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.**

Acetobacter sp. Method 3
Note: Incubate at 25°C in CO_2 for 3-4 days.
Achromobacter sp. Method 1
Acinetobacter sp. Method 1
Actinobacillus sp. Method 3
Actinomyces sp. Method 4
Aerococcus sp. Method 1
Aeromonas sp. Method 2
Note: *A. hydrophila* should be incubated at 30°C. *A. salmoncida* should be incubated at 25°C.
Aggregatibacter sp. Method 3
Alcaligenes sp. Method 1
Alicyclobacillus sp. Method 12
Note: *A. acidoterrestris*, MicroBioLogics #0265, should be incubated at 45°C.
Alloiococcus sp. Method 2
Amylomyces sp. Method 5
Aneurinibacillus sp. Method 1
Aquaspirillum sp. Method 1
Note: Incubate at 25°C for 6 days.
Arcanobacterium sp. Method 2
Arthrobacter sp. Method 1
Note: Incubate at 25° C.
Aspergillus sp. Method 5
Note: *A. flavus* does not grow well on Standard Methods Agar (Plate Count Agar).
Bacillus sp**.** Method 1
Note: Some *Bacillus* sp. demonstrate better recovery on subculture when the stock organism growth is maintained at room temperature rather than 2-8° C.
Bacteroides sp. Method 4
Note: *B. ureolyticus* 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.
Bifidobacterium sp. Method 4
Bordetella bronchiseptica Method 20
Bordetella parapertussis Method 21
Bordetella pertussis Method 21
Brevibacillus sp. Method 1
Brevundimonas sp. Method 1
Brochothrix sp. Method 1
Note: Incubate at 25°C.
Budvicia sp. Method 1
Note: Incubate at 25°C.
Burkholderia sp. Method 1

Campylobacter sp. Method 6
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.
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
Note: *C. difficile, C. sordellii, and C. tetani* will only grow on Anaerobic Blood Agar.
Corynebacterium sp. Method 1
***Note*:** Use Method 2 to grow C. urealyticum.
Cronobacter sp. Method 1
Curtobacterium sp. Method 1
Cryptococcus sp. Method 5
Note: *Cryptococcus* MUST be incubated at 25°C to assure growth.
Deinococcus sp. Method 1
Delftia sp. Method 1
Desulfotomaculum sp. Method 22
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
Finegoldia sp. Method 4
Note: Incubate 72 to 96 hours in anaerobic atmosphere.
Flavobacterium sp. Method 1
Note: Incubate at 30°C.
Fluoribacter sp. Method 8
Fusarium sp. Method 5
Fusobacterium sp. Method 4
Gardnerella sp. Method 9
Gemella sp. Method 4
Geobacillus sp. Method 1
Note: *G. stearothermophilus* strains must be incubated at 55°C. *G. stearothermophilus*, MicroBiologics #0137, does not grow on Sheep Blood Agar.
Geotrichum sp. Method 5
Haemophilus sp. Method 3
Hafnia sp. Method 1
Issatchenkia sp. Method 5
Kingella sp. Method 2
Note: Incubate in 5-10% CO_2.
Klebsiella sp. Method 1
Kloeckera sp. Method 5
Kocuria sp. Method 1
Note: *K. rosea* should be incubated at 25°C.
Lactobacillus sp. Method 11
Lactococcus sp. Method 2
Leclercia sp. Method 1
Legionella sp. Method 8
Listeria sp. Method 1
Lysinibacillus sp. Method 1
Macrococcus sp. Method 1
Malassezia sp. Method 16
Mannheimia sp. Method 1

Methylobacterium 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.
Microbacterium sp. Method 1
Note: Incubate at 30°C.
Micrococcus 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.
Microsporum sp. Method 5
Note: *M. canis* grows poorly on Sabouraud Dextrose Agar.
Moraxella sp. Method 2
Morganella sp. Method 1
Mycobacterium sp. Method 7
Note: *M. gordonae, M. terrae* and *M. tuberculosis* may require up to one month incubation.
Note: *M. haemophilium* should be grown on Middlebrook 7H11 agar and incubated at 30°C in 5 to 7% CO2 for 3 to 4 weeks. An X factor strip must be place on the agar in order for the organism to grow.
Mycoplama gallisepticum Method 17
Mycoplasma hominis Method 13
Mycoplasma hyorhinis Method 18
Mycoplasma orale Method 15
Mycoplasma pneumoniae Method 14
Mycoplasma synoviae Method 19
Myroides sp. Method 2
Neisseria sp. Method 3
Note: Chocolate agar is the best medium for the initial growth of *Neisseria* species. DO NOT open the inoculated agar medium petri plate for the first 48 hours if using a candle jar.
Nocardia sp. Method 1
Novosphingobium sp. Method 1
Note: Incubate at 25° C.
Ochrobactrum sp. Method 1
Oligella sp. Method 2
Paecilomyces sp. Method 5
Paenibacillus sp. Method 1
Note: *P. larvae* should be incubated aerobically at 30° C.
Parabacteroides sp. Method 4
Parvimonas sp. Method 4
Note: *P. micra* requires 5 to 7 days of anaerobic incubation.
Pasteurella sp. Method 2
Pediococcus sp. Method 11
Note: *P. damnosus* may be grown in MRS broth at 25ºC for 48 to 72 hours. Subculture the broth to MRS agar when it becomes cloudy. Incubate the agar at 25ºC in 5 to 7% CO2 for 72 to 96 hours. Alternatively, the lyophilized microorganism may be grown directly on MRS Agar at 25ºC in 5 to 7% CO2 for 5 to 7 days.
Penicillium sp. Method 5
Peptoniphilus sp. Method 4
Note: Incubate 72 to 96 hours in anaerobic atmosphere.
Peptostreptococcus sp. Method 4
Plesiomonas sp. Method 1
Porphyromonas sp. Method 4
Note: 5 to 7 days of anaerobic incubation is required.
Prevotella sp. Method 4
Note: 5 to 7 days of anaerobic incubation is required.
Propionibacterium sp. Method 4
Note: 3 to 5 days of anaerobic incubation is required.
Proteus sp. Method 1
Note: *P. hauseri* grows best on Blood and Tryptic Soy Agar.
Prototheca sp. Method 5
Providencia sp. Method 1
Pseudomonas sp. Method 1
Note: *P. fluorescens* should be incubated at 25°C. *Pseudomonas* species, MicroBioLogics #0162, should be incubated at 30°C.
Ralstonia sp. Method 1

Raoultella sp. Method 1

Rhizopus sp. Method 5
Rhodococcus sp. Method 2
Rhodotorula sp. Method 5
Saccharomyces sp. Method 5
Note: Sabouraud Dextrose Emmons Agar is the best medium for growth of *Saccharomyces* sp.
Salmonella sp. Method 1
Scopulariopsis sp. Method 5
Serratia sp. Method 1
Shewanella sp. Method 10
Shigella sp. Method 1
Sordaria sp. Method 5
Note: *Sordaria* sp. sporulates better on Sabouraud Dextrose Emmons Agar than on Sabouraud Dextrose Agar.
Sphingobacterium sp. Method 1
Sphingomonas sp. Method 1
Note: Incubate at 25°C.
Sporidobolus sp. Method 5
Staphylococcus sp. Method 1
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 Brian Heart Infusion Agar with 4mcg/mL Vancomycin.
Stenotrophomonas sp. Method 1
Note: Incubate at 30°C.
Streptococcus sp. Method 2
Note: *S. cricetus* must be incubated in a microaerophilic environment. *Streptococcus sp.,* MicroBioLogics #0978, should be grown in CO_2.
Streptomyces sp. Method 5
Note: *Streptomyces* sp. does not grow on Potato Dextrose Agar.
Thermoanaerobacterium sp. Method 4
Note: Primary growth medium for *T. thermosaccharolyticum,* 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.
Trichophyton sp. Method 5
Trichosporon sp. Method 5
Ureaplasma sp. Method 13
Veillonella sp. Method 4
Vibrio sp. Method 10
Virgibacillus sp. Method 1
Yarrowia sp. Method 5
Yersinia sp. Method 1
Zygosaccharomyces sp. Method 5
Note: *Z. bailii*, 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 72 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 pneumonia*e and other streptococcal species of the viridians group.

Method 3
- Chocolate Agar - 35°C in 5% to 7% CO_2 – 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 to 7% CO_2 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 7% CO_2 – 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 either Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood. Incubate at 35°C in 5 to 7% CO_2 for 48 hrs. 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 to 7% CO_2 for 48 hrs.

Method 12

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

Method 13

- Rehydrate I 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 7% CO_2. 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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.

Method 22

- Inoculate ISF (modified Infant Soy Formula) Broth with one LYFO DISK® or KWIK-STIK®. Make two dilutions, 1:10 and 1:100. Plate undiluted sample and 1:10 and 1:100 dilutions. It is necessary to plate the diluted samples because at higher concentrations the colonies are pinpoint which makes colony characteristics difficult to see. Grow at 55° in anaerobic conditions for 48 hours. The broth will turn grey, indicating growth. Sub with a swab to Sulfite Agar. Incubate the agar in anaerobic environment at 55°C for 7 days.

Method 23

- Inoculate Mycoplasma Broth with LYFO DISK® or KWIK-STIK®. Prepare serial dilutions of 1:10, 1:100, and 1:1000 using the broth. Incubate at 35°C for 48 hours. Then plate 0.2 mL of the turbid broth culture to Mycoplasma Agar. Incubate agar in 5 to 7% CO2 at 35° for 3 to 7 days. DO NOT use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.