

Recommended Culture Methods for Microorganisms

Selection of Growth Requirements

1. Primary growth on a nonselective agar medium is preferred. Primary growth in a fluid medium should only occur in special instances or when recommended. Because of the manipulations required during hydration, it is difficult to obtain purity of a lyophilized strain in a fluid medium. A contaminant may completely overgrow and obscure the presence of the lyophilized strain.
2. The following information lists which method should be used to grow the various microorganism species. Descriptions of methods follow the microorganism list.

Microorganism	Method	Notes
Acetobacter species	Method 36	
Achromobacter species	Method 1	
Acinetobacter species	Method 1	
Actinobacillus species	Method 3	
Actinomyces species	Method 4	
Aerococcus species	Method 1	
Aeromonas species	Method 2	Exceptions are <i>Aeromonas hydrophila</i> , Microbiologics 0870 and <i>Aeromonas salmonicida</i> .
Aeromonas hydrophila, Microbiologics 0870	Method 31	
Aeromonas salmonicida	Method 32	
Aggregatibacter species	Method 3	
Alcaligenes species	Method 1	
Alicyclobacillus species	Method 12	An exception is <i>Alicyclobacillus acidoterrestris</i> .
Alicyclobacillus acidoterrestris	Method 45	
Alloiococcus species	Method 2	
Alternaria species	Method 5	
Amylomyces species	Method 5	
Aneurinibacillus species	Method 1	
Aquaspirillum species	Method 20	.
Arcanobacterium species	Method 34	
Arthrobacter species	Method 21	
Aspergillus species	Method 5	An exception is <i>Aspergillus flavus</i> .
Aspergillus flavus	Method 46	
Aureobasidium species	Method 5	
Bacillus species	Method 49	
Bacteroides species	Method 4	
Bifidobacterium species	Method 4	An exception is <i>Bifidobacterium animalis</i> subsp. <i>animalis</i> .
Bifidobacterium animalis subsp. animalis.	Method 39	
Bordetella bronchiseptica	Method 15	

Microorganism	Method	Notes
<i>Bordetella parapertussis</i>	Method 16	
<i>Bordetella pertussis</i>	Method 16	An exception is <i>Bordetella pertussis</i> , Microbiologics catalog number 0843.
<i>Bordetella pertussis</i> Microbiologics 0843	Method 57	
<i>Brevibacillus</i> species	Method 1	
<i>Brevundimonas</i> species	Method 1	
<i>Brochothrix</i> species	Method 21	
<i>Budvicia</i> species	Method 21	
<i>Burkholderia</i> species	Method 1	
<i>Campylobacter</i> species	Method 6	
<i>Candida</i> species	Method 5	
<i>Capnocytophaga</i> species	Method 3	
<i>Cedecea</i> species	Method 1	
<i>Cellulosimicrobium</i> species	Method 1	
<i>Chaetomium</i> species	Method 5	
<i>Chryseobacterium</i> species	Method 1	An exception is <i>Chryseobacterium shigense</i> .
<i>Chryseobacterium shigense</i>	Method 22	
<i>Citrobacter</i> species	Method 1	
<i>Cladosporium</i> species	Method 5	
<i>Clostridioides difficile</i>	Method 4	
<i>Clostridium</i> species	Method 40	Exceptions are <i>Clostridium perfringens</i> and <i>Clostridium sordellii</i> .
<i>Clostridium perfringens</i>	Method 41	An exception is <i>Clostridium perfringens</i> , Microbiologics 0318.
<i>Clostridium perfringens</i> Microbiologics 0318	Method 63	
<i>Clostridium sordellii</i>	Method 4	
<i>Corynebacterium</i> species	Method 1	An exception is <i>Corynebacterium urealyticum</i> .
<i>Corynebacterium urealyticum</i>	Method 2	
<i>Cronobacter</i> species	Method 1	
<i>Cryptococcus</i> species	Method 62	An exception is <i>Cryptococcus gattii</i> , Microbiologics 01051.
<i>Cryptococcus gattii</i>	Method 47	
<i>Curtobacterium</i> species	Method 1	
<i>Cutibacterium</i> species	Method 44	
<i>Deinococcus</i> species	Method 1	An exception is <i>Deinococcus radiophilus</i> , Microbiologics 01184.
<i>Deinococcus radiophilus</i> Microbiologics 01184	Method 23	
<i>Delftia</i> species	Method 1	
<i>Desulfotomaculum</i> species	Method 17	
<i>Edwardsiella</i> species	Method 1	
<i>Eggerthella</i> species	Method 4	

Microorganism	Method	Notes
<i>Eikenella</i> species	Method 3	
<i>Elizabethkingia</i> species	Method 1	
<i>Enterobacter</i> species	Method 1	
<i>Enterococcus</i> species	Method 1	
<i>Erysipelothrix</i> species	Method 2	
<i>Escherichia coli</i>	Method 1	
<i>Eurotium rubrum</i>	Method 5	
<i>Exiguobacterium</i> species	Method 1	
<i>Finegoldia</i> species	Method 42	
<i>Fluoribacter</i> species	Method 8	
<i>Fusarium</i> species	Method 5	
<i>Fusobacterium</i> species	Method 4	An exception is <i>Fusobacterium mortiferum</i> , Microbiologics 01191.
<i>Fusobacterium mortiferum</i> Microbiologics 01191	Method 39	
<i>Gardnerella</i> species	Method 9	
<i>Gemella</i> species	Method 4	
<i>Geobacillus</i> species	Method 24	
<i>Geotrichum</i> species	Method 5	
<i>Haemophilus</i> species	Method 3	
<i>Hafnia</i> species	Method 1	
<i>Hanseniaspora</i> species	Method 5	
<i>Herminiimonas</i> species	Method 20	
<i>Issatchenkia</i> species	Method 5	
<i>Kingella</i> species	Method 33	
<i>Klebsiella</i> species	Method 1	
<i>Kloeckera</i> species	Method 5	
<i>Kocuria</i> species	Method 1	An exception is <i>Kocuria rosea</i> .
<i>Kocuria rosea</i>	Method 21	
<i>Lactobacillus</i> species	Method 65	Exceptions are <i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus gasseri</i> , and <i>Lactobacillus leichmannii</i> .
<i>Lactobacillus acidophilus</i>	Method 11	
<i>Lactobacillus casei</i>	Method 11	
<i>Lactobacillus gasseri</i>	Method 11	
<i>Lactobacillus leichmannii</i>	Method 11	
<i>Lactococcus</i> species	Method 2	
<i>Leclercia</i> species	Method 1	
<i>Legionella</i> species	Method 8	
<i>Listeria</i> species	Method 1	
<i>Lysinibacillus</i> species	Method 1	
<i>Macrococcus</i> species	Method 1	
<i>Malassezia</i> species	Method 14	

Microorganism	Method	Notes
Mannheimia species	Method 1	
Methylobacterium species	Method 25	An exception is <i>Methylobacterium extorquens</i> .
Methylobacterium extorquens	Method 26	
Meyerozyma guilliermondii	Method 5	
Microbacterium species	Method 22	
Micrococcus species	Method 1	An exception is <i>Micrococcus luteus</i> , Microbiologics 0337 and <i>Micrococcus luteus</i> , Microbiologics 0689.
Micrococcus luteus, Microbiologics 0337	Method 27	
Micrococcus luteus, Microbiologics 0689	Method 67	
Microsporium species	Method 5	Exceptions are <i>Microsporium canis</i> and <i>Microsporium gypseum</i> .
Microsporium canis	Method 48	
Microsporium gypseum	Method 73	
Moraxella species	Method 2	
Morganella species	Method 1	
Mucor racemosus	Method 5	
Mycobacterium species	Method 13	Exceptions are <i>Mycobacterium fortuitum</i> , <i>Mycobacterium peregrinum</i> and <i>Mycobacterium haemophilum</i> , and <i>Mycobacterium smegmatis</i> .
Mycobacterium fortuitum	Method 7	
Mycobacterium haemophilum	Method 18	
Mycobacterium peregrinum	Method 7	
Mycobacterium smegmatis	Method 7	
Mycoplasma bovis	Method 60	
Mycoplasma hominis	Method 58	
Mycoplasma pneumoniae	Method 59	
Myroides species	Method 2	
Neisseria species	Method 37	
Nocardia species	Method 81	
Novosphingobium species	Method 21	
Ochrobactrum species	Method 1	
Oligella species	Method 2	
Paecilomyces species	Method 5	
Paenibacillus species	Method 1	An exception is <i>Paenibacillus larvae</i> subsp. <i>larvae</i> .
Paenibacillus larvae subsp. larvae	Method 82	
Parabacteroides species	Method 4	
Parvimonas species	Method 43	
Pasteurella species	Method 2	
Pediococcus species	Method 11	
Penicillium species	Method 5	

Microorganism	Method	Notes
<i>Peptoniphilus</i> species	Method 42	
<i>Peptostreptococcus</i> species	Method 4	
<i>Plesiomonas</i> species	Method 1	
<i>Pluralibacter gergoviae</i>	Method 1	
<i>Porphyromonas</i> species	Method 43	
<i>Prevotella</i> species	Method 43	
<i>Propionibacterium</i> species	Method 44	
<i>Proteus</i> species	Method 1	An exception is <i>Proteus hauseri</i> .
<i>Proteus hauseri</i>	Method 27	
<i>Prototheca</i> species	Method 5	
<i>Providencia</i> species	Method 1	
<i>Pseudomonas</i> species	Method 1	Exceptions are <i>Pseudomonas aeruginosa</i> , Microbiologics 0484, <i>Pseudomonas benneri</i> , <i>Pseudomonas fluorescens</i> , <i>Pseudomonas mosselii</i> , <i>Pseudomonas protegens</i> , and <i>Pseudomonas putida</i> .
<i>Pseudomonas aeruginosa</i>, Microbiologics 0484	Method 28	
<i>Pseudomonas brenneri</i>	Method 21	
<i>Pseudomonas fluorescens</i>	Method 21	
<i>Pseudomonas mosselii</i>	Method 21	
<i>Pseudomonas protegens</i>	Method 21	
<i>Pseudomonas putida</i>	Method 22	
<i>Ralstonia</i> species	Method 1	An exception is <i>Ralstonia pickettii</i> , Microbiologics 01197.
<i>Ralstonia pickettii</i>, Microbiologics 01197	Method 29	
<i>Raoultella</i> species	Method 1	
<i>Rhizopus</i> species	Method 5	
<i>Rhodococcus</i> species	Method 2	
<i>Rhodotorula</i> species	Method 5	
<i>Saccharomyces</i> species	Method 50	
<i>Salmonella</i> species	Method 1	
<i>Scopulariopsis</i> species	Method 5	
<i>Serratia</i> species	Method 1	
<i>Shewanella</i> species	Method 1	Exceptions are <i>Shewanella haliotis</i> and <i>Shewanella putrefaciens</i> .
<i>Shewanella haliotis</i>	Method 74	
<i>Shewanella putrefaciens</i>	Method 75	
<i>Shigella</i> species	Method 1	
<i>Sphingobacterium</i> species	Method 1	
<i>Sphingomonas</i> species	Method 21	
<i>Sporidiobolus</i> species	Method 5	
<i>Staphylococcus</i> species	Method 1	An exception is <i>Staphylococcus aureus</i> , Microbiologics 0158.

Microorganism	Method	Notes
<i>Staphylococcus aureus</i>, Microbiologics 0158	Method 30	
<i>Stenotrophomonas</i> species	Method 22	
<i>Streptococcus</i> species	Method 34	Exceptions are <i>Streptococcus</i> species, Microbiologics 0978, and <i>Streptococcus pneumoniae</i> .
<i>Streptococcus pneumoniae</i>	Method 66	
<i>Streptococcus</i> species, Microbiologics 0978	Method 66	
<i>Streptomyces</i> species	Method 5	
<i>Talaromyces</i> species	Method 5	
<i>Thermoanaerobacterium</i> species	Method 56	
<i>Trichoderma</i> species	Method 5	
<i>Trichophyton</i> species	Method 51	
<i>Trichosporon</i> species	Method 5	
<i>Trueperella pyogenes</i>	Method 34	
<i>Ureaplasma</i> species	Method 61	
<i>Veillonella</i> species	Method 4	
<i>Vibrio</i> species	Method 10	An exception is <i>Vibrio alginolyticus</i> .
<i>Vibrio alginolyticus</i>, Microbiologics 0819	Method 54	
<i>Virgibacillus</i> species	Method 1	
<i>Wallemia mellicola</i>	Method 83	
<i>Yarrowia</i> species	Method 5	
<i>Yersinia</i> species	Method 1	An exception is <i>Yersinia ruckeri</i> , Microbiologics 0785.
<i>Yersinia ruckeri</i>, Microbiologics 0785	Method 21	
<i>Zygosaccharomyces</i> species	Method 5	Exceptions are <i>Zygosaccharomyces rouxii</i> , Microbiologics 0803 and <i>Zygosaccharomyces parabailii</i> , Microbiologics 01011.
<i>Zygosaccharomyces rouxii</i>, Microbiologics 0803	Method 53	
<i>Zygosaccharomyces parabailii</i>, Microbiologics 01011	Method 52	

3. The following information lists methods for growing microorganisms. When possible, more than one type of agar medium per method is listed.

Method 1

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 2

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Method 3

Media	Chocolate Agar
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	24 to 48 hours

Method 4

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Note: Some obligate anaerobes may require 5-7 days to demonstrate sufficient growth.

Method 5

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.

Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 6

Media	Chocolate Agar
Temperature	35°C
Atmosphere	Microaerophilic
Growth Time	48 to 72 hours

Note: Do not open the inoculated agar medium petri plate for the first 48 hours.

Method 7

Media	Lowenstein Jensen Agar or Middlebrook Agar
Temperature	35°C
Atmosphere	Aerobic or 5 to 7% Carbon Dioxide
Growth Time	2 to 30 days

Note: *M. fortuitum* subsp. *fortuitum*, *M. smegmatis*, *M. peregrinum* will also grow on Tryptic Soy Agar (Soybean Casein Digest Agar) as well as Lowenstein Jensen and Middlebrook Agar but additional incubation time may be required.

Method 8

Media	Buffered Charcoal Yeast Extract Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	3 to 5 days

Method 9

Media	V Agar or Chocolate Agar
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	48 hours

Method 10

Media	Tryptic Soy Agar (Soybean Casein Digest Agar) or Marine Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 35°C for 24 to 48 hours.

Note: Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery when grown on the recommended media.

Method 11

Media	Phase 1: MRS (Man, Rogosa, Sharpe) Broth Phase 2: Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
Growth Time	Phase 1: 48 hours Phase 2: 48 hours

Note: For Phase 1, the primary growth medium is MRS (Man, Rogosa, Sharpe) Broth. Incubate at 35°C in aerobic atmosphere for 48 hours. For Phase 2, transfer to either Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood using a sterile swab or pipette. Incubate at 35°C in 5 to 7% carbon dioxide for 48 hours.

Method 12

Media	Potato Dextrose Agar
Temperature	55°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 13

Media	Lowenstein Jensen Agar or Middlebrook Agar
Temperature	35°C
Atmosphere	Aerobic or 5 to 7% Carbon Dioxide
Growth Time	May require up to one month of incubation.

Method 14

Media	Leeming Notman Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	72 hours

Method 15

Media	Chocolate agar, Nonselective Sheep Blood Agar, Tryptic Soy Agar, and Bordet Gengou Agar with 15% Defibrinated Sheep Blood
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Standard Methods Agar (Plate Count Agar) or Nutrient Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 16

Media	Chocolate agar or Bordet Gengou Agar with 15% Defibrinated Sheep Blood
Temperature	35°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Method 17

Media	Phase 1: ISF (modified Infant Soy Formula) Broth Phase 2: Sulfite Agar
Temperature	Phase 1: 55°C Phase 2: 55°C
Atmosphere	Phase 1: Anaerobic Phase 2: Anaerobic
Growth Time	Phase 1: 48 hours Phase 2: 48 hours to 7 days

Prepare and use ISF (modified Infant Soy Formula) Broth using the following steps:

1. Fill tubes with 10 ml Infant Soy Formula. Infant Soy Formula may be purchased at a grocery store.
2. Place a four-penny nail in each tube. A four-penny nail is approximately 1.5 inches, or 38 mm, in length. It should contain steel or iron.
3. Sterilize the broth.
4. Inoculate ISF Broth with one LYFO DISK™ or KWIK-STIK™.
5. Grow at 55°C in anaerobic conditions for 48 hours. The broth will turn grey, indicating growth.
6. Make two dilutions, 1:10 and 1:100.
7. Subculture with a swab to Sulfite Agar. Plate the undiluted sample and the 1:10 and 1:100 dilutions. It is necessary to plate the diluted samples because at higher concentrations the

colonies are pin-point which makes colony characteristics difficult to see. Sulfite Agar is used for detecting thermophilic anaerobes which produce sulfite.

8. Incubate the agar in anaerobic environment at 55°C for 48 hours to 7 days.

Method 18

Media	Middlebrook 7H11 Agar
Temperature	30°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	3 to 4 weeks

Note: An X factor strip must be placed on the agar in order for the organism to grow.

Method 19

Media	Sheep Blood Agar supplemented with Pyridoxal
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	24 to 48 hours

Method 20

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	6 days

Method 21

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 22

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 23

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 24

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	55°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 25

Media	Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	5 days

Method 26

Media	Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	5 days

Note: Alternatively, *M. extorquens* may be grown on R2A Agar in 72 hours at 30°C.

Method 27

Media	Tryptic Soy Agar (Soybean Casein Digest Agar) or Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 28

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, or Standard Methods Agar (Plate Count Agar)
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 29

Media	Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar), or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 30

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C

Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: The degree of resistance of *S. aureus*, Microbiologics 0158, to Vancomycin tends to decrease depending on age of culture, type of media, and number of subcultures. For best results, propagate strain on Brain Heart Infusion Agar with 4mcg/ml Vancomycin.

Method 31

Media	Nonselective Sheep Blood Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Method 32

Media	Nonselective Sheep Blood Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Method 33

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	5 to 10% Carbon Dioxide
Growth Time	24 to 72 hours

Method 34

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Note: *Streptococcus* will also recover well on Columbia CNA Agar with 5% Sheep Blood.

Note: Growth of some species such as *Streptococcus*, *Arcanobacterium* and *Trueperella* is enhanced by enrichment of the incubation atmosphere with carbon dioxide. 5% Carbon Dioxide is recommended for the culture of *Streptococcus pneumoniae* and other streptococcal species of the viridans group.

Method 35

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Microaerophilic
Growth Time	24 to 72 hours

Method 36

Media	Chocolate Agar
Temperature	25°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	3 to 4 days

Method 37

Media	Chocolate Agar
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	24 to 48 hours

Note: Do not open the inoculated agar medium petri plate for the first 48 hours if using a candle jar.

Method 38

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	2 to 5 days

Note: *B. ureolyticus* colonies are very small. Several subculture plates may need to be inoculated to have adequate quantity of the microorganism for testing.

Method 39

Media	Anaerobic Blood Agar, Tryptic Soy Agar (Soybean Casein Digest Agar)
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Method 40

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Note: Nutrient Agar, Tryptic Soy Agar (Soybean Casein Digest Agar), and Standard Methods Agar (Plate Count Agar) are appropriate alternatives for some *Clostridium* species together with an additional period (24 hours) of incubation. *Clostridium* species may have reduced recovery when using the alternative agars.

Method 41

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	24 hours

Note: Tryptic Soy Agar (Soybean Casein Digest Agar) and Standard Methods Agar are appropriate alternatives together with an additional period (24 hours) of incubation. Using alternative agars may result in reduced recovery for *Clostridium* species.

Method 42

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	3 to 4 days

Method 43

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	5 to 7 days

Method 44

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	3 to 5 days

Method 45

Media	Potato Dextrose Agar
Temperature	45°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 46

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.

Note: Nutrient Agar, Tryptic Soy Agar, and Potato Dextrose Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 47

Media	Sabouraud Dextrose Emmons Agar or Malt Extract Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Method 48

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.

Note: Nutrient Agar, Tryptic Soy Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 49

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Some *Bacillus* spp. demonstrate better recovery on subculture when the stock organism growth is maintained at room temperature rather than 2° to 8°C.

Method 50

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Method 51

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	7 to 14 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.

Note: Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 52

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Potato Dextrose Agar and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 53

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.

Note: Nutrient Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 54

Media	Marine Agar; See notes below for rehydration instructions
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 35°C for 24 to 48 hours.

Note: Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery.

Method 55

Media	See note below for important directions. Phase 1: MRS (Man, Rogosa, Sharpe) Broth Phase 2: MRS Agar
Temperature	Phase 1: 25°C Phase 2: 25°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
Growth Time	Phase 1: 48 to 72 hours Phase 2: 72 to 96 hours.

Note: For Phase 1, *P. damnosus* may be grown in MRS broth at 25°C for 48 to 72 hours. When the broth becomes cloudy, begin Phase 2 by subculturing the broth to MRS agar using a sterile swab or pipette. Incubate the MRS agar at 25°C in 5 to 7% Carbon Dioxide for 72 to 96 hours.

Note: Alternatively, the lyophilized microorganism may be grown directly on MRS Agar at 25°C in 5 to 7% carbon dioxide for 5 to 7 days.

Method 56

Media	See note below for important directions. Phase 1: Cooked Meat Medium Phase 2: Anaerobic Blood Agar
Temperature	Phase 1: 45°C Phase 2: 45°C
Atmosphere	Phase 1: Aerobic Phase 2: Anaerobic
Growth Time	Phase 1: 72 hours Phase 2: 3 to 5 days

Note: Primary growth medium for *T. thermosaccharolyticum*, Microbiologics 0728, is Cooked Meat Medium. During Phase 1, incubation at 45°C for 72 hours is required. During Phase 2, the organism is transferred to Anaerobic Blood Agar which is incubated anaerobically at 45°C for 3 to 5 days.

Method 57

Media	Bordet Gengou Agar with 15% Defibrinated Sheep Blood
Temperature	35°C
Atmosphere	Aerobic
Growth Time	2 days to 1 week

Method 58

Media	See note below for important directions. Phase 1: Mycoplasma Broth Phase 2: Mycoplasma Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
Growth Time	Phase 1: 48 hours Phase 2: 4 to 6 days

Note: Inoculate Broth with LYFO DISK™. For Phase 1, prepare a 1:10 serial dilution using Mycoplasma Broth. Incubate the broth aerobically at 35°C for 48 hours. After incubation, begin Phase 2 by plating 0.2 ml of the broth culture to Mycoplasma Agar. Incubate agar in 5 to 7% Carbon Dioxide at 35°C for 3 to 7 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 59

Media	See note below for important directions. Phase 1: SP4 Glucose Broth Phase 2: SP4 Glucose Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: Carbon Dioxide (Candle Jar)
Growth Time	Phase 1: 7 to 28 days Phase 2: 5 to 15 days

Note: Inoculate Broth with LYFO DISK™. For Phase 1, prepare a 1:10 serial dilution using SP4 Glucose Broth. Incubate broth aerobically at 35°C for 7 to 28 days until the broth turns yellow. Then plate 0.2 ml of the broth culture to SP4 Glucose Agar. Incubate agar in a candle jar for 5 to 15 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 60

Media	See note below for important directions. Phase 1: Mycoplasma Broth Phase 2: Mycoplasma Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
Growth Time	Phase 1: 48 hours Phase 2: 3 to 7 days

Note: Inoculate Broth with LYFO DISK™. For Phase 1, prepare a 1:10 serial dilution using Mycoplasma Broth. Incubate the broth aerobically at 35°C for 48 hours. For Phase 2, plate 0.2 ml of the broth culture to Mycoplasma Agar. Incubate agar in 5 to 7% Carbon Dioxide at 35°C for 3 to 7 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 61

Media	See note below for important directions. Phase 1: SP4 Urea Broth Phase 2: A8 Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: Anaerobic
Growth Time	Phase 1: 24 to 96 hours Phase 2: 4 to 6 days

Note: Inoculate Broth with LYFO DISK™. For Phase 1, prepare a 1:10 serial dilution using SP4 Urea Broth. Incubate the broth aerobically at 35°C for 24 to 96 hours. As soon as the SP4 Urea Broth turns red, begin Phase 2 by plating 0.1 ml of the broth to A8 Agar and streak for isolation. Incubate A8 agar anaerobically at 35°C for 4 to 6 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 62

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: *Cryptococcus* species grow poorly on Nonselective Sheep Blood Agar.

Note: Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 63

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Note: Tryptic Soy Agar (Soybean Casein Digest Agar) is an appropriate alternative together with an additional period (24 hours) of incubation time. Using the alternative agar may result in reduced recovery for *Clostridium* species.

Method 64

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	55°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 65

Media	Nonselective Sheep Blood Agar or Columbia CNA with 5% Sheep Blood
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	48 hours

Note: Alternatively, the strain may be grown in MRS (Man, Rogosa, Sharpe) Broth in an aerobic atmosphere for 48 hours. Transfer to either Columbia CNA with Sheep Blood or Nonselective Sheep Blood Agar. Incubate at 35°C in 5 to 7% Carbon Dioxide for 48 hours.

Method 66

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	5% Carbon Dioxide
Growth Time	24 to 72 hours

Note: *Streptococcus* will also recover well on Columbia CNA with 5% Sheep Blood.

Method 67

Media	Tryptic Soy Agar (Soybean Casein Agar) or Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Alternatively, the microorganism may be grown on Standard Methods Agar (Plate Count Agar) for a minimum of 72 hours.

Method 68

Media	Chocolate Agar
Temperature	35°C
Atmosphere	Microaerophilic
Growth Time	48 hours

Method 69

Media	Malt Extract Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	5 to 7 days

Method 70

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 71

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	7-14 days

Method 72

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 hours

Method 73

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 4 weeks

Note: Nonselective Sheep Blood Agar, Malt Agar, Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives.

Method 74

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar or Standard Methods Agar (Plate Count Agar)
Temperature	30°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 30°C for 24 to 48 hours.

Note: Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery when transferred to the recommended media.

Method 75

Media	Tryptic Soy Agar (Soybean Casein Agar) or Nonselective Sheep Blood Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: For optimal recovery, rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 25°C for 24 to 48 hours.

Note: Rehydration with water or the fluid provided in the KWIK-STIK™ unit may result in decreased or no recovery.

Method 76

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	One week

Method 77

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 4 days

Method 78

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	48 to 72 hours

Method 79

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	3 to 7 days

Method 80

Media	Nutrient
Temperature	25°C
Atmosphere	Aerobic
Growth Time	3 to 5 days

Method 81

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	4 to 5 days

Note: Very small colonies develop within 48 hours. The colony morphology does not fully develop until 4 to 5 days.

Method 82

Media	Columbia Blood Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	48 to 72 hours

Note: Using alternative agars such as Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar may result in reduced recovery.

Method 83

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Nutrient Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.