytogenes* or other *Listeria* species may be present in urine (e.g., after abortion due to these agents). *Listeria* will produce blue to blue-green colonies that are PYR negative, mimicking *Streptococcus agalactiae*. Therefore, it may be useful to perform a Gram stain of organisms producing small, blue to blue-green colonies on this medium that are PYR negative. The presence of gram-positive bacilli may be indicative of *Listeria* species, but additional biochemical tests are necessary to confirm their identification.

Very rarely, isolates of *Aeromonas hydrophila* may produce rose colonies. They may be differentiated from *E. coli* with the oxidase test (*Aeromonas* = positive; *E. coli* = negative).

This medium will not support the growth of fastidious organisms, such as *Neisseria* spp., *Haemophilus* spp. or *Mycoplasma* spp. Use of this medium for non-clinical or clinical specimens other than urine has not been documented.

Minimize exposure of BBL CHROMagar Orientation medium to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.

XII PERFORMANCE CHARACTERISTICS

Clinical studies have demonstrated that BBL CHROMagar Orientation medium has advantages over other differential media used in the isolation, differentiation and enumeration of UTI pathogens, such as CLED Agar or a combination of Blood and MacConkey Agars.²⁻⁴ Presumptive identification of *S. saprophyticus*, *S. agalactiae*, *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups is possible by means of colony morphology, pigmentation and medium discoloration.

Further testing must be performed for confirmation. (See Table 1)

BBL CHROMagar Orientation medium allows for the differentiation and identification of *E. coli* and enterococci without confirmatory testing, based on the criteria for identification established by the CLSI standard M35-A, "Abbreviated Identification of Bacteria and Yeast; Approved Guideline."⁹ In a blinded internal study which included testing of over 900 bacterial strains seeded in urine, the sensitivity and specificity of BBL CHROMagar Orientation identification of *E. coli*, based on

colony color and morphology only, were 97% and 99%, respectively; for *Enterococcus* the sensitivity and specificity of identification were 99% and 97%, respectively (see table).

| Organism | Sensitivity % (95% Confidence Interval) | Specificity % (95% Confidence Interval) |
|---------------------|--|--|
| <i>E. coli</i> | 277/286 96.9% (94.1-98.6%) | 638/645 98.9% (97.8-99.6%) |
| <i>Enterococcus</i> | 319/324 98.5% (96.4-99.5%) | 603/622 97% (95.3-98.2%) |

XIII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 254102 | BBL™ CHROMagar™ Orientation, Pkg. of 20 plates |
| 215081 | BBL™ CHROMagar™ Orientation, Ctn. of 100 plates |

XIV REFERENCES

1. Isenberg, H.D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology. Washington, D.C.
2. Merlino, J., S. Siarakas, G.J. Robertson, G.R. Funnell, T. Gottlieb, and R. Bradbury. 1996. Evaluation of **CHROMagar** Orientation for differentiation and presumptive identification of gram-negative bacilli and *Enterococcus* species. J. Clin. Microbiol. 34:1788-1793.
3. Hengstler, K.A., R. Hammann, and A.M. Fahr. 1997. Evaluation of **BBL CHROMagar** Orientation medium for detection and presumptive identification of urinary tract pathogens. J. Clin. Microbiol. 35:2773-2777.
4. Samra, Z., M. Heifetz, J. Talmor, E. Bain, and J. Bahar. 1998. Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. J. Clin. Microbiol. 36:990-994.
5. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
6. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
7. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
9. Clinical and Laboratory Standards Institute. 2002. Approved Guideline M35-A. Abbreviated identification of bacteria and yeast, CLSI, Wayne, PA.

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**BBL™ CHROMagar™ Orientation and
BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)—I Plate™**
8083714 • Rev. 01 • October 2008

QUALITY CONTROL PROCEDURES

I INTRODUCTION

BBL™ CHROMagar™ Orientation is a nonselective medium for the isolation, differentiation and enumeration of urinary tract pathogens.

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood is used for the growth of fastidious organisms and for the visualization of hemolytic reactions.

II PERFORMANCE TEST PROCEDURE

A. BBL CHROMagar Orientation

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak inoculate with 10³-10⁴ CFUs of all organisms.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth and color formation.
3. Expected Results

| Organisms | ATCC™ | Recovery | Colony Color |
|-------------------------------------|-------|---|--|
| * <i>Enterobacter cloacae</i> | 13047 | Fair to heavy growth | Dark blue to medium blue with or without violet halos in the surrounding medium |
| * <i>Enterococcus faecalis</i> | 29212 | Fair to heavy growth of small size colonies | Blue-green |
| * <i>Escherichia coli</i> | 25922 | Fair to heavy growth of medium to large size colonies | Transparent, dark rose to pink, with or without halos |
| <i>Klebsiella pneumoniae</i> | 33495 | Fair to heavy growth | Medium blue to dark blue, mucoid |
| * <i>Proteus mirabilis</i> | 43071 | Fair to heavy growth of medium size colonies. Swarming is partially to completely inhibited | Transparent, pale beige to brown, surrounded by a brown halo. In areas of dense growth, the medium may be completely orange-brown. |
| * <i>Staphylococcus aureus</i> | 25923 | Fair to heavy growth of small to medium size colonies | White to cream (natural pigmentation) |
| <i>Staphylococcus epidermidis</i> | 12228 | Fair to heavy growth | White to cream (natural pigmentation) |
| <i>Staphylococcus saprophyticus</i> | 15305 | Fair to heavy growth | Light pink to rose |
| * <i>Streptococcus agalactiae</i> | 12386 | Fair to heavy growth of pinpoint to small size colonies | Light blue-green to light blue with or without halos |

*Recommended organism strain for User Quality Control.

B. Trypticase Soy Agar with 5% Sheep Blood

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak inoculate with 10³-10⁴ CFUs of all organisms.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth and hemolytic reactions.
3. Expected Results

| CLSI Organisms | ATCC™ | Recovery |
|-----------------------------------|-------|-------------------------|
| * <i>Streptococcus pyogenes</i> | 19615 | Growth, beta hemolysis |
| * <i>Streptococcus pneumoniae</i> | 6305 | Growth, alpha hemolysis |
| * <i>Staphylococcus aureus</i> | 25923 | Growth |
| * <i>Escherichia coli</i> | 25922 | Growth |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.9 ± 0.2 (**CHROMagar** Orientation) and 7.4 ± 0.2 (**Trypticase** Soy Agar with 5% Sheep Blood).
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ Orientation medium is a nonselective differentiated medium for the isolation, differentiation and enumeration of urinary tract pathogens. **BBL CHROMagar** Orientation medium allows for the differentiation and identification of *Escherichia coli* and *Enterococcus* without confirmatory testing.

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

U.S. Patent Nos. 5,716,799; 5,962,251

V SUMMARY AND EXPLANATION

A. BBL CHROMagar Orientation

Escherichia coli, enterococci, the *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups are frequently encountered organisms in urinary tract infections (UTI). Most UTIs are caused by *E. coli* alone, or in combination with enterococci. *Staphylococcus saprophyticus* and *Streptococcus agalactiae* may be isolated from females, although less frequently.

Due to the different antimicrobial susceptibility patterns of the microorganisms involved, identification to the species level is necessary for effective antimicrobial therapy. The most frequently isolated species or organism groups produce characteristic enzymes. Thus, it is possible to identify these organisms to the species level with a limited number of substrate fermentation or utilization tests.¹

Some of the organisms encountered in UTIs produce enzymes either for the metabolism of lactose or glucosides or both. Other organisms produce none of these enzymes. For example, *E. coli* contains enzymes for lactose metabolism but is β -glucosidase negative. Some members of the family *Enterobacteriaceae* are β -glucosidase positive but do not contain enzymes necessary for lactose fermentation; others may contain both types of enzymes or none of them. β -glucosidases are also found in gram-positive cocci, such as *S. agalactiae* and the enterococci. Tryptophan deaminase (TDA) is an enzyme characteristically found in the *Proteus-Morganella-Providencia* group.

CHROMagar Orientation medium was developed by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

B. BBL Trypticase Soy Agar with 5% Sheep Blood

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% Sheep Blood is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiating characteristics for bacteria, especially *Streptococcus* species.

VI PRINCIPLES OF THE PROCEDURE

A. BBL CHROMagar Orientation

Specially selected peptones supply the nutrients in **BBL CHROMagar** Orientation medium. The chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests. *Proteus* swarming is partially to completely inhibited.

B. BBL Trypticase Soy Agar with 5% Sheep Blood

The combination of casein and soy peptones in the **Trypticase** Soy Agar base render the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium.

Defibrinated sheep blood is the most widely used blood for enriching agar base media.² Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

Trypticase Soy Agar with 5% Sheep Blood (TSA II) provides excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provides excellent growth and appropriate hemolytic reactions with other fastidious organisms. It is suitable for use with low concentration (0.04 unit) bacitracin discs (**Taxo™** A) for presumptive identification of group A streptococci (*S. pyogenes*).

VII REAGENTS

BBL CHROMagar Orientation

Approximate Formula* Per Liter Purified Water

| | |
|---------------------|--------|
| Chromopeptone | 16.1 g |
| Chromogen Mix | 1.3 g |
| Agar | 15.0 g |

*Adjusted and/or supplemented as required to meet performance criteria.

BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II)

Approximate Formula* Per Liter Purified Water

| | | | |
|-------------------------------------|--------|--------------------------------|--------|
| Pancreatic Digest of Casein | 14.5 g | Agar | 14.0 g |
| Papaic Digest of Soybean Meal | 5.0 g | Growth Factors | 1.5 g |
| Sodium Chloride | 5.0 g | Defibrinated Sheep Blood | 5% |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be

inoculated up to the expiration date and incubated for recommended incubation time. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{3,4} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

IX PROCEDURE

Material Provided: BBL CHROMagar Orientation and BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II)–I Plate

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

A dilution of the specimen on the plate (by using calibrated loops or other techniques commonly used for plating urine specimens) is required to obtain isolated colonies with typical colors and morphology. Incubate plates aerobically at 35 ± 2°C for not less than 20–24 h in an inverted position (agar-side up). Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation as light may destroy the chromogens. Once the colony color develops, exposure to light is permissible.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

A. BBL CHROMagar Orientation

After incubation, the plates should show isolated colonies in the areas where the inoculum was diluted appropriately. Table 1 and Scheme 1 should be used for identification or differentiation and as a guideline for additional confirmatory reactions. A Gram stain and microscopic examination can be used to confirm results.

Confirmatory Tests: BBL CHROMagar Orientation has been validated as an acceptable medium for both identification and antimicrobial susceptibility testing on the BD Phoenix™ System.

Do not apply any detection reagents directly onto the colonies growing on the medium. Perform the tests on filter paper with growth from the respective colonies.

For *E. coli* colonies that are dark rose to pink, but are pinpoint to small in size, do not use Kovacs' indole reagent, as the colony color may interfere with the red color of a positive indole test. Use only dimethylaminocinnamaldehyde (DMACA) indole reagent.

If other confirmatory tests or biochemical identification systems are used, follow the instructions accompanying the identification systems.

Perform confirmatory testing for *Enterococcus* only if speciation beyond the genus level is required.

Table 1: Guidelines for Identification Based on Different Colony Colors

| Organism | Appearance on BBL CHROMagar Orientation Medium | Confirmatory Tests (Necessary for further differentiation) |
|--|--|---|
| <i>E. coli</i> * | Dark rose to pink, transparent colonies, medium to large size, with or without halos in the surrounding medium | |
| KES group | Medium-blue to dark blue colonies | BBL™ Crystal™ E/NF for differentiation within the genera |
| PMP group | Pale to beige colonies surrounded by brown halos** | Indole, H ₂ S, ODC, BBL Crystal E/NF for differentiation within the genera |
| <i>Enterococcus</i> | Blue-green small colonies | |
| <i>S. agalactiae</i> * | Light blue-green to light blue, pinpoint to small colonies, with or without halos | PYR |
| <i>S. saprophyticus</i> (most strains) | Light pink to rose, small opaque colonies with or without halos | 5 µg Novobiocin disc |
| Other including yeasts | Natural (cream) pigmentation | Appropriate biochemical or serological identification methods |

Scheme 1: Guidelines for the Performance of Identification Tests on Select Organisms

Colony Appearance

| | | | | |
|--|------------------------|----------------------------|---|---|
| Small, rose, opaque | ⇒ Novobiocin 5 µg disc | ⇒ sensitive ⇒ resistant | ⇒ <i>S. intermedius</i> ⇒ <i>S. xylosum</i> ⇒ <i>S. saprophyticus</i> | ⇒ Identify species with biochemical tests |
| Colorless to beige colonies, orange-brown medium | ⇒ PMP group | ⇒ DMACA | ⇒ green (positive) ↓ H ₂ S positive ⇒ <i>P. vulgaris</i> H ₂ S negative ⇒ <i>Providencia</i> spp. H ₂ S negative ⇒ <i>Morganella</i> spp. ⇒ colorless to rose (negative) ↓ ODC positive ⇒ <i>P. mirabilis</i> ODC negative ⇒ <i>P. penneri</i> | |

* See "Limitations of the Procedure."

** About 50% of *P. vulgaris* strains produce blue colonies on a brownish halo.

Key: KES = *Klebsiella-Enterobacter-Serratia* group; PMP = *Proteus-Morganella-Providencia* group; ODC = Conventional ornithine decarboxylase test; H₂S = Conventional hydrogen sulfide test; DMACA = Indole test performed with DMACA (dimethylaminocinnamaldehyde) reagent.

B. BBL Trypticase Soy Agar with 5% Sheep Blood

Typical results on **Trypticase Soy Agar with 5% Sheep Blood** are as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2–4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.)
2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
4. *Listeria*. Small zones of beta hemolysis are produced. They may be distinguished by their rod shape in stains, and by motility at room temperature.
5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

XI LIMITATIONS OF THE PROCEDURE**A. BBL CHROMagar Orientation**

As this medium is nonselective, other UTI pathogens will grow. Colonies that show their natural color and do not react with the chromogenic substrates must be further differentiated with appropriate biochemical or serological tests to confirm identification.

E. coli colonies that are dark rose to pink but are pinpoint to small in size, require additional confirmatory tests such as spot indole (DMACA indole reagent).

Gram-negative organisms other than those belonging to the KES group may produce large blue colonies and thus require other biochemical tests for identification.

In very rare cases, *Listeria monocytogenes* or other *Listeria* species may be present in urine (e.g., after abortion due to these agents). *Listeria* will produce blue to blue-green colonies that are PYR negative, mimicking *Streptococcus agalactiae*. Therefore, it may be useful to perform a Gram stain of organisms producing small, blue to blue-green colonies on this medium that are PYR negative. The presence of gram-positive bacilli may be indicative of *Listeria* species, but additional biochemical tests are necessary to confirm their identification.

Very rarely, isolates of *Aeromonas hydrophila* may produce rose colonies. They may be differentiated from *E. coli* with the oxidase test (*Aeromonas* = positive; *E. coli* = negative).

This medium will not support the growth of fastidious organisms, such as *Neisseria* spp., *Haemophilus* spp. or *Mycoplasma* spp. Use of this medium for non-clinical or clinical specimens other than urine has not been documented.

Minimize exposure of **BBL CHROMagar Orientation** medium to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.

B. Both Media

Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate texts for detailed information and recommended procedures.^{3,4,9-11}

XII PERFORMANCE CHARACTERISTICS**A. BBL CHROMagar Orientation**

Clinical studies have demonstrated that **BBL CHROMagar Orientation** medium has advantages over other differential media used in the isolation, differentiation and enumeration of UTI pathogens, such as CLED Agar or a combination of Blood and MacConkey Agars.¹²⁻¹⁴ Presumptive identification of *S. saprophyticus*, *S. agalactiae*, *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups is possible by means of colony morphology, pigmentation and medium discoloration.

Further testing must be performed for confirmation. (See Table 1)

BBL CHROMagar Orientation medium allows for the differentiation and identification of *E. coli* and enterococci without confirmatory testing, based on the criteria for identification established by the CLSI standard M35-A, "Abbreviated Identification of Bacteria and Yeast; Approved Guideline."¹⁵ In a blinded internal study which included testing of over 900 bacterial strains seeded in urine, the sensitivity and specificity of **BBL CHROMagar Orientation** identification of *E. coli*, based on colony color and morphology only, were 97% and 99%, respectively; for *Enterococcus* the sensitivity and specificity of identification were 99% and 97%, respectively (see table).

| Organism | Sensitivity % (95% Confidence Interval) | Specificity % (95% Confidence Interval) |
|---------------------|--|--|
| <i>E. coli</i> | 277/286 96.9% (94.1-98.6%) | 638/645 98.9% (97.8-99.6%) |
| <i>Enterococcus</i> | 319/324 98.5% (96.4-99.5%) | 603/622 97% (95.3-98.2%) |

B. BBL Trypticase Soy Agar with 5% Sheep Blood

Trypticase Soy Agar with 5% Sheep Blood was used as a control in a study using broth-enhanced culture (Todd Hewitt) and Optical Immunoassay method for the diagnosis of β -hemolytic streptococcal infection. Five hundred two (502) specimens were tested. TSA with 5% Sheep Blood had a sensitivity and specificity of 92.5% and 99.4%, respectively.¹⁶ Nguyen et al. used **Trypticase Soy Agar with 5% Sheep Blood** as the 'gold standard' for the detection of group B *Streptococcus* from the lower genital tract of pregnant women.¹⁷ In another study, Rossmann et al. successfully reisolated *Lautropia mirabilis* on **Trypticase Soy Agar with 5% Sheep Blood** from the oral cavities of human immunodeficiency virus infected children.¹⁸ Of the 85 children evaluated in this study, 35 (41.4%) were positive for *L. mirabilis*. Isenberg et al. used **Trypticase Soy Agar with 5% Sheep Blood** as a control to evaluate the recovery of *Enterococcus* from a selective medium under study.¹⁹ Two hundred fifty (250) group D streptococcal strains isolated from clinical material and 8 strains obtained from the National Communicable Disease Center (Atlanta, Ga.) were used.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 222239 | BBL™ CHROMagar™ Orientation and BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)–I Plate™ , Ctn. of 100 plates |

XIV REFERENCES

1. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.
2. Vera, H.D., and D.A. Power. 1980. Culture media, p. 969. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
6. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
7. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
9. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. J.B. Lippincott Company, Philadelphia.
10. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
11. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
12. Merlino, J., S. Siarakas, G.J. Robertson, G.R. Funnell, T. Gottlieb, and R. Bradbury. 1996. Evaluation of **CHROMagar Orientation** for differentiation and presumptive identification of gram-negative bacilli and *Enterococcus* species. J. Clin. Microbiol. 34:1788-1793.
13. Hengstler, K.A., R. Hammann, and A.M. Fahr. 1997. Evaluation of **BBL CHROMagar Orientation** medium for detection and presumptive identification of urinary tract pathogens. J. Clin. Microbiol. 35:2773-2777.
14. Samra, Z., M. Heifetz, J. Talmor, E. Bain, and J. Bahar. 1998. Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. J. Clin. Microbiol. 36:990-994.
15. Clinical and Laboratory Standards Institute. 2002. Approved Guideline M35-A. Abbreviated identification of bacteria and yeast, CLSI, Wayne, PA.
16. Fries, S.M. 1995. Diagnosis of group A streptococcal pharyngitis in a private clinic: comparative evaluation of an optical immunoassay method and culture. J. Pediatr. 126: 933-936.
17. Nguyen, T.M., et al. 1998. Detection of group B streptococcus: comparison of an optical immunoassay with direct plating and broth-enhanced culture methods. J. Matern. Fetal. Med. 7:172-176.
18. Rossmann, S.N. et al. 1998. Isolation of *Lautropia mirabilis* from oral cavities of human immunodeficiency virus-infected children. J. Clin. Microbiol. 36:1756-1760.
19. Isenberg, H.D., D. Goldberg, and J. Sampson, 1970. Laboratory studies with a selective medium. Appl. Microbiol. 20: 433-436.

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QUALITY CONTROL PROCEDURES

*See footnote below

I INTRODUCTION

BBL™ CHROMagar™ Salmonella is a selective and differential medium for the isolation and presumptive identification of *Salmonella* species.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak inoculate with 10³-10⁴ CFUs of *S. Typhimurium* and *S. Typhi* and 10⁴-10⁵ CFUs of all other organisms.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)** plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth, and up to 48 h for color formation of *Salmonella*.
3. Expected Results

| Organisms | ATCC™ | Recovery | Colony Color |
|--|-------|----------------------------------|--------------------------------|
| <i>Escherichia coli</i> | 25922 | Inhibition (partial to complete) | Blue to blue-green |
| * <i>Citrobacter freundii</i> | 8090 | Fair to heavy growth | Light blue-green to blue-green |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhi | 19430 | Fair to heavy growth | Mauve |
| * <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium | 14028 | Fair to heavy growth | Light mauve to mauve |
| * <i>Staphylococcus aureus</i> | 25923 | Inhibition (partial to complete) | Cream |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification 7.6 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ Salmonella is a selective and differential medium for the isolation and presumptive identification of *Salmonella* species from other coliform and non-coliform bacteria in clinical stool samples and a variety of food samples.

BBL CHROMagar Salmonella has been validated by the AOAC™ Research Institute under the Performance Tested MethodsSM program only for the analysis of raw ground beef, raw chicken, raw fish, lettuce and shell eggs. ISO, USDA FSIS and FDA BAM methods were used for method comparison testing.¹⁻³ **BBL CHROMagar Salmonella** was found to be equivalent to the plated media recommended in the ISO, FDA and USDA methods.

U.S. Patent Nos. 5,098,832; 5,194,374

V SUMMARY AND EXPLANATION

Salmonella is ubiquitous in animal populations and is generally isolated from the intestinal tract of animals and humans. It is one of the most prevalent organisms associated with foodborne illnesses, which is often linked to animal origin.⁴ Illnesses caused by *Salmonella* have been associated with poultry, beef, chocolate, dairy and vegetable products.⁵

BBL CHROMagar Salmonella is intended for the isolation and differentiation of *Salmonella* species. The addition of chromogenic substrates in the medium facilitates detection of *Salmonella* species from other flora.

BBL CHROMagar Salmonella was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar Salmonella** prepared plated medium using the **Difco™ CHROMagar Salmonella** dehydrated culture medium formulation.

VI PRINCIPLES OF THE PROCEDURE

Specially selected peptones supply the nutrients. Gram-positive organisms are generally inhibited as a result of the selective medium base. The addition of an antifungal agent prevents the growth of *Candida* species and other antimicrobial agents are used to inhibit the growth of gram-negative, non-glucose fermenting bacteria and *Proteus* species, which could potentially overgrow *Salmonella* colonies. A chromogenic mixture is included in the medium. Due to metabolic differences in the presence of selected chromogens, colonies of *Salmonella* species appear mauve (rose to purple) in color, whereas undesired bacteria are either inhibited, or produce blue-green or colorless colonies.

*PRODUCER-SUPPLIED SAMPLES OF THIS TEST KIT MODEL WERE INDEPENDENTLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AND WERE FOUND TO PERFORM TO THE PRODUCER'S SPECIFICATIONS AS STATED IN THE TEST KIT'S DESCRIPTIVE INSERT. THE PRODUCER CERTIFIES THIS KIT CONFORMS IN ALL RESPECTS TO THE SPECIFICATIONS ORIGINALLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AS DETAILED IN *Performance Tested MethodsSM* CERTIFICATE NUMBER 020502.

VII REAGENTS

BBL CHROMagar Salmonella

Approximate Formula* Per Liter Purified Water

| | |
|-------------------------|--------|
| Chromopeptone | 22.0 g |
| Chromogenic Mix | 0.34 g |
| Inhibitory Agents | 0.02 g |
| Agar | 15.0 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Protect from light during drying. See Storage Instructions.

To become familiar with the expected chromogenic (color) reactions produced by *Salmonella*, it is recommended that the user inoculate representative strains commonly observed in their institution. The following strains are suggested: *Salmonella* ser. Typhimurium, ATCC™ 14028; *Salmonella* ser. Dublin, ATCC 15480; *Salmonella* ser. Typhi, ATCC 19430; and *Salmonella enterica* subsp. *arizonae*, ATCC 12323.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and original cardboard box until time of inoculation. Plates may be used up until the expiration date. Minimize the exposure of BBL CHROMagar Salmonella to light both before and during incubation, as light may destroy the chromogen.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying or cracking.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of sample or specimen collection and handling procedures.¹⁻⁵

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁶⁻⁹ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

IX PROCEDURE

Material Provided: BBL CHROMagar Salmonella

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required for the specific laboratory procedure in use, such as ISO 7218, USDA FSIS MLG, FDA BAM or your specific laboratory procedure.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. Allow the medium to warm to room temperature before inoculation.

For clinical specimens: As soon as possible after receipt in the laboratory, inoculate the specimen onto a BBL CHROMagar Salmonella plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2 °C in an inverted position (agar-side up) for 24 h. If negative at 24 h, reincubate for an additional 24 h to report final results. Once the colony color develops, exposure to light is permissible. Typical colonies of *Salmonella* should be subjected to confirmatory biochemical or serological testing.

For food samples: Follow sample preparation methodology as outlined in USDA FSIS's *Microbiology Laboratory Guidebook: Isolation and Identification of Salmonella from Meat, Poultry, and Egg Products*, FDA BAM's chapter on *Salmonella*, ISO guidelines or the procedure guidelines appropriate to sample type and geographic location.

Inoculate the incubated enrichment broth sample onto a BBL CHROMagar Salmonella plate. Streak for isolation, incubate plates aerobically at 35 ± 2°C in an inverted position (agar side up) for 24 h. If negative at 24 h, reincubate for an additional 24 h to report final results. Typical colonies of *Salmonella* growing on BBL CHROMagar Salmonella should be subjected to confirmatory testing as outlined in ISO, USDA FSIS and FDA BAM procedures.¹⁻³

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that clinical users refer to pertinent Clinical Laboratory and Standards Institute (CLSI) (formerly known as NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After proper incubation, read plates against a white background. *Salmonella* Typhimurium and other *Salmonella* species will appear as light mauve to mauve-colored colonies, with the exception of *Salmonella enterica* subspecies *arizonae* and other *Salmonella* species positive for lactose and beta-glucosidase. Those isolates will appear as blue-violet or purple colonies. *Citrobacter* and other coliforms will appear as light blue-green to blue-green colored colonies. Some organisms that do not hydrolyze any of the chromogenic compounds may appear as colorless colonies.

XI LIMITATIONS OF THE PROCEDURE

Occasionally strains of *Aeromonas hydrophila*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *P. putida*, *Acinetobacter* species, or *Candida* species may not be completely inhibited and colonies may exhibit light mauve to mauve pigmentation.

Confirmatory tests that use mauve or purple as an indicator color reaction may be difficult to interpret due to the actual colony color.

Rare strains of the following organisms: *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium*, *S. Choleraesuis*, *S. Minnesota*, *S. enterica* subsp. *arizonae*, and *S. Pullorum* may fail to grow or have reduced growth on this medium. This is strain specific and the majority of the strains tested of each of these serovars were recovered.

BBL CHROMagar Salmonella is not designed for the isolation of intestinal pathogens other than *Salmonella*. When testing some samples, a purple discoloration of the medium, without detectable colony growth, may be observed. This should be considered a negative result.

Minimize exposure of **BBL CHROMagar** Salmonella to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period. Incubation in CO₂ is not recommended.

XII EXPECTED VALUES

The following organisms were isolated during internal and external evaluations of clinical and industrial samples:

| | | |
|---|-------------------------------------|---|
| <i>Salmonella</i> ser. Abony | <i>Salmonella</i> ser. Gallinarum | <i>Salmonella</i> ser. Oranienburg |
| <i>Salmonella</i> ser. Adelaide | <i>Salmonella</i> ser. Gaminara | <i>Salmonella</i> ser. Panama |
| <i>Salmonella</i> ser. Agona | <i>Salmonella</i> ser. Hadar | <i>Salmonella</i> ser. Paratyphi A |
| <i>Salmonella</i> ser. Anatum | <i>Salmonella</i> ser. Hartford | <i>Salmonella</i> ser. Paratyphi B |
| <i>Salmonella</i> ser. Bareilly | <i>Salmonella</i> ser. Heidelberg | <i>Salmonella</i> ser. Pomona |
| <i>Salmonella</i> ser. Berta | <i>Salmonella</i> ser. Illinois | <i>Salmonella</i> ser. Poona |
| <i>Salmonella</i> ser. Brandenburg | <i>Salmonella</i> ser. Infantis | <i>Salmonella</i> ser. Potsdam |
| <i>Salmonella</i> ser. California | <i>Salmonella</i> ser. Iverness | <i>Salmonella</i> ser. Pullorum |
| <i>Salmonella</i> ser. Cerro | <i>Salmonella</i> ser. Javiana | <i>Salmonella</i> ser. Rubislaw |
| <i>Salmonella enterica</i> subsp. <i>arizonae</i> | <i>Salmonella</i> ser. Johannesburg | <i>Salmonella</i> ser. Schwarzengrund |
| <i>Salmonella</i> ser. Choleraesuis | <i>Salmonella</i> ser. Kentucky | <i>Salmonella</i> ser. Senftenberg |
| <i>Salmonella</i> ser. Cubana | <i>Salmonella</i> ser. London | <i>Salmonella</i> ser. St. Paul |
| <i>Salmonella</i> ser. Derby | <i>Salmonella</i> ser. Mbandaka | <i>Salmonella</i> ser. Thompson |
| <i>Salmonella enterica</i> subsp. <i>diarizonae</i> | <i>Salmonella</i> ser. Michigan | <i>Salmonella</i> ser. Typhi |
| <i>Salmonella</i> ser. DT 104 | <i>Salmonella</i> ser. Minnesota | <i>Salmonella</i> ser. Typhimurium |
| <i>Salmonella</i> ser. Dublin | <i>Salmonella</i> ser. Montevideo | <i>Salmonella</i> ser. Typhimurium (lactose positive) |
| <i>Salmonella</i> ser. Enteritidis | <i>Salmonella</i> ser. Muenster | <i>Salmonella</i> ser. Weltevreden |
| <i>Salmonella</i> ser. Essen | <i>Salmonella</i> ser. Newport | <i>Salmonella</i> 8, (20):-:26 |

XIII PERFORMANCE CHARACTERISTICS

Clinical Testing:

BBL CHROMagar Salmonella was tested at a large diagnostic laboratory. A total of 150 known negative stool specimens and 110 known positive stool specimens were tested on **BBL CHROMagar** Salmonella and compared to the performance of XLD and Hektoen Enteric media. The sensitivity and specificity of **BBL CHROMagar** Salmonella medium after 18-24 h of incubation were 76% and 99%, respectively; and after 48 h of incubation were 90% and 94%, respectively. The sensitivity and specificity increased to 99% and 97%, respectively, when using Selenite F broth. Comparative sensitivity and specificity results for XLD medium were 71% and 97% at 18-24 h incubation, and 78% and 95% at 48 h incubation; sensitivity and specificity results for Hektoen Enteric medium were 71% and 94% at 18-24 h, and 79% and 93% at 48 h incubation.

Agrifood Testing:

USDA and FDA Methods

BBL CHROMagar Salmonella was evaluated for the recovery of *Salmonella* in raw chicken, raw ground beef, raw fish, lettuce, and shell eggs in internal and AOAC approved external laboratories. The raw chicken and ground beef were processed according to the USDA FSIS reference methods. The raw fish, lettuce and shell eggs were processed according to the FDA BAM procedures. **BBL CHROMagar** Salmonella was compared to the reference method media for the selective recovery of *Salmonella*. A total of 16 positive cultures were obtained from the raw chicken, 17 in the raw ground beef, 18 in the raw fish and lettuce and 11 in the shell egg samples. **BBL CHROMagar** Salmonella produced comparable results with the reference methods on all matrices resulting in a method agreement of 100%.

Twenty spiked raw chicken samples were tested according to the USDA FSIS reference method. The chicken was seeded with a low inoculum of 8 CFU/25 g and a high inoculum level of 50 CFU/25 g of sample. **BBL CHROMagar** Salmonella recovered 100% (20/20) of the low and high spiked level of *Salmonella*. At inoculum levels less than 1 CFU/25 g, fractional recovery was obtained. Naturally contaminated chicken was tested. Recovery of *Salmonella* was 100% and the Most Probable Number (MPN)/g was 0.23.

One hundred eighteen (118) *Salmonella* isolates of foodborne origin including various serotypes were cultured in Lactose Broth for 24 h and then subcultured to Tetrathionate Broth for 24 h. Tetrathionate Broths were subcultured to **BBL CHROMagar** Salmonella and incubated at 35°C. If mauve colonies were not recovered at 24 h, plates were incubated for an additional 24 h. **BBL CHROMagar** Salmonella recovered 111 isolates. Four strains were inhibited by Tetrathionate Broth and were not recovered on **BBL CHROMagar** Salmonella or on a nonselective control plate. Other isolates of the same serotypes as the four negatives did produce typical colonies so the lack of positive reaction was strain, not serotype, specific. Overall sensitivity for **BBL CHROMagar** Salmonella was 94% (111 of 118 isolates).

Sixty-five (65) isolates of non-*Salmonella* were cultured in Brain Heart Infusion Broth at 35°C for 24 h and subcultured to **BBL CHROMagar** Salmonella at 35°C for 24 h. Negative plates were incubated for a total of 48 h. Sixty one (61) of the 65 isolates did not exhibit mauve coloration on **BBL CHROMagar** Salmonella for a specificity of 94%. The four non-*Salmonella* strains that exhibited mauve coloration were inoculated into Tetrathionate Broth (recommended enrichment broth for USDA and FDA methods). Following 24 h incubation, Tetrathionate Broths were subcultured to **BBL CHROMagar** Salmonella. After 48 h of incubation, one strain grew mauve colonies and three strains were inhibited. Based on the use of Tetrathionate Broth enrichment, the overall specificity of **BBL CHROMagar** Salmonella in this study was 98%.

ISO Method

BBL CHROMagar Salmonella was compared to the reference method media for the selective recovery of *Salmonella* in raw chicken, raw ground beef, raw fish, lettuce, and shell eggs. All matrices were processed according to the ISO culture method. A total of 16 positive cultures were obtained from the raw chicken, 17 in the raw ground beef, 9 in the raw fish, 19 in the lettuce and egg shell samples. **BBL CHROMagar** Salmonella produced comparable results with the reference methods on all matrices resulting in a method agreement of 100%.

Twenty (20) samples of raw ground beef, raw fish, lettuce and shell eggs were spiked with a low level inoculum of *Salmonella* and analyzed according to the ISO culture procedure. Twenty (20) samples of naturally contaminated raw chicken were analyzed according to the ISO culture procedure. **BBL CHROMagar** Salmonella was added to the battery of reference media for

each food matrix tested. The method agreement of **BBL CHROMagar** Salmonella and the other reference media tested was 100%. The low inoculum levels ranged from 0.0036 to 0.23 MPN/g.

One hundred twenty seven (127) *Salmonella* isolates of foodborne origin were cultured in Buffered Peptone Water for 18 h and then subcultured to Rappaport-Vassiliadis with Soya (RVS) medium for 24 h. RVS broths were subcultured to **BBL CHROMagar** Salmonella and incubated at 35°C for 24 h. If mauve colonies were not recovered at 24 h, plates were incubated for an additional 24 h. **BBL CHROMagar** Salmonella recovered 123 isolates. Recovery of the 4 strains that did not grow on **BBL CHROMagar** Salmonella was poor on the nonselective control media. Overall sensitivity for **BBL CHROMagar** Salmonella was 96.8% (123 of 127 isolates).¹⁰

XIV AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 214983 | BBL™ CHROMagar™ Salmonella, Pkg. of 20 plates |

XV REFERENCES

1. Rose, Bonnie E. 2001. Isolation and identification of *Salmonella* from meat, poultry and egg products. *In* Microbiology laboratory guidebook, 3rd ed., Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C.
2. U.S. Food and Drug Administration. 2003. Bacteriological analytical manual (online), AOAC International, Gaithersburg, MD.
3. International Organization for Standards (ISO). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp., 4th Edition, ISO 6579:2002.
4. Bopp, Brenner, Fields, Wells and Strockbine. 2003. *Escherichia*, *Shigella*, and *Salmonella*. *In* Murray, Baron, Jorgensen, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. D'Aoust, Mauer and Bailey. 2001. *Salmonella* species. *In* Doyle, Beuchat and Montville (ed.), Food microbiology: fundamentals and frontiers, 2nd ed. American Society for Microbiology, Washington, D.C.
6. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
7. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
8. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
9. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
10. Data on file, BD Diagnostic Systems.

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QUALITY CONTROL PROCEDURES

*See footnote below

I INTRODUCTION

BBL™ CHROMagar™ Staph aureus is a selective medium for the isolation, enumeration and identification of *Staphylococcus aureus*.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak inoculate with 10³-10⁴ CFUs of *S. aureus* and 10⁴-10⁵ CFUs of all other organisms.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)** plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth and color formation.
3. Expected Results

| Organisms | ATCC™ | Recovery | Colony Color |
|---------------------------------------|-------|----------------------------------|-----------------------|
| <i>Candida albicans</i> | 60193 | Inhibition (partial to complete) | Mauve |
| <i>Enterococcus faecalis</i> | 29212 | Fair to heavy growth | Blue |
| * <i>Staphylococcus aureus</i> | 25923 | Fair to heavy growth | Mauve |
| <i>Staphylococcus aureus</i> | 33862 | Fair to heavy growth | Mauve |
| <i>Staphylococcus aureus</i> | 6538 | Fair to heavy growth | Mauve to orange mauve |
| <i>Staphylococcus epidermidis</i> | 12228 | Inhibition (partial to complete) | White |
| * <i>Staphylococcus saprophyticus</i> | 15305 | Fair to heavy growth | Light blue to green |
| * <i>Proteus mirabilis</i> | 12453 | Inhibition (partial to complete) | NA |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification 6.8 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ Staph aureus is a selective medium for the isolation, enumeration and identification of *Staphylococcus aureus* from clinical and food sources. Confirmatory testing of typical isolates from clinical sources is not required.

BBL CHROMagar Staph aureus (prepared plated medium) has been validated by the AOAC™ Research Institute under the Performance Tested MethodsSM Program for the analysis of shell eggs, smoked salmon and cooked roast beef when using AOAC and ISO methods.^{1,2} Confirmatory testing of mauve-colored colonies obtained from the food matrices mentioned above is required.

U.S. Patent No. 6,548,268

V SUMMARY AND EXPLANATION

S. aureus is a well documented pathogen. It is responsible for infections ranging from superficial to systemic.^{3,4} Due to the prevalence of this organism and its clinical implications, detection is of utmost importance.

Staphylococcal food poisoning caused by *S. aureus* is one of the most common types of foodborne illness worldwide. Its detection and enumeration help provide information about the potential health hazard of food, as well as being an indicator of poor hygiene.⁵ It is also recommended that this organism be used as an indicator of water quality.⁶

BBL CHROMagar Staph aureus is intended for the isolation, enumeration and identification of *S. aureus* based on the formation of mauve-colored colonies. The addition of chromogenic substrates to the medium facilitates the differentiation of *S. aureus* from other organisms.

An advantage **BBL CHROMagar Staph aureus** has over some traditional media, such as Baird-Parker Agar, is the ability to identify *S. aureus* in 24 h as opposed to 48 h.

VI PRINCIPLES OF THE PROCEDURE

BBL CHROMagar Staph aureus was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar Staph aureus** prepared plated medium.

Specially selected **Difco™** peptones supply nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and some gram-positive cocci. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by specific enzymes. This facilitates the detection and differentiation of *S. aureus* from other organisms. *S. aureus* utilizes one of the chromogenic substrates, producing mauve-colored colonies. The growth of mauve-colored colonies at 24 h is considered positive for *S. aureus* on **BBL CHROMagar Staph aureus**. Bacteria other than *S. aureus* may utilize other chromogenic substrates resulting in blue, blue-green, or if no chromogenic substrates are utilized, natural colored colonies.

*PRODUCER-SUPPLIED SAMPLES OF THIS TEST KIT MODEL WERE INDEPENDENTLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AND WERE FOUND TO PERFORM TO THE PRODUCER'S SPECIFICATIONS AS STATED IN THE TEST KIT'S DESCRIPTIVE INSERT. THE PRODUCER CERTIFIES THIS KIT CONFORMS IN ALL RESPECTS TO THE SPECIFICATIONS ORIGINALLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AS DETAILED IN *Performance Tested MethodsSM* CERTIFICATE NUMBER 100503.

VII REAGENTS

BBL CHROMagar Staph aureus

Approximate Formula* Per Liter Purified Water

| | | | |
|-----------------------|--------|-------------------------|--------|
| Chromopeptone | 40.0 g | Inhibitory Agents | 0.07 g |
| Sodium Chloride | 25.0 g | Agar | 14.0 g |
| Chromogen Mix | 0.5 g | | |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and the bottom of the plate during incubation. Protect from light during drying. See Storage Instructions.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁷⁻¹⁰ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

After use, prepared plates, specimen or sample containers and other contaminated materials should be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and original cardboard box until time of inoculation. Avoid freezing and overheating. Do not open until ready to use. Minimize the exposure of BBL CHROMagar Staph aureus to light before and during incubation, as light may destroy the chromogens. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts or standards for details in specimen/sample collection and handling procedures.

IX PROCEDURE

Material Provided: BBL CHROMagar Staph aureus

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

For clinical specimens, as soon as possible after receipt in the laboratory, inoculate onto a BBL CHROMagar Staph aureus plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 20–24 h in an inverted position (agar-side up).

For food samples, consult appropriate references and follow applicable standard methods. Inoculate the homogenized food samples onto BBL CHROMagar Staph aureus using the spread plate technique. Incubate plates aerobically at 35–37°C for 20–28 h in an inverted position (agar-side up).

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the clinical user refer to pertinent Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines for appropriate Quality Control practices.

X RESULTS

After proper incubation, read plates against a white background. *S. aureus* will produce mauve to orange/mauve colored colonies on the BBL CHROMagar medium. Most gram-positive organisms, if not inhibited, will produce blue, blue-green or natural color (colorless, white or cream) colonies. Gram-negative organisms and yeasts are partially to completely inhibited.

XI LIMITATIONS OF THE PROCEDURE

Occasionally some strains of staphylococci, other than *S. aureus*, such as: *S. cohnii*, *S. intermedius*, and *S. schleiferi*, as well as corynebacteria and yeasts, may produce mauve-colored colonies at 24 h.¹¹ Differentiation of *S. aureus* from non-*S. aureus* can be accomplished by coagulase, other biochemicals or Gram stain. Resistant gram-negative bacilli, which typically appear as small blue colonies, may also break through.

Incubation beyond 24 h (clinical) and 28 h (food) is not recommended due to an increase in potential false positives. If incubation time is exceeded, mauve-colored colonies should be confirmed prior to reporting as *S. aureus*.

Incubation less than the recommended 20 h may result in a lower percentage of correct results being obtained.

Due to the natural golden pigment of some *S. aureus* strains, colony color may appear orange-mauve.

XII PERFORMANCE CHARACTERISTICS

Clinical Testing

In a field trial conducted at a large metropolitan hospital, 201 throat and sputum specimens from cystic fibrosis patients and 459 nasal specimens from other hospital patients were evaluated on BBL CHROMagar Staph aureus. BBL CHROMagar Staph aureus was compared to blood agar or Mannitol Salt Agar, with isolate confirmation by slide coagulase. *S. aureus* was recovered from 190 combined specimens. BBL CHROMagar Staph aureus detected 9 additional *S. aureus* positive cultures which were not recovered on conventional media. Four potential false positives were also observed on the BBL CHROMagar Staph aureus medium following 24 h incubation: two corynebacteria and two coagulase-negative staphylococci. BBL CHROMagar Staph aureus produced an overall sensitivity of 99.5% and a specificity of 99.2%.¹¹

Agrifood Testing

BBL CHROMagar Staph aureus was validated by the AOAC Research Institute under the Performance Tested Methods Program. The medium was evaluated by an external reference laboratory, as well as internally at BD, for the recovery and enumeration of *S. aureus* in cooked roast beef, smoked salmon and shell eggs. The recovery and enumeration of *S. aureus* on **BBL CHROMagar Staph aureus** was compared to the AOAC and ISO reference plated medium, Baird-Parker Agar, using the recommended diluents at low, medium and high inoculum levels of *S. aureus*. After 24 h incubation, enumeration was performed on **BBL CHROMagar Staph aureus** and after 48 h on Baird-Parker Agar.

Based on statistical analysis, no significant difference was found between the reference methods and the **BBL CHROMagar Staph aureus** method for any food type or contamination level, with the exception of a low-level smoked salmon sample. The low contamination level of smoked salmon demonstrated a statistical difference in internal testing using the ISO method; i.e., the **BBL CHROMagar Staph aureus** method at 24 h recovered more colonies (Log_{10} 2.04) than the ISO reference at 48 h (Log_{10} 1.64). The repeatability precision estimates of the **BBL CHROMagar Staph aureus** method were satisfactory. The correlation coefficients ranged from 92.6% to 99.4%, demonstrating good correlation for all contamination levels in all food types. Data is summarized in Tables 1 and 2.

No false-positive colonies were recovered from the food matrices using **BBL CHROMagar Staph aureus**. All mauve colonies were confirmed as *S. aureus* with no discrepancies.

Additionally, 30 strains of *S. aureus*, including known enterotoxin-producing strains, and 37 non-*S. aureus* isolates were evaluated producing both a sensitivity and specificity of 100% on **BBL CHROMagar Staph aureus**.¹¹

Table 1. Summary of AOAC and ISO External Testing of Cooked Roast Beef and Shell Eggs¹⁻³

| | | AOAC | | |
|-------------------|----------------|---|---|---|
| | Inoculum Level | Paired t-test or One-way ANOVA ^a | Repeatability (standard deviation) ^b | Square of linear correlation coefficient ^c |
| Cooked Roast Beef | Low | Not significant | 0.398 | 96.0% |
| | Medium | Not significant | 0.04 | |
| | High | Not significant | 0.062 | |
| Shell Eggs | Low | Not significant | 0.302 | 95.5% |
| | Medium | Not significant | 0.089 | |
| | High | Not significant | 0.143 | |
| | | ISO | | |
| | Inoculum Level | Paired t-test or One-way ANOVA ^a | Repeatability (standard deviation) ^b | Square of linear correlation coefficient ^c |
| Cooked Roast Beef | Low | Not significant | 0.315 | 94.6% |
| | Medium | Not significant | 0.045 | |
| | High | Not significant | 0.117 | |
| Shell Eggs | Low | Not significant | 0.341 | 92.6% |
| | Medium | Not significant | 0.223 | |
| | High | Not significant | 0.135 | |

Table 2. Summary of AOAC and ISO External and Internal Testing of Smoked Salmon¹⁻³

| | | Paired t-test or One-way ANOVA ^a | | Repeatability (standard deviation) ^b | | Square of linear correlation coefficient ^c | |
|--------------------|----------------|---|--------------------------|---|----------|---|----------|
| | Inoculum Level | External | Internal | External | Internal | External | Internal |
| AOAC Smoked Salmon | Low | Not significant | Not significant | 0.132 | 0.271 | 99.4% | 93.2% |
| | Medium | Not significant | Not significant | 0.055 | 0.095 | | |
| | High | Not significant | Not significant | 0.064 | 0.161 | | |
| ISO Smoked Salmon | Low | Not significant | Significant ^d | 0.158 | 0.227 | 98.7% | 97.4% |
| | Medium | Not significant | Not significant | 0.135 | 0.165 | | |
| | High | Not significant | Not significant | 0.116 | 0.033 | | |

^a Paired t-test and one-way ANOVA analysis used to evaluate comparable performance of **BBL CHROMagar Staph aureus** versus the reference medium by comparing the mean of the log_{10} of the colony counts.

^b Repeatability demonstrates **BBL CHROMagar Staph aureus** produces comparable results between the tests run on the same material and method.

^c Square of linear correlation coefficient is used to evaluate precision of quantitative methods over different *S. aureus* counts.

^d **BBL CHROMagar Staph aureus** recovered more colonies than the ISO reference method.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 214982 | BBL™ CHROMagar™ Staph aureus, Pkg. of 20 plates |

XIV REFERENCES

1. AOAC Official Method 9755.55. *Staphylococcus aureus* in foods. Surface plating method for isolation and enumeration. 1976.
2. International Organization for Standards (ISO). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird Parker agar medium, 1st ed., ISO 6888-1:1999.
3. Jablonski, L.M. and G.A. Bohach. 1997. *Staphylococcus aureus*. In M. Doyle, L. Beuchat and T. Montville (eds.), Food microbiology fundamentals and frontiers. ASM, Washington, DC.
4. Kloos, W.E. and T.L. Bannerman. 1999. *Staphylococcus* and *Micrococcus*. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Tenover (eds.), Manual of clinical microbiology. ASM, Washington DC.
5. Bennett, R.W. and G.A. Lancefield. 1998. *Staphylococcus aureus*. In FDA, Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. Toranzos, G.A., G.A. McFeters and J.J. Borrego. 2002. Detection of microorganisms in environmental freshwaters and drinking waters. In C. Hurst, R. Crawford, G. Knudsen, M. McInerney and L. Stetzenbach (eds.), Manual of environmental microbiology, 2nd ed. ASM Press, Washington, DC.
7. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections. 3rd ed., CLSI, Wayne, Pa.
8. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
9. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, DC.
10. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
11. Data on file, BD Diagnostic Systems.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Brain Heart Infusion (BHI) is a general purpose liquid medium for the growth of a wide variety of bacterial and fungal species. Brain Heart Infusion with 6.5% Sodium Chloride is used to differentiate enterococci from nonenterococcal group D streptococci.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - From 24- to 48-h **Trypticase™** Soy Broth cultures, prepare dilutions for use as inocula.
 - Inoculation of media
 - For BHI, inoculate tubes of the test samples with a dilution of each culture. The dilution must contain 1,000 or less CFU. Fill volumes of greater than 5 mL should be inoculated with 1.0 mL. Fill volumes of 5 mL or less should be inoculated with 0.1 mL.
 - For BHI with 6.5% Sodium Chloride, inoculate tubes of the test samples using 10⁻¹ dilutions of 18- to 24-h **Trypticase** Soy Broth cultures using a 0.01 mL calibrated loop.
 - Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
- Examine tubes of Brain Heart Infusion at 24 and 48 h for growth. Examine tubes of BHI with 6.5% Sodium Chloride at 18–24 h for growth and selectivity.

3. Expected Results

Brain Heart Infusion

| CLSI Organisms | ATCC™ | Recovery |
|--------------------------------|-------|----------|
| * <i>Escherichia coli</i> | 25922 | Growth |
| * <i>Staphylococcus aureus</i> | 25923 | Growth |

Additional Organisms

| | | |
|-------------------------------|-------|--------|
| <i>Pseudomonas aeruginosa</i> | 27853 | Growth |
| <i>Enterococcus faecalis</i> | 29212 | Growth |
| <i>Streptococcus pyogenes</i> | 19615 | Growth |

BHI with 6.5% Sodium Chloride

| Organisms | ATCC | Recovery |
|--------------------------------|-------|-----------|
| * <i>Enterococcus faecalis</i> | 29212 | Growth |
| * <i>Streptococcus bovis</i> | 9809 | No Growth |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under "Product Deterioration."
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Brain Heart Infusion (BHI) is a general-purpose liquid medium used in the cultivation of fastidious and nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials.

The broth medium which contains 6.5% sodium chloride is used to differentiate the enterococci from nonenterococcal group D streptococci.

V SUMMARY AND EXPLANATION

BHI Broth is used for the cultivation of a wide variety of microorganisms, including bacteria, yeasts and molds.¹

BHI with 6.5% Sodium Chloride is used to differentiate the enterococci (e.g., *E. faecalis*, *E. faecium*, *E. durans* and *E. avium*) from the nonenterococcal species (*S. bovis* and *S. equinus*) by the 6.5% salt tolerance test.²

VI PRINCIPLES OF THE PROCEDURE

BHI Broth is a nutritious, buffered culture medium that contains infusions of brain and heart tissue and peptones to supply protein and other nutrients necessary to support the growth of fastidious and nonfastidious microorganisms. In the formulation containing 6.5% sodium chloride, the salt acts as a differential and/or selective agent by interfering with membrane permeability and osmotic and electrokinetic equilibria in salt-intolerant organisms.¹

VII REAGENTS

Brain Heart Infusion

Approximate Formula* Per Liter Purified Water

| | |
|---|--------|
| Brain Heart, Infusion from (solids) | 6.0 g |
| Peptic Digest of Animal Tissue..... | 6.0 g |
| Sodium Chloride | 5.0 g |
| Dextrose..... | 3.0 g |
| Pancreatic Digest of Gelatin | 14.5 g |
| Disodium Phosphate..... | 2.5 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Brain Heart Infusion with 6.5% Sodium Chloride contains 60 g/L of sodium chloride in addition to the ingredients listed above.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"³⁻⁶ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{7,8} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brain Heart Infusion or Brain Heart Infusion with 6.5% Sodium Chloride

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

With liquid specimens, tubed media should be inoculated with one or two drops of the specimen using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media.

Liquid media for anaerobic incubation should be reduced prior to incubation by placing the tubes, with caps loosened, under anaerobic conditions for 18–24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™ EZ** anaerobic system.

Alternatively, liquid media may be reduced immediately prior to use by boiling in a water bath* with caps loosened and cooling to room temperature with tightened caps before inoculation.

Inoculate the 6.5% NaCl broth lightly with one or two colonies of suspect bacteria. Incubate aerobically at 35 ± 2°C overnight. Examine for growth; reincubate negative tests for an additional 24 h.

*NOTE: Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Growth in the tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram staining and subculturing onto appropriate media, e.g., a **Trypticase Soy Agar** with 5% Sheep Blood (TSA II) and/or Chocolate II Agar plate, EMB Agar or MacConkey II Agar plates. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a **GasPak EZ** anaerobic system.

Enterococci will grow in the 6.5% NaCl broth within 24–48 h. Nonenterococcal group D streptococci fail to grow in the medium after 48 h of incubation.²

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.⁷⁻⁹

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

Strains of other catalase-negative gram-positive cocci; i.e., *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Vagococcus*, have been isolated from human infections. Therefore, the presumptive identification of enterococci based on the bile-esculin reaction and growth in 6.5% NaCl broth only cannot be made.¹⁰

XII PERFORMANCE CHARACTERISTICS

Brain Heart Infusion

Prior to release, all lots of Brain Heart Infusion are tested for performance characteristics. Using a sterile pipette, representative samples of the lot are inoculated with 0.1 mL (for fill volumes of 5 mL or less) or 1.0 mL (for fill volumes greater than 5 mL) of **Trypticase** Soy Broth or Thioglycollate Medium, Enriched cultures containing 1,000 Colony Forming Units (CFU) or less of *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615). The tubes are incubated with loosened caps at 35 ± 2°C and read after 18–24 h and 42–48 h for growth. All cultures exhibit moderate to heavy growth within 48 h.

Brain Heart Infusion with 6.5% Sodium Chloride

Prior to release, all lots of Brain Heart Infusion with 6.5% Sodium Chloride are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are tested with **Trypticase** Soy Broth cultures diluted 10⁻¹ of *Enterococcus faecalis* (ATCC 29212) and *Streptococcus bovis* (ATCC 9809). The tubes are incubated at 35 ± 2°C and read after 18–24 h and 42–48 h for growth. *E. faecalis* exhibits moderate to heavy growth while *S. bovis* is completely inhibited.


Additionally, representative samples are tested chemically by silver nitrate titration for sodium chloride content. The calculated percent sodium chloride is 6.0 to 7.0.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 221778 | BBL™ Brain Heart Infusion, 0.5 mL, Ctn. of 100 size K tubes |
| 297769 | BBL™ Brain Heart Infusion, 2 mL, Ctn. of 100 size K tubes |
| 221812 | BBL™ Brain Heart Infusion, 5 mL, Pkg. of 10 size K tubes |
| 221813 | BBL™ Brain Heart Infusion, 5 mL, Ctn. of 100 size K tubes |
| 220837 | BBL™ Brain Heart Infusion, 8 mL, Ctn. of 100 size K tubes |
| 221785 | BBL™ Brain Heart Infusion with 6.5% Sodium Chloride, Pkg. of 10 size K tubes |

XIV REFERENCES

1. MacFaddin, J.F. 1985. Media for the isolation- cultivation-identification-maintenance of medial bacteria, vol. I. Williams & Wilkins, Baltimore.
2. Pratt-Rippin, K., and M. Pezzlo. 1992. Identification of commonly isolated aerobic gram-positive bacteria, p. 1.20.1-1.20.47. *In* H. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
4. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
5. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
6. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262, 17/10/2000, p. 0021-0045.*
7. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
8. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis.
9. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
10. Facklam, R.R., D.F. Sahm, and L.M. Teixeira. 1999. *Enterococcus*, p. 297-305. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Fluid Thioglycollate Medium is a general-purpose medium for the cultivation of anaerobes, microaerophiles and aerobes, and is recommended as one of the media for the sterility testing of biologics.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Before use, loosen the caps and place the tubes in boiling water* for approximately 5 min until the medium is reduced (colorless). Tighten caps immediately after removing from heat. Allow medium to cool to room temperature.
*NOTE: Use of a microwave oven is not recommended.
 - b. From 24- to 48-h **Trypticase™** Soy Broth cultures or Enriched Thioglycollate Medium cultures for the *Bacteroides* and *Clostridium* strains, prepare a dilution containing 100 or less CFU/mL.
 - c. Using sterile 1.0 mL pipettes, inoculate tubes with 0.75 mL of the dilutions.
 - d. Incubate tubes with loosened caps at 30–35°C in an aerobic atmosphere except for CLSI strains (*Bacteroides fragilis* ATCC™ 25285 and *Staphylococcus aureus* ATCC 25923) which should be incubated with tightened caps.
2. Examine tubes of the CLSI-recommended control strains (tightened caps) at 18–24 and 48 h for growth. Examine tubes of the other control strains (USP-recommended) for up to 3 days for growth.
3. Expected Results

| CLSI Organisms | ATCC™ | Recovery |
|--|-------|----------|
| * <i>Bacteroides fragilis</i> | 25285 | Growth |
| * <i>Staphylococcus aureus</i> | 25923 | Growth |
| Additional Organisms (USP Growth Promotion Test) | | |
| ** <i>Staphylococcus aureus</i> | 6538 | Growth |
| ** <i>Pseudomonas aeruginosa</i> | 9027 | Growth |
| ** <i>Clostridium sporogenes</i> | 11437 | Growth |
| ** <i>Clostridium sporogenes</i> | 19404 | Growth |
| ** <i>Bacillus subtilis</i> | 6633 | Growth |
| ** <i>Kocuria rhizophila</i> | 9341 | Growth |
| ** <i>Bacteroides vulgatus</i> | 8482 | Growth |

* Recommended organism strain for User Quality Control.

**For verification of growth promotion for use in USP Sterility Tests.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Fluid Thioglycollate Medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Fluid Thioglycollate Medium (FTM) is used for the sterility testing of biologics and for the cultivation of anaerobes, aerobes and microaerophiles.

V SUMMARY AND EXPLANATION

Fluid Thioglycollate Medium was designed by Brewer for rapid cultivation of anaerobes as well as aerobes.¹ It was first made available in dehydrated form by the Baltimore Biological Laboratory (BBL) in 1940. Incorporation of casein peptone was introduced by Vera in 1944.²

This medium is capable of supporting good growth of a great variety of fastidious organisms, of both pathogenic and nonpathogenic species. A feature of sodium thioglycollate, in addition to lowering the oxidation-reduction potential, is its ability to neutralize the antibacterial activity of mercurial compounds. These characteristics make FTM particularly useful for determining the presence of contamination in biological and other materials. The BBL formula meets the requirements of the USP growth promotion test.³

Fluid Thioglycollate Medium may be used after its preparation until approximately 30% of the medium has been oxidized, as indicated by a pink color of the resazurin at the surface. If oxidation has proceeded further, the broth may be reheated once in steam or boiling water, cooled and used.

VI PRINCIPLES OF THE PROCEDURE

Dextrose, peptone, L-cystine and yeast extract provide the growth factors necessary for bacterial replication. Sodium chloride provides essential ions. Sodium thioglycollate is a reducing agent that prevents the accumulation of peroxides which are lethal to some microorganisms. The L-cystine is also a reducing agent, since it contains sulfhydryl groups which inactivate heavy metal compounds and maintain a low redox potential, thereby supporting anaerobiosis. Resazurin is an oxidation-reduction indicator, being pink when oxidized and colorless when reduced. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium.⁴ The USP lists 5.5 g/L of dextrose in the formulation for Fluid Thioglycollate Medium. The BBL formula contains the anhydrous form of dextrose (5.0 g/L).

VII REAGENTS

Fluid Thioglycollate Medium

Approximate Formula* Per Liter Purified Water

| | | | |
|-----------------------------------|--------|-----------------------------|---------|
| Pancreatic Digest of Casein | 15.0 g | Sodium Chloride..... | 2.5 g |
| L-Cystine | 0.5 g | Sodium Thioglycollate | 0.5 g |
| Dextrose (anhydrous)..... | 5.0 g | Resazurin | 0.001 g |
| Yeast Extract | 5.0 g | Agar | 0.75 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark per label directions. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{9,10} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Fluid Thioglycollate Medium

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Before use, loosen the caps and place the tubes in boiling water* for approximately 5 min until the medium is reduced (colorless). Tighten caps immediately after removing from heat. Allow medium to cool to room temperature.

For general use, inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2°C.

For sterility testing, recommendations of the *USP*³ and various control agencies should be followed.¹¹ These reference sources specify the ratio of medium to product that should be utilized in sterility tests as well as details of sampling and test result interpretation. For sterility testing purposes, it is important that the medium in the test vessels is reduced to a sufficient degree to ensure the replication of obligate anaerobes and microaerophilic organisms. If the test sample renders the medium so turbid that microbial growth cannot be easily recognized, transfers should be made to fresh medium.

*NOTE: Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized (pink) layer. By carefully removing liquid from different levels, it is possible to enhance the ability to separate different species in a mixed culture.

XI LIMITATIONS OF THE PROCEDURE

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.¹²

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{9,10,13}

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Fluid Thioglycollate Medium are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for approximately 5 min. After cooling, the tubes are inoculated with 0.75 mL of cultures of *Bacillus subtilis* (ATCC 6633), *Bacteroides fragilis* (ATCC 25285), *B. vulgatus* (ATCC 8482), *Clostridium sporogenes* (ATCC 11437 and 19404), *Kocuria rhizophila* (ATCC 9341), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538 and ATCC 25923). The inocula for *B. subtilis*, *B. fragilis*, *C. sporogenes*, *K. rhizophila*,


P. aeruginosa and *S. aureus* are diluted to contain 100 or less colony-forming units (CFU) per mL. The inoculum for *B. vulgatus* is prepared from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and adjusted in Thioglycollate Medium without Dextrose and Indicator to obtain 10–100 CFU/mL. The caps are tightened immediately after inoculation for tubes containing *B. fragilis* and *S. aureus*; caps of the remaining tubes are loosened. Tubes are incubated at 35 ± 2°C. Tubes containing *B. fragilis* and *S. aureus* (ATCC 25923) show trace to heavy growth within 48 h incubation. Remaining organisms show moderate to heavy growth within 3 days incubation.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 221195 | BBL™ Fluid Thioglycollate Medium, 8 mL, Pkg. of 10 size K tubes |
| 221196 | BBL™ Fluid Thioglycollate Medium, 8 mL, Ctn. of 100 size K tubes |
| 299802 | BBL™ Fluid Thioglycollate Medium, 8 mL, Ctn. of 100 size K tubes (ink-jet label) |
| 220888 | BBL™ Fluid Thioglycollate Medium, 20 mL, Pkg. of 10 size A tubes |
| 220889 | BBL™ Fluid Thioglycollate Medium, 20 mL, Ctn. of 100 size A tubes |
| 299803 | BBL™ Fluid Thioglycollate Medium, 20 mL, Ctn. of 100 size A tubes (ink-jet label) |
| 298214 | BBL™ Fluid Thioglycollate Medium, 10 mL, Ctn. of 100 size D tubes |

XIV REFERENCES

1. Brewer, J.H. 1940. Clear liquid medium for the "aerobic" cultivation of anaerobes. *J. Am. Med. Assoc.* 115:598-600.
2. Vera, H.D. 1944. A comparative study of materials suitable for the cultivation of clostridia. *J. Bacteriol.* 47:59-70.
3. U.S. Pharmacopeial Convention, Inc. 2009. The U.S. pharmacopeia 32/The national formulary 27. U.S. Pharmacopeial Convention, Inc., Rockville, Md.
4. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
5. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
6. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
7. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
9. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.) 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
10. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis.
11. Horwitz, W. (ed.). 2000. Official methods of analysis of AOAC International, 17th ed, vol.1. AOAC International, Gaithersburg, Md.
12. Reischelderfer and Mangels. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
13. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

SIM (Sulfide Indole Motility) Medium is used for the determination of sulfide production, indole formation and motility of enteric microorganisms.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Loosen caps, boil and cool before use.
 - b. Inoculate the tubes by stabbing a straight needle down the center of the medium to approximately one-half its depth using 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - c. Incubate with loosened caps at 35 ± 2°C in an aerobic atmosphere.
2. Examine tubes after 18–24 and 42–48 h for growth, motility and sulfide.
3. After 48 h, test for indole production. Add 0.2 mL of Kovacs' Reagent down the inside of the tubes. Observe for the production of a pink to red color (positive reaction).
4. Expected Results

| Organisms | ATCC™ | H ₂ S | Indole | Motility |
|--|-------|------------------|--------|----------|
| * <i>Escherichia coli</i> | 25922 | – | + | + |
| * <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium | 13311 | + | – | + |
| * <i>Shigella sonnei</i> | 9290 | – | – | – |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

SIM Medium is used to differentiate enteric bacilli on the basis of sulfide production, indole formation and motility.

V SUMMARY AND EXPLANATION

Hydrogen sulfide production, indole formation and motility are distinguishing characteristics which aid in the identification of the *Enterobacteriaceae*, especially *Salmonella* and *Shigella*. SIM Medium, therefore, is useful in the process of identification of enteric pathogens.

VI PRINCIPLES OF THE PROCEDURE

The ingredients in SIM Medium enable the determination of three activities by which enteric bacteria can be differentiated. Sodium thiosulfate and ferrous ammonium sulfate are indicators of hydrogen sulfide production. The ferrous ammonium sulfate reacts with H₂S gas to produce ferrous sulfide, a black precipitate.¹ The casein peptone is rich in tryptophan, which is attacked by certain microorganisms resulting in the production of indole. The indole is detected by the addition of chemical reagents following the incubation period. Motility detection is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile.

VII REAGENTS

SIM Medium

Approximate Formula* Per Liter Purified Water

| | |
|--------------------------------------|--------|
| Pancreatic Digest of Casein | 20.0 g |
| Peptic Digest of Animal Tissue | 6.1 g |
| Ferrous Ammonium Sulfate | 0.2 g |
| Sodium Thiosulfate | 0.2 g |
| Agar | 3.5 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{2,3} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: SIM Medium

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Loosen caps, boil and cool before use. Using a light inoculum of a pure culture, stab an inoculating needle one-half of the distance to the bottom in the center of the tube. Incubate tubes with loosened caps for 18–48 h at 35 ± 2°C in an aerobic atmosphere.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Following incubation, observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H₂S production (blackening along the stab line). To detect indole production, add three or four drops of Kovács reagent² and observe for a red color (positive reaction).

Consult appropriate references for activities of specific microorganisms.⁴⁻⁶

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.²⁻⁶

XII PERFORMANCE CHARACTERISTICS


Prior to release, all lots of SIM Medium are tested for performance characteristics. Representative samples of the lot are tested with **Trypticase Soy Broth** cultures diluted 10⁻¹ of *Salmonella* Typhimurium (ATCC 13311), *Escherichia coli* (ATCC 25922) and *Shigella sonnei* (ATCC 9290), by stabbing the center of the medium to approximately one-half its depth with an inoculating needle. The tubes are incubated with loosened caps at 35 ± 2°C and read after 18–24 h and 42–48 h for growth, motility, indole formation and sulfide production. Growth of all organisms is moderate to heavy within 48 h. Both *E. coli* and *Salmonella* Typhimurium are motile as is evidenced by the growth pattern of the organism in the medium; i.e., growth originates at the center line of inoculation and spreads evenly throughout the medium. *S. sonnei* is nonmotile; i.e., growth is evident only along the center line of inoculation. Only *Salmonella* Typhimurium is positive for hydrogen sulfide production indicated by blackening of the medium. After 48 h incubation, 0.2 mL of Kovács Reagent is added to each tube. Only *E. coli* is positive for indole formation denoted by a medium pink to dark pink color reaction in the tube.


XIII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 221010 | BBL™ SIM Medium, Pkg. of 10 size K tubes |
| 221011 | BBL™ SIM Medium, Ctn. of 100 size K tubes |

XIV REFERENCES

1. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
2. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.) 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York.
5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
6. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Thioglycollate Medium, Enriched with Vitamin K₁ and Hemin is a general-purpose medium for the cultivation of fastidious and nonfastidious microorganisms.

II PERFORMANCE TEST PROCEDURE

1. Reduce tubes of the medium by boiling* with caps loosened. After boiling, tighten caps immediately and allow tubes to cool to room temperature.

*NOTE: Use of a microwave oven is not recommended.

2. Preparation of inocula

Use a 48- to 72-h culture of Enriched Thioglycollate Medium, Chopped Meat Medium, or colonies from a CDC Anaerobe 5% Sheep Blood Agar plate which have been transferred to a pre-reduced tube of Enriched Thioglycollate Medium, and adjust to a turbidity comparable to a 0.5 McFarland standard.

3. Using a sterile 0.01 mL calibrated loop, inoculate tubes from the standardized inoculum for each organism.

4. Incubate tubes at 35 ± 2°C in an aerobic atmosphere with tightened caps.

5. Examine tubes after 18–24 and 42–48 h for growth.

6. Expected Results

| CLSI Organisms | ATCC™ | Recovery |
|--|-------|----------|
| * <i>Peptostreptococcus anaerobius</i> | 27337 | Growth |
| * <i>Bacteroides vulgatus</i> | 8482 | Growth |
| * <i>Clostridium perfringens</i> | 13124 | Growth |

Additional Organisms

| | | |
|----------------------------|-------|--------|
| <i>Porphyromonas levii</i> | 29147 | Growth |
| <i>Clostridium novyi A</i> | 7659 | Growth |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."

2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.

3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.0 ± 0.2.

4. Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Thioglycollate Medium, Enriched with Vitamin K₁ and Hemin is a general-purpose medium used in qualitative procedures for the cultivation of fastidious as well as nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials.

V SUMMARY AND EXPLANATION

Enriched Thioglycollate Medium is BBL™ Thioglycollate Medium without Indicator-135C supplemented with vitamin K₁ and hemin.¹⁻³ The enriched broth medium is recommended for use in the isolation and cultivation of fastidious or slow growing, obligately anaerobic microorganisms present in clinical materials.^{4,5} It is also recommended for the isolation and cultivation of a wide variety of aerobic and facultatively anaerobic microorganisms. The medium is prepared with an anaerobic head space and is provided in screw-capped tubes in accordance with CDC recommendations.⁴ Vitamin K₁ and hemin have been shown to be required by certain anaerobes for growth.^{6,7}

The isolation of microorganisms from clinical materials frequently requires the use of enriched broth media in addition to the selective, differential and nonselective plated media normally used for primary isolation. The use of liquid "back up" media reduces the possibility of completely missing an etiological agent that is present in low numbers, slow growing on plated media, susceptible to selective agents, or sensitive to unfavorable incubation conditions; i.e., insufficient anaerobiosis for optimal growth of obligate anaerobes.

VI PRINCIPLES OF THE PROCEDURE

Sodium thioglycollate, a reducing agent, maintains a low oxygen tension in the medium. Vitamin K₁ is a growth requirement for some strains of *Prevotella melaninogenica*⁶ and is reported to enhance the growth of some strains of *Bacteroides* species and gram-positive nonsporeformers.⁸ Hemin is the source of the X factor which stimulates the growth of many microorganisms.

VII REAGENTS

Thioglycollate Medium, Enriched with Vitamin K₁ and Hemin

Approximate Formula* Per Liter Purified Water

| | | | |
|-------------------------------------|--------|------------------------------|---------|
| Pancreatic Digest of Casein | 17.0 g | Agar | 0.7 g |
| Papaic Digest of Soybean Meal | 3.0 g | L-Cystine | 0.25 g |
| Dextrose | 6.0 g | Sodium Sulfite | 0.1 g |
| Sodium Chloride | 2.5 g | Hemin | 0.005 g |
| Sodium Thioglycollate | 0.5 g | Vitamin K ₁ | 0.001 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁹⁻¹² and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{13,14} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Thioglycollate Medium, Enriched with Vitamin K₁ and Hemin

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic condition for 18–24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™** EZ anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling*, with caps loosened, and cooling, with tightened caps, to room temperature before inoculation.

Inoculate the specimen into the media of choice as soon as it arrives in the laboratory. With liquid specimens, tubed media should be inoculated with one or two drops of the specimen. Tissue specimens should be minced and ground in sterile, reduced broth such as Enriched Thioglycollate Medium for the cultivation of microorganisms. Inoculation is then performed as for liquid specimens. Swab specimens may be inserted into the broth after inoculation of plated media. Alternatively, the swab may be "scrubbed" in a small volume of sterile, reduced broth such as Enriched Thioglycollate Medium and the broth used to inoculate media as performed with liquid specimens.

Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube. Incubate tubes with tight caps aerobically at 35 ± 2°C or other appropriate temperature.

Broth cultures should be held at least 1 week before discarding as negative.¹⁵

*NOTE: Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

Growth in broth tubes, such as Enriched Thioglycollate Medium, is demonstrated by the appearance of turbidity when compared to an uninoculated control.

If growth is detected, cultures should be examined by Gram staining and subcultured onto selective and nonselective plated media. If anaerobes are suspected, subcultures should also be made to appropriate anaerobic plated media.

XI LIMITATIONS OF THE PROCEDURE

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.¹⁵

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{13,14,16}

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

XII PERFORMANCE CHARACTERISTICS


Prior to release, all lots of Thioglycollate Medium, Enriched with Vitamin K₁ and Hemin are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for a minimum of 10 minutes and cooled. Using a 0.01 mL calibrated loop, tubes are inoculated with cultures that have been adjusted to a 0.5 McFarland turbidity standard. The inocula for *Porphyromonas levii* (ATCC 29147), *Clostridium perfringens* (ATCC 13124) and *Peptostreptococcus anaerobius* (ATCC 27337) are prepared from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and adjusted to the correct inoculum concentration in pre-reduced Thioglycollate Medium, Enriched. The inoculum for *Bacteroides vulgatus* (ATCC 8482) is taken from Thioglycollate Medium, Enriched and the inoculum for *C. novyi* A (ATCC 7659) is taken from Chopped Meat Glucose Broth, PR II. Tubes are inoculated below the surface of the broths as deeply into the medium as possible. The caps are tightened immediately after inoculation and the tubes are incubated aerobically at 35 ± 2°C. Tubes are read for the amount of growth after 18–24 h and 42–48 h. All organisms show trace to heavy growth after 48 h.


XIII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 221741 | BBL™ Thioglycollate Medium, Enriched with Vitamin K ₁ , and Hemin, 5 mL, Pkg. of 10 size K tubes |
| 221742 | BBL™ Thioglycollate Medium, Enriched with Vitamin K ₁ , and Hemin, 5 mL, Ctn. of 100 size K tubes |
| 221787 | BBL™ Thioglycollate Medium, Enriched with Vitamin K ₁ , and Hemin, 8 mL, Pkg. of 10 size K tubes |
| 221788 | BBL™ Thioglycollate Medium, Enriched with Vitamin K ₁ , and Hemin, 8 mL, Ctn. of 100 size K tubes |
| 297289 | BBL™ Thioglycollate Medium, Enriched with Vitamin K ₁ , and Hemin, 10 mL, Pkg. of 10 size D tubes |
| 297292 | BBL™ Thioglycollate Medium, Enriched with Vitamin K ₁ , and Hemin, 10 mL, Ctn. of 100 size D tubes |

XIV REFERENCES

1. Brewer, J.H. 1940. A clear liquid medium for the "aerobic" cultivation of anaerobes. *J. Bacteriol.* 39:10.
2. Vera, H.D. 1944. Comparative study of materials suitable for the cultivation of clostridia. *J. Bacteriol.* 47:59-65.
3. Dowell, V.R., Jr., and T.M. Hawkins. 1987. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta.
4. Dowell, V.R., Jr., G.L. Lombard, F.S. Thompson, and A.Y. Armfield. 1977. Media for isolation, characterization, and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta.
5. Forbes, B.A., and P.A. Granato. 1995. Processing specimens for bacteria, p. 265-281. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
6. Gibbons, R.J. and J.B. MacDonald. 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. *J. Bacteriol.* 80:164-170.
7. Wilkins, T.D., S.L. Chalgren, F. Jimenez-Ulate, C.R. Drake, Jr., and J.L. Johnson. 1976. Inhibition of *Bacteroides fragilis* on blood agar plates and reversal of inhibition by added hemin. *J. Clin. Microbiol.* 3:359-363.
8. Finegold, S.M., V.L. Sutter, H.R. Attebery, and J.E. Rosenblatt. 1974. Isolation of anaerobic bacteria, p. 365-375. In E.H. Lennette, G.H. Spaulding, and J.P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
9. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
10. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
11. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
12. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
13. Murray, P.R., E.J. Baron, J.H. Tenover, M.L. Landry and M.A. Pfaller (ed.). 2007. *Manual of clinical microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
14. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. *Bailey & Scott's diagnostic microbiology*, 12th ed. Mosby, Inc., St. Louis.
15. Reischelderfer, C., and J.I. Mangel. 1994. Culture media for anaerobes, p. 2.3.1-2.3.8. In H.D. Isenberg (ed.), *Clinical microbiology procedures handbook*, vol. 1. American Society for Microbiology, Washington, D.C.
16. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore..

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**BBL™ Thioglycollate Medium,
Enriched (with Vitamin K₁ and Hemin),
with Calcium Carbonate
L007510 • Rev. 04 • April 2009**



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Enriched Thioglycollate Medium with Calcium Carbonate is a general-purpose medium for the cultivation of fastidious and nonfastidious microorganisms and for the maintenance of stock cultures.

II PERFORMANCE TEST PROCEDURE

1. Reduce tubes of the medium by boiling* with caps loosened. After boiling, tighten caps immediately and allow tubes to cool to room temperature.

*NOTE: Use of a microwave oven is not recommended.

2. Preparation of inocula

Use a 48- to 72-h culture of Enriched Thioglycollate Medium, Chopped Meat Medium, or colonies from a CDC Anaerobe 5% Sheep Blood Agar plate which have been transferred to a pre-reduced tube of Enriched Thioglycollate Medium, and adjust to a turbidity comparable to a 0.5 McFarland standard.

3. Using a sterile 0.01 mL calibrated loop, inoculate tubes from the standardized inoculum for each organism.

4. Incubate tubes at 35 ± 2°C in an aerobic atmosphere with tightened caps.

5. Examine tubes after 18–24 and 42–48 h for growth.

6. Expected Results

| Organisms | ATCC™ | Recovery |
|--|-------|----------|
| * <i>Peptostreptococcus anaerobius</i> | 27337 | Growth |
| * <i>Bacteroides vulgatus</i> | 8482 | Growth |
| * <i>Clostridium perfringens</i> | 13124 | Growth |
| <i>Clostridium novyi A</i> | 7659 | Growth |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."

2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.

3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.0 ± 0.2.

4. Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Enriched Thioglycollate Medium with Calcium Carbonate is a general-purpose medium used in qualitative procedures for the cultivation of fastidious as well as nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials. It is also recommended for the maintenance of stock cultures.

V SUMMARY AND EXPLANATION

Enriched Thioglycollate Medium is BBL Thioglycollate Medium without Indicator-135C supplemented with vitamin K₁ and hemin.¹⁻³ The enriched broth medium is recommended for use in the isolation and cultivation of fastidious or slow growing, obligately anaerobic microorganisms present in clinical materials.^{4,5} It is also recommended for the isolation and cultivation of a wide variety of aerobic and facultatively anaerobic microorganisms. The medium is prepared with an anaerobic head space and is provided in screw-capped tubes in accordance with CDC recommendations.⁴ Vitamin K₁ and hemin have been shown to be required by certain anaerobes for growth.^{6,7}

Calcium carbonate enhances the maintenance of stock cultures by neutralizing acids produced during growth.⁸

The isolation of microorganisms from clinical materials frequently requires the use of enriched broth media in addition to the selective, differential and nonselective plated media normally used for primary isolation. The use of liquid "back up" media reduces the possibility of completely missing an etiological agent that is present in low numbers, slow growing on plated media, susceptible to selective agents, or sensitive to unfavorable incubation conditions; i.e., insufficient anaerobiosis for optimal growth of obligate anaerobes.

VI PRINCIPLES OF THE PROCEDURE

Casein and soy peptones provide amino acids and other nitrogenous substances to support bacterial growth. Yeast extract provides the B-complex vitamins. Sodium chloride provides essential ions. Dextrose is source of energy.

The reducing action provided by sodium thioglycollate and sodium sulfite binds molecular oxygen, thereby removing it from the medium to maintain a low Eh.⁹ A small amount of agar is added to retard the absorption of oxygen by reducing convection currents in the medium.⁹

Vitamin K₁ is a growth requirement for some strains of *Prevotella melaninogenica*⁶ and is reported to enhance the growth of some strains of *Bacteroides* species and gram-positive nonsporeformers.¹⁰ Hemin is the source of the X factor which stimulates the growth of many microorganisms.

The incorporation of calcium carbonate is recommended because otherwise fastidious organisms may grow and then die off rapidly; it serves to neutralize acid produced during growth.^{8,11}

VII REAGENTS

Enriched Thioglycollate Medium with Calcium Carbonate

Approximate Formula* Per Liter Purified Water

| | | | |
|-------------------------------------|--------|------------------------------|------------|
| Pancreatic Digest of Casein | 12.0 g | Agar | 0.7 g |
| Papaic Digest of Soybean Meal | 3.0 g | L-Cystine | 0.25 g |
| Dextrose | 6.0 g | Sodium Sulfite | 0.1 g |
| Yeast Extract | 5.0 g | Hemin | 0.005 g |
| Sodium Chloride | 2.5 g | Vitamin K ₁ | 0.001 g |
| Sodium Thioglycollate | 0.5 g | Marble Chip | 1 per tube |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹²⁻¹⁵ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{16,17} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Enriched Thioglycollate Medium with Calcium Carbonate

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic condition for 18–24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™ EZ** anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling*, with caps loosened, and cooling, with tightened caps, to room temperature before inoculation.

Inoculate the specimen into the media of choice as soon as it arrives in the laboratory. With liquid specimens, tubed media should be inoculated with one or two drops of the specimen. Tissue specimens should be minced and ground in sterile, reduced broth such as Enriched Thioglycollate Medium with Calcium Carbonate for the cultivation of microorganisms. Inoculation is then performed as for liquid specimens. Swab specimens may be inserted into the broth after inoculation of plated media. Alternatively, the swab may be "scrubbed" in a small volume of sterile, reduced broth and the broth used to inoculate media as performed with liquid specimens.

Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube. Incubate tubes with tight caps aerobically at 35 ± 2°C or other appropriate temperature.

Broth cultures should be held at least 1 week before discarding as negative.⁸

*NOTE: Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

Growth in broth tubes, such as Enriched Thioglycollate Medium with Calcium Carbonate, is demonstrated by the appearance of turbidity when compared to an uninoculated control.

If growth is detected, cultures should be examined by Gram staining and subcultured onto selective and nonselective plated media. If anaerobes are suspected, subcultures should also be made to appropriate anaerobic plated media.

XI LIMITATIONS OF THE PROCEDURE

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.⁸

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.¹⁶⁻¹⁸

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and

the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

XII PERFORMANCE CHARACTERISTICS


Prior to release, all lots of Enriched Thioglycollate Medium with Calcium Carbonate are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for a minimum of 10 minutes and cooled. Using a 0.01 mL calibrated loop, tubes are inoculated with cultures that have been adjusted to a 0.5 McFarland turbidity standard. The inocula for *Porphyromonas levii* (ATCC 29147), *Clostridium perfringens* (ATCC 13124) and *Peptostreptococcus anaerobius* (ATCC 27337) are prepared from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and adjusted to the correct inoculum concentration in pre-reduced Thioglycollate Medium, Enriched. The inoculum for *Bacteroides vulgatus* (ATCC 8482) is taken from Thioglycollate Medium, Enriched and the inoculum for *C. novyi* (ATCC 7659) is taken from Chopped Meat Glucose Broth, PR II. Tubes are inoculated below the surface of the broths as deeply into the medium as possible. The caps are tightened immediately after inoculation and the tubes are incubated aerobically at 35 ± 2°C. Tubes are read for the amount of growth after 18–24 h and 42–48 h. All organisms show trace to heavy growth after 48 h.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 297264 | BBL™ Enriched Thioglycollate Medium with Calcium Carbonate, Ctn. of 100 size K tubes |

XIV REFERENCES

1. Brewer, J.H. 1940. A clear liquid medium for the "aerobic" cultivation of anaerobes. *J. Bacteriol.* 39:10.
2. Vera, H.D. 1944. Comparative study of materials suitable for the cultivation of clostridia. *J. Bacteriol.* 47:59-65.
3. Dowell, V.R., Jr., and T.M. Hawkins. 1987. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta.
4. Dowell, V.R., Jr., G.L. Lombard, F.S. Thompson, and A.Y. Armfield. 1977. Media for isolation, characterization, and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta.
5. Forbes, B.A., and P.A. Granato. 1995. Processing specimens for bacteria, p. 265-281. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
6. Gibbons, R.J. and J.B. MacDonald. 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. *J. Bacteriol.* 80:164-170.
7. Wilkins, T.D., S.L. Chalgren, F. Jimenez-Ulate, C.R. Drake, Jr., and J.L. Johnson. 1976. Inhibition of *Bacteroides fragilis* on blood agar plates and reversal of inhibition by added hemin. *J. Clin. Microbiol.* 3:359-363.
8. Reischelderfer, C., and J.I. Mangels. 1994. Culture media for anaerobes, p. 2.3.1-2.3.8. *In* H.D. Isenberg (ed.), *Clinical microbiology procedures handbook*, vol. 1. American Society for Microbiology, Washington, D.C.
9. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore.
10. Finegold, S.M., V.L. Sutter, H.R. Attebery, and J.E. Rosenblatt. 1974. Isolation of anaerobic bacteria, p. 365-375. *In* E.H. Lennette, G.H. Spaulding, and J.P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
11. Vera, H.D. 1944. A comparative study of materials suitable for the cultivation of clostridia. *J. Bacteriol.* 47:59-70.
12. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
13. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
14. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
15. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
16. Murray, P.R., E.J. Baron, J.H. Tenover, M.L. Tenover and M.A. Pfaller (ed.) 2007. *Manual of clinical microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
17. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. *Bailey & Scott's diagnostic microbiology*, 12th ed. Mosby, Inc., St. Louis.
18. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Thioglycollate Medium without Indicator-135C is a general-purpose medium for the cultivation of microorganisms, especially obligate anaerobes.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Loosen the caps and place tubes in boiling water* for 2–5 min. Tighten the caps immediately after removing from the heat and allow the medium to cool to room temperature prior to use.
*NOTE: Use of a microwave oven is not recommended.
 - b. Using sterile 1.0 mL pipettes, inoculate tubes of Thioglycollate Medium with 1.0 mL of dilutions of 18- to 24-h broth cultures. Use Chopped Meat Carbohydrate Broth for *Bacteroides fragilis* and Trypticase™ Soy Broth for *Staphylococcus aureus*. The dilution used for *S. aureus* should be 1,000 or less CFU/mL; the dilution for *B. fragilis* should contain 10⁵–10⁶ CFU/mL.
 - c. Incubate tubes with tightened caps at 35 ± 2°C in an aerobic atmosphere.

2. Examine tubes at 18–24 and 48 h for growth.

3. Expected Results

| CLSI Organisms | ATCC™ | Recovery |
|--------------------------------|-------|----------|
| * <i>Bacteroides fragilis</i> | 25285 | Growth |
| * <i>Staphylococcus aureus</i> | 25923 | Growth |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.0 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Thioglycollate Medium without Indicator-135C is an enriched general-purpose medium for the recovery of a wide variety of microorganisms, particularly obligate anaerobes, from clinical specimens and other materials.

V SUMMARY AND EXPLANATION

Thioglycollate Medium was originally described by Brewer¹ as a medium favoring the growth of obligately anaerobic as well as aerobic organisms. The original thioglycollate medium was modified to have the nutritional quality of Trypticase Soy Broth. As a result the improved formula, 135C, has a broad growth spectrum of both pathogenic and nonpathogenic fastidious microorganisms.² The original formula also contained methylene blue, but no Eh indicator is now used. This avoids any possible toxicity of the indicator and also facilitates early detection of growth.

Thioglycollate Medium-135C is characterized by its superior ability to support growth, from minimal inocula, of a wide variety of aerobic and anaerobic organisms. The more strictly aerobic species grow at the top, while anaerobic types grow in the depths of the medium.

Thioglycollate Medium-135C is, therefore, recommended for use as a general utility medium, and for examination of blood cultures and all other materials in which the presence of a variety of aerobic, facultative or anaerobic organisms is possible.

The incorporation of the casein and soy peptones makes possible the growth of certain aerobic organisms, such as members of the genus *Brucella*, which do not grow readily in Fluid Thioglycollate Medium. Both media support growth of strictly anaerobic species, such as *Clostridium novyi*, *C. acetobutylicum*, *Actinomyces bovis* and *Bacteroides*, as well as facultative pneumococci, streptococci, lactobacilli and other bacteria.

The broth may be used with 10% added serum for cultivation of *Trichomonas vaginalis*, the Reiter spirochete and other organisms.

It is for this reason that the medium is satisfactorily used as an enrichment culture for various types of specimens and also as a transportation medium. When used for such purposes, it is recommended that CaCO₃ be incorporated, because otherwise fastidious organisms may grow and then die off rapidly; the CaCO₃ serves to neutralize acid produced during growth. Rapid growth and death, in the absence of CaCO₃ may occur, for example, with cultures of pneumococci, gram-negative cocci, *C. perfringens* and other acid-sensitive bacteria.

VI PRINCIPLES OF THE PROCEDURE

The casein and soybean meal peptones, dextrose and cystine supply nitrogenous and carbonaceous compounds, fermentable carbohydrate and trace ingredients. Sodium chloride provides essential ions. Sulfur is provided by sodium sulfite. Sodium thioglycollate, a reducing agent, lowers the Eh potential, thus enabling obligate anaerobic organisms to grow in the depths of the medium. The relatively small amount of agar aids in the prevention of convection currents in the medium and thus contributes to the maintenance of anaerobiosis.³

VII REAGENTS

Thioglycollate Medium without Indicator-135C

Approximate Formula* Per Liter Purified Water

| | | | |
|-------------------------------------|--------|-----------------------------|--------|
| Pancreatic Digest of Casein | 17.0 g | Sodium Thioglycollate | 0.5 g |
| Papaic Digest of Soybean Meal | 3.0 g | Agar | 0.7 g |
| Dextrose..... | 6.0 g | L-Cystine..... | 0.25 g |
| Sodium Chloride | 2.5 g | Sodium Sulfite | 0.1 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁴⁻⁷ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{3,8} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Thioglycollate Medium without Indicator-135C

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18–24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™** EZ anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling*, with caps loosened, and cooling, with tightened caps, to room temperature before inoculation.

Inoculate the specimen into the medium as soon as possible after it is received in the laboratory. With liquid specimens, tubed media should be inoculated with one or two drops of the specimen. Tissue specimens should be minced and ground in sterile, reduced broth for the cultivation of microorganisms. Inoculation is then performed as for liquid specimens. Swab specimens may be inserted into the broth after inoculation of plated media. Alternatively, the swab may be "scrubbed" in a small volume of sterile, reduced broth and the broth used to inoculate media as performed with liquid specimens.

Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Incubate tubes with tight caps aerobically at 35 ± 2°C, or other appropriate temperature depending on the type of organism being cultured, and inspect daily for up to 7 days before discarding as negative, unless special circumstances exist that warrant longer incubation.^{3,9}

*NOTE: Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

Growth in broth tubes is indicated by the presence of turbidity compared to an uninoculated control. Subcultures to appropriate solid media should be made to obtain pure cultures of isolates which can then be further tested and identified.

XI LIMITATIONS OF THE PROCEDURE

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.⁹

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{3,8,9}

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Thioglycollate Medium without Indicator-135C are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for 2–5 min. After cooling, the tubes are


inoculated with cultures of *Bacteroides fragilis* (ATCC 25285), *Clostridium novyi* (ATCC 7659) and *Staphylococcus aureus* (ATCC 25923). The inoculum (1 mL) for *S. aureus* is taken from a broth culture adjusted to contain 1,000 or less colony-forming units (CFU) per mL. The inoculum (1 mL) for *B. fragilis* is taken from a broth culture adjusted to contain 10^5 – 10^6 CFU per mL. The inoculum (0.01 mL loop) for *C. novyi* is taken from an undiluted broth culture; the inoculum is placed in the bottom of the tube. The caps are tightened immediately after inoculation and the tubes are incubated at $35 \pm 2^\circ\text{C}$. Tubes are read for the amount of growth after 18–24 h and 42–48 h. All organisms show trace to heavy growth after 48 h.


XIII AVAILABILITY

| Cat. No. | Description |
|----------|---|
| 221199 | BBL™ Thioglycollate Medium without Indicator-135C, 8 mL, Pkg. of 10 size K tubes |
| 221200 | BBL™ Thioglycollate Medium without Indicator-135C, 8 mL, Ctn. of 100 size K tubes |
| 221797 | BBL™ Thioglycollate Medium without Indicator-135C, 10 mL, Pkg. of 10 size D tubes |
| 221798 | BBL™ Thioglycollate Medium without Indicator-135C, 10 mL, Ctn. of 100 size D tubes |
| 221047 | BBL™ Thioglycollate Medium without Indicator-135C, 20 mL, Ctn. of 100 size A tubes |

XIV REFERENCES

1. Brewer, J.H. 1940. A clear liquid medium for the "aerobic" cultivation of anaerobes. *J. Bacteriol.* 39:10.
2. Vera, H.D. 1944. Comparative study of materials suitable for the cultivation of clostridia. *J. Bacteriol.* 47:59-65.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. *Bailey & Scott's diagnostic microbiology*, 12th ed. Mosby, Inc., St. Louis.
4. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
5. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
6. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
7. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
8. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. *Manual of clinical microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
9. Reischelderfer, C., and J.I. Mangels. 1994. Culture media for anaerobes, p. 2.3.1-2.3.8. *In* H.D. Isenberg (ed.), *Clinical microbiology procedures handbook*, vol. 1. American Society for Microbiology, Washington, D.C.

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BBL™ Trypticase™ Soy Broth

BBL™ Trypticase™ Soy Broth with 6.5% Sodium Chloride



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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Trypticase™ Soy Broth is a general purpose medium for the cultivation of fastidious and nonfastidious microorganisms. With the addition of 6.5% sodium chloride, differentiation between salt-tolerant and salt-intolerant species is achieved.

II PERFORMANCE TEST PROCEDURE

A. Trypticase Soy Broth

- Inoculate representative samples with the cultures listed below.
 - Using sterile 1.0 mL pipettes, inoculate tubes with dilutions of 18- to 48-h **Trypticase** Soy Broth cultures. Containers with fills over 5.0 mL should be inoculated with 0.75 mL of culture. Tubes with fills of 5.0 mL and below should be inoculated with 0.075 mL of culture.
 - For *Escherichia coli*, *Staphylococcus aureus*, and the organisms specified in *The United States Pharmacopeia (USP)* for growth promotion testing of sterility test media (*Bacillus*, *Candida* and *Aspergillus* strains), the dilution used should contain 100 or less CFU/mL.
 - For *Streptococcus pneumoniae*, the dilution should contain 1,000 or less CFU/mL.
 - Incubate the *E. coli* and *S. aureus* inoculated tubes and the *S. pneumoniae* tubes with loosened caps at 30–35°C. Incubate the *B. subtilis*, *C. albicans* and *A. brasiliensis* tubes with loosened caps at 20–25°C.

NOTE: Work with *A. brasiliensis* (ATCC™ 16404) in a biological safety cabinet.

- Examine *S. pneumoniae* at 18–24 h and *E. coli* and *S. aureus* cultures after 24–48 h of incubation. Examine *B. subtilis*, *C. albicans* and *A. brasiliensis* at 18–24 h and 66–72 h. *C. albicans* and *A. brasiliensis* may be incubated up to 5 days.

3. Expected Results

| CLSI Organisms | ATCC | Recovery |
|------------------------------------|-------|------------------|
| * <i>Escherichia coli</i> | 25922 | Growth by 48 h |
| * <i>Staphylococcus aureus</i> | 25923 | Growth by 48 h |
| Additional Organisms | | |
| ** <i>Bacillus subtilis</i> | 6633 | Growth by 3 days |
| ** <i>Candida albicans</i> | 10231 | Growth by 5 days |
| ** <i>Aspergillus brasiliensis</i> | 16404 | Growth by 5 days |
| <i>Streptococcus pneumoniae</i> | 6305 | Growth by 24 h |

* Recommended organism strain for User Quality Control.

**For verification of growth promotion for use in *USP* Sterility Tests.

B. Trypticase Soy Broth with 6.5% Sodium Chloride

- Inoculate representative samples with the cultures listed below.
 - Inoculate the tubes using a 0.01 mL loop with a 10⁻¹ dilution of 18- to 24-h **Trypticase** Soy Broth cultures.
 - Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.

- Examine tubes at 18–24 h and 42–48 h for growth and selectivity.

3. Expected Results

| Organisms | ATCC | Recovery |
|--------------------------------|-------|-----------|
| * <i>Enterococcus faecalis</i> | 29212 | Growth |
| * <i>Streptococcus bovis</i> | 9809 | No Growth |

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under "Product Deterioration."
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- For **Trypticase** Soy Broth, determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2.
- Incubate uninoculated representative tubes at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Trypticase Soy Broth conforms with specifications of *The United States Pharmacopeia (USP)*.

Trypticase Soy Broth (Soybean-Casein Digest Medium) is a general-purpose medium used in qualitative procedures for the cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens.

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate *Enterococcus* species from the *Streptococcus bovis* group of streptococci.

V SUMMARY AND EXPLANATION

Trypticase Soy Broth (TSB) is a nutritious medium that will support the growth of a wide variety of microorganisms, including common aerobic, facultative and anaerobic bacteria and fungi.¹⁻⁴ Because of its capacity for growth promotion, this formulation is included in the *USP* as a sterility test medium.⁵

TSB is also recommended, because of growth promotion, for use as the inoculum broth for disc diffusion and agar-dilution antimicrobial susceptibility testing as standardized by the Clinical and Laboratory Standards Institute (formerly NCCLS).^{6,7} **Trypticase Soy Broth with 6.5% Sodium Chloride** is used to differentiate the enterococcal species from the *S. bovis* group of streptococci by the 6.5% NaCl tolerance test.⁸

VI PRINCIPLES OF THE PROCEDURE

Enzymatic digests of casein and soybean meal provide amino acids and other complex nitrogenous substances. Dextrose is an energy source. Sodium chloride maintains the osmotic equilibrium. Dibasic potassium phosphate acts as a buffer to control pH. The addition of 6.5% sodium chloride to **Trypticase Soy Broth** permits the differentiation of salt-tolerant enterococci, which are resistant to the high salt content, from the salt-intolerant *S. bovis* group and other streptococcal species. At this concentration, the sodium chloride is a selective agent that interferes with membrane permeability and osmotic and electrokinetic equilibria.¹

VII REAGENTS

Trypticase Soy Broth

Approximate Formula* Per Liter Purified Water

| | |
|-------------------------------------|--------|
| Pancreatic Digest of Casein | 17.0 g |
| Papaic Digest of Soybean Meal | 3.0 g |
| Sodium Chloride | 5.0 g |
| Dipotassium Phosphate | 2.5 g |
| Dextrose..... | 2.5 g |

*Adjusted and/or supplemented as required to meet performance criteria.

Trypticase Soy Broth with 6.5% Sodium Chloride contains 60 g/L of sodium chloride in addition to the ingredients listed above.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁹⁻¹² and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{3,13} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: **Trypticase Soy Broth** or **Trypticase Soy Broth with 6.5% Sodium Chloride**

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Inoculate the medium as soon as possible after the specimens arrive at the laboratory. Swab specimens may be inserted into the medium after inoculation of appropriate plated media. For liquid specimens, use a sterile inoculating loop to transfer a loopful of the specimen to the broth medium. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Incubate the tubes with loosened caps at 35 ± 2°C aerobically with or without supplementation with carbon dioxide. Tubed media intended for the cultivation of anaerobes should be incubated under anaerobic conditions. An efficient and easy way to obtain suitable anaerobic conditions is through the use of a **BD GasPak™ EZ** anaerobic system or equivalent alternative system. Examine for growth after 18–24 h and 42–48 h of incubation.

For use in sterility testing, consult the *USP* for procedural details and specifications for volume of medium relative to container size.⁵

For use in the preparation of standardized inocula for antimicrobial susceptibility testing, refer to the CLSI standards.^{6,7}

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media (**Trypticase Soy Broth** only).

X RESULTS

Growth in broth media is indicated by the presence of turbidity compared to an uninoculated control. Broth cultures should be held for at least a week before discarding as negative.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{3,13,14}

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides, and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

Strains of other catalase-negative, gram-positive cocci, i.e., *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Vagococcus*, have been isolated from human infections. Therefore, the presumptive identification of enterococci based on the bile-esculin reaction and growth in 6.5% NaCl broth only cannot be made.⁸

XII PERFORMANCE CHARACTERISTICS

Trypticase Soy Broth

Trypticase Soy Broth was used as a control in the evaluation of bile as a culture medium for the isolation of *Salmonella* from blood cultures. Sixty-eight percent (68%) of the **Trypticase Soy Broth** cultures were positive for *Salmonella*.¹⁵ In another study, growth patterns of *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, group B β -hemolytic *Streptococcus* (GBS), *Klebsiella pneumoniae* and *Staphylococcus epidermidis* were compared using cerebrospinal fluid (CSF), **Trypticase Soy Broth** (TSB), and a phosphate buffer. *S. aureus*, GBS, *L. monocytogenes*, *K. pneumoniae* and *S. epidermidis* grew within 24 h in TSB; the growth of these bacteria in CSF and phosphate buffer remained within one log of the starting point with the exception of GBS and *S. epidermidis* whose growth continued to decline. All three fluids supported the growth of *E. coli* to varying degrees for 24 h.¹⁶

Trypticase Soy Broth with 6.5% Sodium Chloride

Prior to release, all lots of **Trypticase Soy Broth with 6.5% Sodium Chloride** are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are tested with **Trypticase Soy Broth** cultures diluted to 10⁻¹ of *Enterococcus faecalis* (ATCC 29212) and *Streptococcus bovis* (ATCC 9809). The tubes are incubated at 35 ± 2°C and read after 18–24 h and 42–48 h for growth. *E. faecalis* exhibits moderate to heavy growth while *S. bovis* is completely inhibited.


Additionally, representative samples are tested chemically by silver nitrate titration for sodium chloride content. The calculated percent sodium chloride is 6.0–7.0.

XIII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 297294 | BBL™ Trypticase™ Soy Broth , 0.5 mL, Ctn. of 100 size K tubes |
| 295634 | BBL™ Trypticase™ Soy Broth , 1 mL, Ctn. of 100 size K tubes |
| 299647 | BBL™ Trypticase™ Soy Broth , 1 mL, Ctn. of 100 size K tubes (Filtered) |
| 221815 | BBL™ Trypticase™ Soy Broth , 2 mL, Ctn. of 100 size K tubes |
| 297979 | BBL™ Trypticase™ Soy Broth , 3 mL, Ctn. of 100 size K tubes |
| 299783 | BBL™ Trypticase™ Soy Broth , 3 mL, Ctn. of 100 size E tubes |
| 297482 | BBL™ Trypticase™ Soy Broth , 4 mL, Ctn. of 100 size D tubes |
| 221715 | BBL™ Trypticase™ Soy Broth , 5 mL, Pkg. of 10 size K tubes |
| 221716 | BBL™ Trypticase™ Soy Broth , 5 mL, Ctn. of 100 size K tubes |
| 297342 | BBL™ Trypticase™ Soy Broth , 5 mL, Ctn. of 100 size D tubes |
| 221092 | BBL™ Trypticase™ Soy Broth , 8 mL, Pkg. of 10 size K tubes |
| 221093 | BBL™ Trypticase™ Soy Broth , 8 mL, Ctn. of 100 size K tubes |
| 297354 | BBL™ Trypticase™ Soy Broth , 10 mL, Ctn. of 100 size D tubes |
| 221823 | BBL™ Trypticase™ Soy Broth , 15 mL, Ctn. of 100 size A tubes |
| 299749 | BBL™ Trypticase™ Soy Broth , 20 mL, Ctn. of 100 size A tubes |
| 297811 | BBL™ Trypticase™ Soy Broth , 21 mL, Pkg. of 10 size A tubes |
| 221351 | BBL™ Trypticase™ Soy Broth with 6.5% Sodium Chloride , Ctn. of 100 size K tubes |

XIV REFERENCES

1. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
2. Wehr, H.M. and J.F. Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis.
4. Fredette, V., and A. Forget. 1961. The sensitivity of several media to small inocula. Extract from a paper presented at the Canadian Society of Microbiology Annual Meeting, June 12-15. Kingston, Ontario.
5. U.S. Pharmacopeial Convention, Inc. 2009. The U.S. pharmacopeia 32/The national formulary 27. U.S. Pharmacopeial Convention, Inc., Rockville, Md.
6. Clinical and Laboratory Standards Institute. 2009. Approved standard: M2-A10. Performance standards for antimicrobial disk susceptibility tests, 10th ed. CLSI, Wayne, Pa.
7. Clinical and Laboratory Standards Institute. 2009. Approved standard: M7-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th ed. CLSI, Wayne, Pa.
8. Facklam, R.R., D.F. Sahm, and L.M. Teixeira. 1999. *Enterococcus*, p. 297-305. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
9. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
10. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
11. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
12. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). 8 Official Journal L262, 17/10/2000, p. 0021-0045.
13. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
14. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
15. Kaye, D. et al. 1966. Comparison of bile and **Trypticase** soy broth for isolation of *Salmonella* from blood. Am. J. Clin. Pathol. 46:408-410.
16. Agbayani, M.M. et al. 1981. Effect of CSF on bacterial growth. Arch. Neurol. 38: 43-45.

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