

## 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
<i>Acetobacter</i> species	Method 80	
<i>Acinetobacter</i> species	Method 1	
<i>Actinobacillus</i> species	Method 3	
<i>Actinomyces</i> species	Method 4	
<i>Aerococcus</i> species	Method 1	
<i>Aeromonas</i> species	Method 2	Exceptions are <i>Aeromonas hydrophila</i> and <i>Aeromonas salmonicida</i> .
<i>Aeromonas hydrophila</i>	Method 31	
<i>Aeromonas salmonicida</i>	Method 32	
<i>Aggregatibacter</i> species	Method 3	
<i>Alcaligenes</i> species	Method 1	
<i>Alicyclobacillus</i> species	Method 12	An exception is <i>Alicyclobacillus acidoterrestris</i> .
<i>Alicyclobacillus acidoterrestris</i>	Method 45	
<i>Alternaria</i> species	Method 5	
<i>Aneurinibacillus</i> species	Method 1	
<i>Arcanobacterium</i> species	Method 34	
<i>Arthrobacter</i> species	Method 21	
<i>Aspergillus</i> species	Method 5	An exception is <i>Aspergillus flavus</i> .
<i>Aspergillus flavus</i>	Method 46	
<i>Aureobasidium</i> species	Method 5	
<i>Bacillus</i> species	Method 49	
<i>Bacteroides</i> species	Method 4	
<i>Bifidobacterium</i> species	Method 4	An exception is <i>Bifidobacterium animalis</i> subsp. <i>animalis</i> .
<i>Bifidobacterium animalis</i> subsp. <i>animalis</i>	Method 39	
<i>Bordetella bronchiseptica</i>	Method 15	
<i>Bordetella parapertussis</i>	Method 16	

Microorganism	Method	Notes
<i>Bordetella pertussis</i>	Method 16	An exception is <i>Bordetella pertussis</i> derived from ATCC® 9797™*.
<i>Bordetella pertussis</i> derived from ATCC® 9797™*	Method 57	
<i>Brevibacillus</i> species	Method 1	
<i>Brevundimonas</i> species	Method 1	
<i>Brucella</i> species	Method 1	
<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>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	An exception is <i>Clostridium perfringens</i> .
<i>Clostridium perfringens</i>	Method 41	An exception is <i>Clostridium perfringens</i> derived from ATCC® 13124™*.
<i>Clostridium perfringens</i> derived from ATCC® 13124™*	Method 63	
<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> .
<i>Cryptococcus gattii</i>	Method 47	
<i>Curtobacterium</i> species	Method 1	
<i>Cutaneotrichosporon</i> species	Method 5	
<i>Cutibacterium</i> species	Method 44	
<i>Delftia</i> species	Method 1	
<i>Deinococcus radiophilus</i>	Method 23	
<i>Edwardsiella</i> species	Method 1	
<i>Eggerthella</i> species	Method 4	
<i>Eikenella</i> species	Method 3	
<i>Elizabethkingia</i> species	Method 1	
<i>Enterobacter</i> species	Method 1	
<i>Enterococcus</i> species	Method 1	

Microorganism	Method	Notes
<i>Erysipelothrix</i> species	Method 2	
<i>Escherichia coli</i>	Method 1	
<i>Eurotium rubrum</i>	Method 5	
<i>Finegoldia</i> species	Method 42	
<i>Fusarium</i> species	Method 5	
<i>Fusobacterium</i> species	Method 4	
<i>Galactomyces</i> species	Method 4	
<i>Gardnerella</i> species	Method 9	
<i>Geobacillus</i> species	Method 24	
<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>Klebsiella</i> species	Method 1	
<i>Kocuria</i> species	Method 1	An exception is <i>Kocuria rosea</i> .
<i>Kocuria rosea</i>	Method 21	
<i>Lacticaseibacillus casei</i>	Method 11	
<i>Lacticaseibacillus paracasei</i>	Method 65	
<i>Lacticaseibacillus rhamnosus</i>	Method 65	
<i>Lactiplantibacillus</i> species	Method 65	
<i>Lactobacillus</i> species	Method 65	Exceptions are <i>Lactobacillus acidophilus</i> , <i>Lactobacillus gasseri</i> , and <i>Lactobacillus delbrueckii subsp. lactis</i> .
<i>Lactobacillus acidophilus</i>	Method 11	
<i>Lactobacillus gasseri</i>	Method 11	
<i>Lactobacillus delbrueckii subsp. lactis</i>	Method 11	
<i>Lactococcus</i> species	Method 2	
<i>Legionella</i> species	Method 8	
<i>Levilactobacillus</i> species	Method 65	
<i>Listeria</i> species	Method 1	
<i>Malassezia</i> species	Method 14	
<i>Mammaliicoccus</i> species	Method 1	
<i>Methylobacterium</i> species	Method 25	
<i>Methylorubrum extorquens</i>	Method 26	
<i>Meyerozyma guilliermondii</i>	Method 5	
<i>Microbacterium</i> species	Method 22	
<i>Micrococcus</i> species	Method 1	An exception is <i>Micrococcus luteus</i> derived from ATCC® 10240™*.

Microorganism	Method	Notes
<i>Micrococcus luteus</i> derived from ATCC® 10240™*	Method 67	
<i>Microsporium canis</i>	Method 48	
<i>Microsporium gypseum</i>	Method 73	
<i>Moraxella</i> species	Method 2	
<i>Morganella</i> species	Method 1	
<i>Mucor racemosus</i>	Method 5	
<i>Mycobacterium</i> species	Method 13	Exceptions are <i>Mycobacterium fortuitum</i> , <i>Mycobacterium haemophilum</i> , and <i>Mycobacterium smegmatis</i> .
<i>Mycobacterium fortuitum</i>	Method 7	
<i>Mycobacterium haemophilum</i>	Method 18	
<i>Mycobacterium smegmatis</i>	Method 7	
<i>Mycoplasma bovis</i>	Method 60	
<i>Metamycoplasma hominis</i>	Method 58	
<i>Mycoplasma pneumoniae</i>	Method 59	
<i>Myroides</i> species	Method 2	
<i>Neisseria</i> species	Method 37	
<i>Niallia</i> species	Method 49	
<i>Nocardia</i> species	Method 81	
<i>Ochrobactrum</i> species	Method 1	
<i>Oligella</i> species	Method 2	
<i>Paenibacillus</i> species	Method 1	An exception is <i>Paenibacillus larvae</i> subsp. <i>larvae</i> .
<i>Paenibacillus larvae</i> subsp. <i>larvae</i>	Method 82	
<i>Paeniclostridium sordellii</i>	Method 4	
<i>Papiliotrema</i> species	Method 62	
<i>Parabacteroides</i> species	Method 4	
<i>Paraclostridium</i> species	Method 40	
<i>Parvimonas</i> species	Method 43	
<i>Pasteurella</i> species	Method 2	
<i>Pediococcus</i> species	Method 11	
<i>Penicillium</i> species	Method 5	
<i>Peptostreptococcus</i> species	Method 4	
<i>Phocaeicola</i> species	Method 4	
<i>Pluralibacter gergoviae</i>	Method 1	
<i>Porphyromonas</i> species	Method 43	
<i>Prevotella</i> species	Method 43	
<i>Priestia</i> species	Method 49	
<i>Proteus</i> species	Method 1	An exception is <i>Proteus hauseri</i> .
<i>Proteus hauseri</i>	Method 27	

Microorganism	Method	Notes
<b><i>Prototheca</i> species</b>	Method 5	
<b><i>Providencia</i> species</b>	Method 1	
<b><i>Pseudomonas</i> species</b>	Method 1	Exceptions are <i>Pseudomonas paraeruginosa</i> , <i>Pseudomonas brenneri</i> , <i>Pseudomonas fluorescens</i> , <i>Pseudomonas mosselii</i> , <i>Pseudomonas protegens</i> , <i>Pseudomonas putida</i> , and <i>Pseudomonas</i> sp. derived from ATCC® 31483™*.
<b><i>Pseudomonas paraeruginosa</i></b>	Method 28	
<b><i>Pseudomonas brenneri</i></b>	Method 21	
<b><i>Pseudomonas fluorescens</i></b>	Method 21	
<b><i>Pseudomonas mosselii</i></b>	Method 21	
<b><i>Pseudomonas protegens</i></b>	Method 21	
<b><i>Pseudomonas putida</i></b>	Method 22	
<b><i>Pseudomonas</i> sp. derived from ATCC® 31483™*</b>	Method 22	
<b><i>Ralstonia</i> species</b>	Method 1	An exception is <i>Ralstonia pickettii</i>
<b><i>Ralstonia pickettii</i></b>	Method 29	
<b><i>Raoultella</i> species</b>	Method 1	
<b><i>Rhizopus</i> species</b>	Method 5	
<b><i>Rhodococcus</i> species</b>	Method 2	
<b><i>Rhodotorula</i> species</b>	Method 5	
<b><i>Saccharomyces</i> species</b>	Method 50	
<b><i>Salmonella</i> species</b>	Method 1	
<b><i>Schaalia</i> species</b>	Method 4	
<b><i>Serratia</i> species</b>	Method 1	
<b><i>Shewanella haliotis</i></b>	Method 74	
<b><i>Shigella</i> species</b>	Method 1	
<b><i>Sphingobacterium</i> species</b>	Method 1	
<b><i>Sphingomonas</i> species</b>	Method 21	
<b><i>Sporidiobolus</i> species</b>	Method 5	
<b><i>Staphylococcus</i> species</b>	Method 1	An exception is <i>Staphylococcus aureus</i> .
<b><i>Staphylococcus aureus</i></b>	Method 30	
<b><i>Stenotrophomonas</i> species</b>	Method 22	
<b><i>Streptococcus</i> species</b>	Method 34	Exceptions are <i>Streptococcus</i> species derived from ATCC® 12392™* and <i>Streptococcus pneumoniae</i> .
<b><i>Streptococcus pneumoniae</i></b>	Method 66	
<b><i>Streptococcus</i> species derived from ATCC® 12392™*</b>	Method 66	
<b><i>Streptomyces</i> species</b>	Method 5	
<b><i>Talaromyces</i> species</b>	Method 5	

Microorganism	Method	Notes
<i>Thermoanaerobacterium</i> species	Method 56	
<i>Trichophyton</i> species	Method 51	
<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>	Method 54	
<i>Wallemia mellicola</i>	Method 83	
<i>Weizmannia</i> species	Method 49	
<i>Yarrowia</i> species	Method 5	
<i>Yersinia</i> species	Method 1	
<i>Zygosaccharomyces rouxii</i>	Method 53	
<i>Zygosaccharomyces parabailii</i>	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

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

#### Method 2

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

#### Method 3

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

**Method 4**

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	48 to 72 hours

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

**Method 5**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Chocolate Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Microaerophilic
<b>Growth Time</b>	48 to 72 hours

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

**Method 7**

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

Note: *Mycobacterium fortuitum* subsp. *fortuitum* and *Mycobacterium smegmatis* 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**

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

**Method 9**

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

**Method 10**

<b>Media</b>	Tryptic Soy Agar (Soybean Casein Digest Agar) or Marine Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

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

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

**Method 13**

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

**Method 14**

<b>Media</b>	Leeming Notman Agar
<b>Temperature</b>	30°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	72 hours

## Method 15

<b>Media</b>	Chocolate agar, Nonselective Sheep Blood Agar, Tryptic Soy Agar, and Bordet Gengou Agar with 15% Defibrinated Sheep Blood
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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

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

## Method 17

<b>Media</b>	Phase 1: ISF (modified Infant Soy Formula) Broth Phase 2: Sulfite Agar
<b>Temperature</b>	Phase 1: 55°C Phase 2: 55°C
<b>Atmosphere</b>	Phase 1: Anaerobic Phase 2: Anaerobic
<b>Growth Time</b>	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**

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

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

**Method 19**

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

**Method 20**

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

**Method 21**

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

**Method 22**

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

**Method 23**

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

**Method 24**

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

**Method 25**

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

**Method 26**

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

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

**Method 27**

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

**Method 28**

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

**Method 29**

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

**Method 30**

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

Note: The degree of resistance of *Staphylococcus aureus* derived from ATCC® 700699™\* 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**

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

**Method 32**

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

**Method 33**

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

**Method 34**

<b>Media</b>	Nonselective Sheep Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

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

**Method 36**

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

**Method 37**

<b>Media</b>	Chocolate Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	5 to 7% Carbon Dioxide
<b>Growth Time</b>	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**

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	2 to 5 days

**Method 39**

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

**Method 40**

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	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

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	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

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	3 to 4 days

## Method 43

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	5 to 7 days

## Method 44

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	3 to 5 days

## Method 45

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

## Method 46

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

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

**Method 48**

<b>Media</b>	Potato Dextrose Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

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

**Method 51**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Marine Agar; See notes below for rehydration instructions
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

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

**Method 56**

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

Note: Primary growth medium for *Thermoanaerobacterium thermosaccharolyticum* 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**

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

**Method 58**

<b>Media</b>	See note below for important directions. Phase 1: Mycoplasma Broth Phase 2: Mycoplasma Agar
<b>Temperature</b>	Phase 1: 35°C Phase 2: 35°C
<b>Atmosphere</b>	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
<b>Growth Time</b>	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**

<b>Media</b>	See note below for important directions. Phase 1: SP4 Glucose Broth Phase 2: SP4 Glucose Agar
<b>Temperature</b>	Phase 1: 35°C Phase 2: 35°C
<b>Atmosphere</b>	Phase 1: Aerobic Phase 2: Carbon Dioxide (Candle Jar)
<b>Growth Time</b>	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**

<b>Media</b>	See note below for important directions. Phase 1: Mycoplasma Broth Phase 2: Mycoplasma Agar
<b>Temperature</b>	Phase 1: 35°C Phase 2: 35°C
<b>Atmosphere</b>	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
<b>Growth Time</b>	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. Examine plates microscopically to see colonies.

**Method 61**

<b>Media</b>	See note below for important directions. Phase 1: SP4 Urea Broth Phase 2: A8 Agar
<b>Temperature</b>	Phase 1: 35°C Phase 2: 35°C
<b>Atmosphere</b>	Phase 1: Aerobic Phase 2: Anaerobic
<b>Growth Time</b>	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. Examine plates microscopically to see colonies.

**Method 62**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Anaerobic Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Anaerobic
<b>Growth Time</b>	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**

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

**Method 65**

<b>Media</b>	Nonselective Sheep Blood Agar or Columbia CNA with 5% Sheep Blood
<b>Temperature</b>	35°C
<b>Atmosphere</b>	5 to 7% Carbon Dioxide
<b>Growth Time</b>	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**

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

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

**Method 67**

<b>Media</b>	Tryptic Soy Agar (Soybean Casein Agar) or Nonselective Sheep Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Chocolate Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Microaerophilic
<b>Growth Time</b>	48 hours

**Method 69**

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

**Method 70**

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

**Method 71**

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

**Method 72**

<b>Media</b>	Nonselective Sheep Blood Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	24 hours

**Method 73**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar or Standard Methods Agar (Plate Count Agar)
<b>Temperature</b>	30°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Tryptic Soy Agar (Soybean Casein Agar) or Nonselective Sheep Blood Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Potato Dextrose Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	One week

**Method 77**

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

**Method 78**

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

**Method 79**

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

**Method 80**

<b>Media</b>	Nutrient
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	3 to 5 days

**Method 81**

<b>Media</b>	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
<b>Temperature</b>	35°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Columbia Blood Agar
<b>Temperature</b>	30°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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**

<b>Media</b>	Sabouraud Dextrose Emmons Agar
<b>Temperature</b>	25°C
<b>Atmosphere</b>	Aerobic
<b>Growth Time</b>	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.