

## Recommended Growth Requirements for LYFO DISK® and KWIK-STIK™ Microorganisms

### Selection Of Growth Requirements

- 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.
- The following information lists which method should be used to grow the various microorganism species. Descriptions of methods follow the microorganism list.

<i>Acetobacter sp.</i>	Method 3	<b>Note:</b> Incubate at 25°C in CO <sub>2</sub> for 3-4 days.
<i>Achromobacter sp.</i>	Method 1	
<i>Acinetobacter sp.</i>	Method 1	
<i>Actinobacillus sp.</i>	Method 3	
<i>Actinomyces sp.</i>	Method 4	
<i>Aerococcus sp.</i>	Method 1	
<i>Aeromonas sp.</i>	Method 2	<b>Note:</b> <i>A. hydrophila</i> should be incubated at 30°C. <i>A. salmonicida</i> should be incubated at 25°C.
<i>Aggregatibacter sp.</i>	Method 3	
<i>Alcaligenes sp.</i>	Method 1	
<i>Alicyclobacillus sp.</i>	Method 12	<b>Note:</b> <i>A. acidoterrestris</i> , Microbiologics O265, should be incubated at 45°C.
<i>Alloiococcus sp.</i>	Method 2	
<i>Amylomyces sp.</i>	Method 5	
<i>Aneurinibacillus sp.</i>	Method 1	
<i>Aquaspirillum sp.</i>	Method 1	<b>Note:</b> Incubate at 25°C for 6 days.
<i>Arcanobacterium sp.</i>	Method 2	
<i>Arthrobacter sp.</i>	Method 1	<b>Note:</b> Incubate at 25°C.
<i>Aspergillus sp.</i>	Method 5	<b>Note:</b> <i>A. flavus</i> does not grow well on Standard Methods Agar (Plate Count Agar).
<i>Aureobasidium sp.</i>	Method 5	
<i>Bacillus sp.</i>	Method 1	<b>Note:</b> Some <i>Bacillus sp.</i> demonstrate better recovery on subculture when the stock organism growth is maintained at room temperature rather than 2°C-8°C.
<i>Bacteroides sp.</i>	Method 4	<b>Note:</b> <i>B. ureolyticus</i> should be incubated 5 days. The colonies are very small. Several subculture plates may need to be inoculated in order to have sufficient quantity of the microorganism for testing.
<i>Bifidobacterium sp.</i>	Method 4	<b>Note:</b> <i>B. animalis</i> subsp. <i>animalis</i> only grows well on Anaerobic Blood Agar or Tryptic Soy Agar.
<i>Bordetella bronchiseptica</i>	Method 15	
<i>Bordetella parapertussis</i>	Method 16	
<i>Bordetella pertussis</i>	Method 16	
<i>Brevibacillus sp.</i>	Method 1	
<i>Brevundimonas sp.</i>	Method 1	
<i>Brochothrix sp.</i>	Method 1	<b>Note:</b> Incubate at 25°C.
<i>Budvicia sp.</i>	Method 1	<b>Note:</b> Incubate at 25°C.

*Burkholderia* sp. Method 1  
*Chaetomium* sp. Method 5  
*Campylobacter* sp. Method 6

*Candida* sp. Method 5  
*Capnocytophaga* sp. Method 3  
*Cedecea* sp. Method 1  
*Cellulosimicrobium* sp. Method 1  
*Citrobacter* sp. Method 1  
*Cladosporium* sp. Method 5  
*Clostridium* sp. Method 4

*Corynebacterium* sp. Method 1  
*Cronobacter* sp. Method 1  
*Curtobacterium* sp. Method 1  
*Cryptococcus* sp. Method 5

*Deinococcus* sp. Method 1  
*Delftia* sp. Method 1  
*Desulfotomaculum* sp. Method 17  
*Edwardsiella* sp. Method 1  
*Eggerthella* sp. Method 4  
*Eikenella* sp. Method 3  
*Elizabethkingia* sp. Method 1  
*Enterobacter* sp. Method 1  
*Enterococcus* sp. Method 1  
*Erysipelothrix* sp. Method 2  
*Escherichia coli* Method 1  
*Exiguobacterium* sp. Method 1  
*Fingoldia* sp. Method 4

*Flavobacterium* sp. Method 1  
*Fluoribacter* sp. Method 8  
*Fusarium* sp. Method 5  
*Fusobacterium* sp. Method 4  
*Gardnerella* sp. Method 9  
*Gemella* sp. Method 4  
*Geobacillus* sp. Method 1

*Geotrichum* sp. Method 5  
*Granulicatella adiacens* Method 19  
*Haemophilus* sp. Method 3  
*Hafnia* sp. Method 1

**Note:** Chocolate agar is the best medium for the primary growth of *C. jejuni*. Do not open the inoculated agar medium petri plate for the first 48 hours.

**Note:** *C. difficile*, *C. sordellii*, and *C. tetani* will only grow on Anaerobic Blood Agar. *C. perfringens* may not grow well on Nutrient Agar.

**Note:** Use Method 2 to grow *C. urealyticum*.

**Note:** *Cryptococcus* must be incubated at 25°C to assure growth. *C. gattii* grows best on Malt Extract Agar or Sabouraud Dextrose Emmons Agar.

**Note:** *Cryptococcus* grows poorly on non-selective Sheep Blood agar.

**Note:** Incubate 72 to 96 hours in anaerobic atmosphere.

**Note:** Incubate at 30°C.

**Note:** *G. stearothermophilus* strains must be incubated at 55°C. *G. stearothermophilus*, Microbiologics 0137, does not grow on Sheep Blood Agar.

<i>Issatchenkia</i> sp.	Method 5	
<i>Kingella</i> sp.	Method 2	<b>Note:</b> Incubate in 5-10% CO <sub>2</sub> .
<i>Klebsiella</i> sp.	Method 1	
<i>Kloeckera</i> sp.	Method 5	
<i>Kocuria</i> sp.	Method 1	<b>Note:</b> <i>K. rosea</i> should be incubated at 25°C.
<i>Lactobacillus</i> sp.	Method 11	
<i>Lactococcus</i> sp.	Method 2	
<i>Leclercia</i> sp.	Method 1	
<i>Legionella</i> sp.	Method 8	
<i>Listeria</i> sp.	Method 1	
<i>Lysinibacillus</i> sp.	Method 1	
<i>Macrococcus</i> sp.	Method 1	
<i>Malassezia</i> sp.	Method 14	
<i>Mannheimia</i> sp.	Method 1	
<i>Methylobacterium</i> sp.	Method 1	<b>Note:</b> Incubate at 25°C for 5 days. Grows best on Standard Methods Agar (Plate Count Agar). Does not grow on Tryptic Soy Agar (Soybean Casein Digest Agar) or nonselective Sheep Blood Agar.
<i>Microbacterium</i> sp.	Method 1	<b>Note:</b> <i>M. extorquens</i> obtains good growth on R2A agar in 72 hours at 30°C.
<i>Micrococcus</i> sp.	Method 1	<b>Note:</b> Incubate at 30°C.
		<b>Note:</b> Tryptic Soy Agar (Soybean Casein Digest Agar) or nonselective Sheep Blood Agar is the best medium for growth of Microbiologics 0337 and 0689.
<i>Microsporium</i> sp.	Method 5	<b>Note:</b> <i>M. canis</i> grows poorly on Sabouraud Dextrose Agar.
<i>Moraxella</i> sp.	Method 2	<b>Note:</b> <i>M. gordonae</i> , <i>M. terrae</i> and <i>M. tuberculosis</i> may require up to one month incubation.
<i>Morganella</i> sp.	Method 1	<b>Note:</b> <i>M. haemophilium</i> should be grown on Middlebrook 7H11 agar and incubated at 30°C in 5 to 7% CO <sub>2</sub> for 3 to 4 weeks. An X factor strip must be placed on the agar in order for the organism to grow.
<i>Mycobacterium</i> sp.	Method 7	
<i>Mycoplasma</i> sp.	Method 18	<b>Note:</b> Chocolate agar is the best medium for the initial growth of <i>Neisseria</i> species. Do not open the inoculated agar medium petri plate for the first 48 hours if using a candle jar.
<i>Myroides</i> sp.	Method 2	
<i>Neisseria</i> sp.	Method 3	
<i>Nocardia</i> sp.	Method 1	<b>Note:</b> Incubate at 25°C.
<i>Novosphingobium</i> sp.	Method 1	
<i>Ochrobactrum</i> sp.	Method 1	
<i>Oligella</i> sp.	Method 2	
<i>Paecilomyces</i> sp.	Method 5	
<i>Paenibacillus</i> sp.	Method 1	<b>Note:</b> <i>P. larvae</i> should be incubated aerobically at 30°C.
<i>Parabacteroides</i> sp.	Method 4	

<i>Parvimonas</i> sp.	Method 4	<b>Note:</b> <i>P. micra</i> requires 5 to 7 days of anaerobic incubation.
<i>Pasteurella</i> sp.	Method 2	
<i>Pediococcus</i> sp.	Method 11	<b>Note:</b> <i>P. damnosus</i> may be grown in MRS broth at 25°C for 48 to 72 hours. Subculture the broth to MRS agar when it becomes cloudy. Incubate the agar at 25°C in 5-7% CO <sub>2</sub> for 72 to 96 hours. Alternatively, the lyophilized microorganism may be grown directly on MRS Agar at 25°C in 5 to 7% CO <sub>2</sub> for 5 to 7 days.
<i>Penicillium</i> sp.	Method 5	
<i>Peptoniphilus</i> sp.	Method 4	<b>Note:</b> Incubate 72 to 96 hours in anaerobic atmosphere.
<i>Peptostreptococcus</i> sp.	Method 4	
<i>Plesiomonas</i> sp.	Method 1	
<i>Porphyromonas</i> sp.	Method 4	<b>Note:</b> 5 to 7 days of anaerobic incubation is required.
<i>Prevotella</i> sp.	Method 4	<b>Note:</b> 5 to 7 days of anaerobic incubation is required.
<i>Propionibacterium</i> sp.	Method 4	<b>Note:</b> 3 to 5 days of anaerobic incubation is required.
<i>Proteus</i> sp.	Method 1	<b>Note:</b> <i>P. hauseri</i> grows best on Blood and Tryptic Soy Agar.
<i>Prototheca</i> sp.	Method 5	
<i>Providencia</i> sp.	Method 1	
<i>Pseudomonas</i> sp.	Method 1	<b>Note:</b> <i>P. fluorescens</i> and <i>P. protogens</i> should be incubated at 25°C. <i>Pseudomonas</i> species, Microbiologics 0162, should be incubated at 30°C. <b>Note:</b> <i>P. aeruginosa</i> , Microbiologics 0484, grows poorly on Nutrient agar.
<i>Ralstonia</i> sp.	Method 1	
<i>Raoultella</i> sp.	Method 1	
<i>Rhizopus</i> sp.	Method 5	
<i>Rhodococcus</i> sp.	Method 2	
<i>Rhodotorula</i> sp.	Method 5	
<i>Saccharomyces</i> sp.	Method 5	<b>Note:</b> Sabouraud Dextrose Emmons Agar is the best medium for growth of <i>Saccharomyces</i> sp.
<i>Salmonella</i> sp.	Method 1	
<i>Scopulariopsis</i> sp.	Method 5	
<i>Serratia</i> sp.	Method 1	
<i>Shewanella</i> sp.	Method 10	
<i>Shigella</i> sp.	Method 1	
<i>Sphingobacterium</i> sp.	Method 1	
<i>Sphingomonas</i> sp.	Method 1	<b>Note:</b> Incubate at 25°C.
<i>Sporidobolus</i> sp.	Method 5	
<i>Staphylococcus</i> sp.	Method 1	<b>Note:</b> The degree of resistance of <i>S. aureus</i> , 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 Brian Heart Infusion Agar with 4mcg/ml Vancomycin.
<i>Stenotrophomonas</i> sp.	Method 1	<b>Note:</b> Incubate at 30°C.

<i>Streptococcus</i> sp.	Method 2	<b>Note:</b> <i>S. criceti</i> must be incubated in a microaerophilic environment. <i>Streptococcus</i> sp., Microbiologics 0978, should be grown in CO <sub>2</sub> .
<i>Streptomyces</i> sp.	Method 5	<b>Note:</b> <i>Streptococcus</i> will also recover well on Columbia CNA agar with 5% Sheep Blood.
<i>Thermoanaerobacterium</i> sp.	Method 4	<b>Note:</b> Primary growth medium for <i>T. thermosaccharolyticum</i> , Microbiologics 0728, is Cooked Meat Medium. Incubation at 45°C for 72 hours is required. After initial growth, organism may be grown on Anaerobic Blood Agar which is incubated at 45°C for 72 hours in anaerobic atmosphere.
<i>Trichoderma</i> sp.	Method 5	
<i>Trichophyton</i> sp.	Method 5	
<i>Trichosporon</i> sp.	Method 5	
<i>Ureaplasma</i> sp.	Method 13	
<i>Veillonella</i> sp.	Method 4	
<i>Vibrio</i> sp.	Method 10	<b>Note:</b> <i>V. alginolyticus</i> , Microbiologics 0819, does not recover well on Tryptic Soy Agar (Soybean Casein Digest Agar). For best results, grow on Marine Agar.
<i>Virgibacillus</i> sp.	Method 1	
<i>Yarrowia</i> sp.	Method 5	
<i>Yersinia</i> sp.	Method 1	
<i>Zygosaccharomyces</i> sp.	Method 5	<b>Note:</b> <i>Z. bailii</i> , Microbiologics 01011, does not grow well on nonselective Sheep Blood Agar, Nutrient Agar, or Tryptic Soy Agar (Soybean Casein Digest Agar).

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

#### Method 1

- Tryptic Soy Agar (Soybean Casein Digest Agar), nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar at 35°C in aerobic atmosphere for 24 to 48 hours.

#### Method 2

- Nonselective Sheep Blood Agar at 35°C in aerobic atmosphere for 24 to 72 hours. Growth of some species such as *Streptococcus* and *Arcanobacterium* are enhanced by CO<sub>2</sub> enrichment of the incubation atmosphere. 5% CO<sub>2</sub> is recommended for the culture of *Streptococcus pneumoniae* and other streptococcal species of the viridians group.

#### Method 3

- Chocolate Agar at 35°C in 5-7% CO<sub>2</sub> for 24 to 48 hours.

#### Method 4

- Anaerobic Blood Agar at 35°C in anaerobic environment for 48 to 72 hours.
- Some obligate anaerobes may require 5 to 7 days to demonstrate sufficient growth.
- Fresh prepared 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.

#### Method 5

- Sabouraud Dextrose Emmons Agar at 25°C in aerobic atmosphere for 2 to 7 days.
- Nonselective Sheep Blood Agar is an appropriate alternative.
- Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Plate Count Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

#### Method 6

- Chocolate Agar at 35°C in microaerophilic environment for 48 to 72 hours.

#### Method 7

- Lowenstein Jensen Agar or Middlebrook Agar at 35°C in 5-7% CO<sub>2</sub> or aerobic atmosphere. Incubation times may vary from 2 to 30 days. *M. fortuitum* subsp. *fortuitum*, *M. peregrinum* and *M. 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

- Buffered Charcoal Yeast Extract Agar at 35°C in aerobic atmosphere for 3 to 5 days.

#### Method 9

- V Agar or Chocolate Agar at 35°C in 5-7% CO<sub>2</sub> for 48 hours.

#### Method 10

- Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth (Soybean Casein Digest Agar), or 0.85% Saline. Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery.
- Grow on Tryptic Soy Agar (Soybean Casein Digest Agar) at 35°C in aerobic atmosphere for 24 to 48 hours. *Vibrio* sp. also grows on Marine Agar.

#### Method 11

- The primary growth medium is MRS (Man, Rogosa, Sharpe) Broth. Incubate at 35°C in aerobic atmosphere for 48 hours. Transfer to either Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood. Incubate at 35°C in 5-7% CO<sub>2</sub> for 48 hours. A few *Lactobacilli* species, such as *L. fermentum*, *L. paracasei* subsp. *paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. sakei* do not need to be started in Lactobacilli MRS broth. They may be plated directly to Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood and incubated at 35°C in 5-7% CO<sub>2</sub> for 48 hrs.

#### Method 12

- Potato Dextrose Agar at 55°C in aerobic atmosphere for 24 to 48 hours.

#### Method 13

- Rehydrate 1 pellet of *Ureaplasma* sp. in SP4 Urea Broth. Alternatively, inoculate broth with a KWIK-STIK™. Make serial dilutions (for example, 1:10, 1:100, 1:1000, 1:10,000). Incubate at 35°C in aerobic atmosphere. As soon as the SP4 Urea Broth turns red (24 to 96 hours), sub 0.1 ml of broth to A-8 Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate A-8 agar at 35°C in anaerobic conditions for 4 to 6 days. In order to see colonies, examine plates microscopically.

#### Method 14

- Leeming Notman Agar at 30°C in aerobic atmosphere for 72 hours.

#### Method 15

- Chocolate agar, Sheep Blood Agar, Tryptic Soy Agar, and Bordet Gengou Agar with 15% Defibrinated Sheep Blood at 35°C in aerobic atmosphere for 24 to 48 hours. Standard Methods Agar (Plate Count Agar) or Nutrient Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

**Method 16**

- Chocolate or Bordet Gengou Agar with 15% Defibrinated Sheep Blood at 35°C in aerobic atmosphere for 2 days to 1 week. *B. pertussis*, Microbiologics 0100, and *B. pertussis*, Microbiologics 0843, require Bordet Gengou Agar with 15% Defibrinated Sheep Blood.

**Method 17**

- 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) Sub 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**

- Inoculate Broth with LYFO DISK® or KWIK-STIK™. Prepare a 1:10 serial dilution using the broth. Incubate broth according to the table below. Then plate 0.2 ml of the broth culture to Agar. Incubate agar according to the table below. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Recommended Broth and Agar for Growth of Mycoplasma Species					
Catalog Number	Microorganism	Broth	Broth Incubation Temperature/ ATM /Time	Agar	Agar Incubation Temperature/ ATM/Time
0156	<i>Mycoplasma hominis</i>	Mycoplasma	35°C / O <sub>2</sub> / 48 hours	Mycoplasma	35°C / 5-7% CO <sub>2</sub> / 4-6 days
0503	<i>Mycoplasma pneumoniae</i>	SP4 Glucose	35°C / O <sub>2</sub> / 7-28 days	SP4 Glucose	35°C / CO <sub>2</sub> (Candle Jar) / 5-15 days
0504	<i>Mycoplasma orale</i>	Mycoplasma	35°C / O <sub>2</sub> / 48 hours	Mycoplasma	35°C / AN/ 3-6 days
01053	<i>Mycoplasma bovis</i>	Mycoplasma	35°C / O <sub>2</sub> / 48 hours	Mycoplasma	35°C / 5-7% CO <sub>2</sub> / 3-7 days
0151	<i>Ureaplasma parvum</i>	SP4 with Urea	35°C / O <sub>2</sub> / 48 hours	A8	35°C / AN / 4-6 days

**Method 19**

- Sheep Blood Agar supplemented with Pyridoxal at 35°C in 5-7% CO<sub>2</sub> for 24 to 48 hours.