



RECOMMENDED GROWTH REQUIREMENTS

LYFO DISK® AND KWIK-STIK™ 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.

<i>Acetobacter</i> sp.	Method 3
Note: Incubate at 25°C in CO ₂ for 3-4 days.	
<i>Achromobacter</i> sp.	Method 1
<i>Acinetobacter</i> sp.	Method 1
<i>Actinobacillus</i> sp.	Method 3
<i>Actinomyces</i> sp.	Method 4
<i>Aerococcus</i> sp.	Method 1
<i>Aeromonas</i> sp.	Method 2
Note: <i>A. hydrophila</i> should be incubated at 30°C. <i>A. salmonicida</i> should be incubated at 25°C.	
<i>Aggregatibacter</i> sp.	Method 3
<i>Alcaligenes</i> sp.	Method 1
<i>Alicyclobacillus</i> sp.	Method 12
Note: <i>A. acidoterrestris</i> , MicroBioLogics #0265, should be incubated at 45°C.	
<i>Alloicoccus</i> sp.	Method 2
<i>Amylomyces</i> sp.	Method 5
<i>Aneurinibacillus</i> sp.	Method 1
<i>Aquaspirillum</i> sp.	Method 1
Note: Incubate at 25°C for 6 days.	
<i>Arcanobacterium</i> sp.	Method 2
<i>Arthrobacter</i> sp.	Method 1
Note: Incubate at 25° C.	
<i>Aspergillus</i> sp.	Method 5
Note: <i>A. flavus</i> does not grow well on Standard Methods Agar (Plate Count Agar).	
<i>Bacillus</i> sp.	Method 1
Note: Some <i>Bacillus</i> sp. demonstrate better recovery on subculture when the stock organism growth is maintained at room temperature rather than 2-8° C.	
<i>Bacteroides</i> sp.	Method 4
Note: <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</i> sp.	Method 4
<i>Bordetella bronchiseptica</i>	Method 20
<i>Bordetella parapertussis</i>	Method 21
<i>Bordetella pertussis</i>	Method 21
<i>Brevibacillus</i> sp.	Method 1
<i>Brevundimonas</i> sp.	Method 1
<i>Brochothrix</i> sp.	Method 1
Note: Incubate at 25°C.	
<i>Budvicia</i> sp.	Method 1
Note: Incubate at 25°C.	
<i>Burkholderia</i> sp.	Method 1

<i>Campylobacter</i> sp.	Method 6
Note: Chocolate agar is the best medium for the primary growth of <i>C. jejuni</i> . DO NOT open the inoculated agar medium petri plate for the first 48 hours.	
<i>Candida</i> sp.	Method 5
<i>Capnocytophaga</i> sp.	Method 3
<i>Cedecea</i> sp.	Method 1
<i>Cellulosimicrobium</i> sp.	Method 1
<i>Citrobacter</i> sp.	Method 1
<i>Cladosporium</i> sp.	Method 5
<i>Clostridium</i> sp.	Method 4
Note: <i>C. difficile</i> , <i>C. sordellii</i> , and <i>C. tetani</i> will only grow on Anaerobic Blood Agar.	
<i>Corynebacterium</i> sp.	Method 1
<i>Cronobacter</i> sp.	Method 1
<i>Cryptococcus</i> sp.	Method 5
Note: <i>Cryptococcus</i> MUST be incubated at 25°C to assure growth.	
<i>Deinococcus</i> sp.	Method 1
<i>Delftia</i> sp.	Method 1
<i>Edwardsiella</i> sp.	Method 1
<i>Eggerthella</i> sp.	Method 4
<i>Eikenella</i> sp.	Method 3
<i>Elizabethkingia</i> sp.	Method 1
<i>Enterobacter</i> sp.	Method 1
<i>Enterococcus</i> sp.	Method 1
<i>Erysipelothrix</i> sp.	Method 2
<i>Escherichia coli</i>	Method 1
<i>Exiguobacterium</i> sp.	Method 1
<i>Finegoldia</i> sp.	Method 4
Note: Incubate 72 to 96 hours in anaerobic atmosphere.	
<i>Flavobacterium</i> sp.	Method 1
Note: Incubate at 30°C.	
<i>Fluoribacter</i> sp.	Method 8
<i>Fusarium</i> sp.	Method 5
<i>Fusobacterium</i> sp.	Method 4
<i>Gardnerella</i> sp.	Method 9
<i>Gemella</i> sp.	Method 4
<i>Geobacillus</i> sp.	Method 1
Note: <i>G. stearothermophilus</i> strains must be incubated at 55°C. <i>G. stearothermophilus</i> , MicroBiologics #0137, does not grow on Sheep Blood Agar.	
<i>Geotrichum</i> sp.	Method 5
<i>Haemophilus</i> sp.	Method 3
<i>Hafnia</i> sp.	Method 1
<i>Issatchenkia</i> sp.	Method 5
<i>Kingella</i> sp.	Method 2
Note: Incubate in 5-10% CO ₂ .	
<i>Klebsiella</i> sp.	Method 1
<i>Kloeckera</i> sp.	Method 5
<i>Kocuria</i> sp.	Method 1
Note: <i>K. rosea</i> should be incubated at 25°C.	
<i>Lactobacillus</i> sp.	Method 2
Note: <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. delbrueckii</i> and <i>L. gasseri</i> must use 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 16
<i>Mannheimia</i> sp.	Method 1
<i>Methylobacterium</i> sp.	Method 1
Note: 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
Note: Incubate at 30°C.	
<i>Micrococcus</i> sp.	Method 1
Note: 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
Note: <i>M. canis</i> grows poorly on Sabouraud Dextrose Agar.	
<i>Moraxella</i> sp.	Method 2
<i>Morganella</i> sp.	Method 1
<i>Mycobacterium</i> sp.	Method 7
Note: <i>M. goodnae</i> , <i>M. terrae</i> and <i>M. tuberculosis</i> may require up to one month incubation.	
<i>Mycoplasma gallisepticum</i>	Method 17
<i>Mycoplasma hominis</i>	Method 13
<i>Mycoplasma hyorhinis</i>	Method 18
<i>Mycoplasma orale</i>	Method 15
<i>Mycoplasma pneumoniae</i>	Method 14
<i>Mycoplasma synoviae</i>	Method 19
<i>Myroides</i> sp.	Method 2
<i>Neisseria</i> sp.	Method 3
Note: 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>Nocardia</i> sp.	Method 1
<i>Novosphingobium</i> sp.	Method 1
Note: Incubate at 25° C.	
<i>Ochrobactrum</i> sp.	Method 1
<i>Oligella</i> sp.	Method 2
<i>Paecilomyces</i> sp.	Method 5
<i>Paenibacillus</i> sp.	Method 1
Note: <i>P. larvae</i> should be incubated aerobically at 30° C.	
<i>Parabacteroides</i> sp.	Method 4
<i>Parvimonas</i> sp.	Method 4
Note: <i>P. micra</i> requires 5 to 7 days of anaerobic incubation.	
<i>Pasteurella</i> sp.	Method 2
<i>Pediococcus</i> sp.	Method 11
Note: <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 to 10% CO ₂ for 72 to 96 hours. Alternatively, the lyophilized microorganism may be grown directly on MRS Agar at 25°C in 5 to 10% CO ₂ for 5 to 7 days.	
<i>Penicillium</i> sp.	Method 5
<i>Peptoniphilus</i> sp.	Method 4
Note: 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
Note: 5 to 7 days of anaerobic incubation is required.	
<i>Prevotella</i> sp.	Method 4
Note: 5 to 7 days of anaerobic incubation is required.	
<i>Propionibacterium</i> sp.	Method 4
Note: 3 to 5 days of anaerobic incubation is required.	
<i>Proteus</i> sp.	Method 1
Note: <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
Note: <i>P. fluorescens</i> should be incubated at 25°C. <i>Pseudomonas</i> species, MicroBioLogics #0162, should be incubated at 30°C.	
<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
Note: 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>Sordaria</i> sp.	Method 5
Note: <i>Sordaria</i> sp. sporulates better on Sabouraud Dextrose Emmons Agar than on Sabouraud Dextrose Agar.	
<i>Sphingobacterium</i> sp.	Method 1
<i>Sphingomonas</i> sp.	Method 1
Note: Incubate at 25°C.	
<i>Sporidobolus</i> sp.	Method 5
<i>Staphylococcus</i> sp.	Method 1
Note: 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
<i>Streptococcus</i> sp.	Method 2
Note: <i>S. cricetus</i> must be incubated in a microaerophilic environment. <i>Streptococcus</i> sp., MicroBioLogics #0978, should be grown in CO ₂ .	
<i>Streptomyces</i> sp.	Method 5
Note: <i>Streptomyces</i> sp. does not grow on Potato Dextrose Agar.	
<i>Thermoanaerobacterium</i> sp.	Method 4
Note: 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>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
<i>Virgibacillus</i> sp.	Method 1
<i>Yarrowia</i> sp.	Method 5
<i>Yersinia</i> sp.	Method 1
<i>Zygosaccharomyces</i> sp.	Method 5
Note: <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 - 35°C in aerobic atmosphere – 24 to 48 hours.

Method 2

- Nonselective Sheep Blood Agar - 35°C in aerobic atmosphere – 24 to 48 hours. Growth of some species such as *Streptococcus* and *Arcanobacterium* are enhanced by CO₂ enrichment of the incubation atmosphere. 5% CO₂ is recommended for the culture of *Streptococcus pneumoniae* and other streptococcal species of the viridians group.

Method 3

- Chocolate Agar - 35°C in 5% to 10% CO₂ – 24 to 48 hours.

Method 4

- Anaerobic Blood Agar 35°C in Anaerobic Environment – 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), 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 - 25°C in aerobic atmosphere – 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 - 35°C in Microaerophilic Environment – 48 to 72 hours.

Method 7

- Lowenstein Jensen Agar or Middlebrook Agar - 35°C in 5-10% CO₂ or aerobic atmosphere – up to one week. *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 - 35°C in aerobic atmosphere – 3 to 5 days.

Method 9

- V Agar or Chocolate Agar - 35°C in 5% to 10% CO₂ – 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) - 35°C in aerobic atmosphere – 24 to 48 hrs. *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 Columbia CNA with Sheep Blood. Incubate at 35°C in 5-10% CO₂ for 48 hrs.

Method 12

- Potato Dextrose Agar - 55°C in aerobic atmosphere - 48 hours.

Method 13

- Rehydrate 1 pellet of *M. hominis* or *Ureaplasma* sp. in 10B Arginine 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 Arginine vial turns pink (24 to 48 hours), sub 0.1 mL of broth to A-8 Agar and streak for isolation. DO NOT use cotton swab or wooden shaft. Incubate mycoplasma at 35°C in 5 to 10% CO₂. Incubate ureaplasma at 35°C anaerobically for up to 96 hours. In order to see colonies, examine plates microscopically.

Method 14

- Rehydrate 1 pellet of *M. pneumoniae* in SP4 Glucose Broth. Alternatively, inoculate broth with a KWIK-STIK. Make serial dilutions (for example, 1:2, 1:4, 1:8, 1:16, 1:32). Incubate at 35°C in aerobic atmosphere. As soon as the broth turns from red to yellow (1-4 weeks), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. DO NOT use cotton swab or wooden shaft. Incubate at 35°C in CO₂ atmosphere, preferably in a candle jar, for 5 to 15 days. In order to see colonies, examine plates microscopically.

Method 15

- Rehydrate 1 pellet of *M. orale* in 10B Arginine Broth. Alternatively, inoculate broth with a KWIK-STIK. Make serial dilutions (for example, 1:10, 1:100, 1:1000). Incubate at 35°C, in aerobic atmosphere. As soon as the broth turns from yellow to pink (48 to 72 hours), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. DO NOT use cotton swab or wooden shaft. Incubate plates at 35°C in anaerobic conditions for 3 to 6 days. In order to see colonies, examine plates microscopically.

Method 16

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

Method 17

- Rehydrate 1 pellet of *M. gallisepticum* in SP4 Glucose Broth. Alternatively, inoculate broth with a KWIK-STIK. Make serial dilutions (for example, 1:2, 1:4). Incubate at 35°C in aerobic atmosphere. As soon as the broth turns from red to yellow (4 days to 2 weeks), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. DO NOT use cotton swab or wooden shaft. Incubate at 35°C in CO₂ atmosphere, preferably in a candle jar, for 3 days to 2 weeks. In order to see colonies, examine plates microscopically.

Method 18

- Rehydrate 1 pellet of *M. hyorhinis* in SP4 Glucose Broth. Alternatively, inoculate broth with a KWIK-STIK. Make serial dilutions (for example, 1:10, 1:100, 1:1000). Incubate at 35°C in aerobic atmosphere. As soon as the broth turns from red to yellow (4 days to 2 weeks), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. DO NOT use cotton swab or wooden shaft. Incubate at 35°C in CO₂ atmosphere, preferably in a candle jar, for 2 to 10 days. In order to see colonies, examine plates microscopically.

Method 19

- Rehydrate 1 pellet of *M. synoviae* in SP4 Glucose Broth. Alternatively, inoculate broth with a KWIK-STIK. Make serial dilutions (for example, 1:2, 1:4, 1:8, 1:16, 1:32). Incubate at 35°C in 5 to 10% CO₂ for 7 days. After 7 days (no color change will be noted), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. DO NOT use cotton swab or wooden shaft. Incubate at 35°C in CO₂ atmosphere, preferably in a candle jar, for 1 to 4 weeks. In order to see colonies, examine plates microscopically.

Method 20

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

Method 21

- Chocolate or Bordet Gengou Agar with 15% Defibrinated Sheep Blood - 35°C in aerobic atmosphere – 2 days to one week. *B. pertussis*, MicroBioLogics #100, and *B. pertussis*, MicroBioLogics #0843, require Bordet Gengou Agar with 15% Defibrinated Sheep Blood.