

# MicroBiologics®

## RECOMMENDED GROWTH REQUIREMENTS

### LYFO DISK® AND KWIK-STIK™ Microorganisms

#### SELECTION OF GROWTH REQUIREMENTS

1. Primary growth on a non-selective, 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 availability of nonselective agar media will differ between laboratories. The following lists alternative agar media selection, if applicable, AND growth requirement selections:

#### Method 1

- Tryptic Soy Agar, Nonselective Sheep Blood Agar, Standard Plate Count Agar or Nutrient Agar - 35°C in ambient atmosphere – 24 to 48 hours.

#### Method 2

- Nonselective Sheep Blood Agar - 35°C in ambient atmosphere – 24 to 48 hours.

#### Method 3

- Chocolate Agar with Hemoglobin/NAD - 35°C in 5% to 10% CO<sub>2</sub> – 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, Standard Plate Count Agar (Standard Methods Agar) are appropriate alternatives for the *Clostridium species* together with an additional period (24 hours) of incubation.

#### Method 5

- Saboraud Dextrose Emmons Agar - 25°C in ambient atmosphere – 3 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 w/ Hemoglobin/NAD - 35°C in Microaerophilic Environment – 48 to 72 hours.

#### Method 7

- Lowenstein Jensen Agar or Middlebrook Agar - 35°C in 5-10% CO<sub>2</sub> or ambient atmosphere – up to one week.

#### Method 8

- Buffered Charcoal Yeast Extract Agar - 35°C in ambient atmosphere – 3 to 5 days.

#### Method 9

- V Agar or Chocolate Agar - 35°C in 5% to 10% CO<sub>2</sub> – 48 hours.

#### Method 10

- Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth 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 non-selective Sheep Blood Agar or Tryptic Soy Agar - 35°C in ambient atmosphere – 24 to 48 hrs.

#### Method 11

- The primary growth medium is MRS (Man, Rogosa, Sharpe) Broth. Incubate at 35°C in ambient atmosphere for 48 hours. Transfer to Columbia CNA with Sheep Blood. Incubate at 35°C in 5-10% CO<sub>2</sub> for 48 hrs.

#### Method 12

- Potato Dextrose Agar - 45°C in ambient atmosphere - 48 to 72 hours.

#### Method 13

- Rehydrate in 10 B Arginine Broth. Make Serial Dilutions. Incubate at 35°C, in ambient atmosphere. As soon as the Arginine vial turns pink, sub broth to A-8 Agar. DO NOT use cotton swab or wooden shaft. Incubate *Mycoplasma* at 35°C in 5-10% CO<sub>2</sub>. Incubate *Ureaplasma* at 35°C anaerobically.

#### Method 14

- Rehydrate in SP4 Glucose Broth. Make serial dilutions. Incubate at 35°C, in ambient atmosphere. As soon as the broth turns from red to yellow, sub broth to SP4 Glucose Agar. Do not use cotton swab or wooden shaft. Incubate at 35°C in CO<sub>2</sub> atmosphere, preferably in a candle jar.

**Method 15**

- Rehydrate in 10B Arginine Broth. Make serial dilutions. Incubate at 35°C, in ambient atmosphere. As soon as the broth turns from yellow to pink, sub broth to SP4 Glucose Agar. Do not use cotton swab or wooden shaft. Incubate plates at 35°C in Anaerobic conditions.

3. The following information lists the growth requirements for the strains listed:

<i>Acetobacter sp.</i>	Method 3
<b>Note:</b> Incubate at 25°C in CO <sub>2</sub> for 3-4 days.	
<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>Aeromonas hydrophila</i> should be incubated at 30°C. <i>Aeromonas salmonicida</i> should be incubated at 25° C.	
<i>Alcaligenes sp.</i>	Method 1
<i>Alicyclobacillus sp.</i>	Method 12
<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
<i>Bacillus sp.</i>	Method 1
<i>Bacteroides sp.</i>	Method 4
<b>Note:</b> <i>Bacteroides 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
<i>Bordetella sp.</i>	Method 3
<b>Note:</b> May require up to 1 week of incubation. May be incubated in Normal Environment.	
<b>Note:</b> <i>Bordetella pertussis</i> ( <i>MicroBioLogics Catalog #0843</i> ) requires Bordet Gengou with 15% Defibrinated Sheep Blood.	
<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.	
<i>Burkholderia sp.</i>	Method 1
<i>Campylobacter sp.</i>	Method 6
<b>Note:</b> Chocolate agar is the best medium for the primary growth of <i>Campylobacter jejuni</i> . DO NOT open the inoculated agar medium petri plate for the first 48 hours.	
<i>Candida sp.</i>	Method 5
<i>Capnocytophaga sp.</i>	Method 3
<i>Cellulosimicrobium sp.</i>	Method 1
<i>Chryseobacterium sp.</i>	Method 1
<i>Citrobacter sp.</i>	Method 1
<i>Clostridium sp.</i>	Method 4
<b>Note:</b> <i>Clostridium baratii</i> only grows on Anaerobic Blood Agar.	

<i>Corynebacterium sp.</i>	Method 1
<i>Cryptococcus sp.</i>	Method 5
<b>Note:</b> <i>Cryptococcus</i> should be incubated at 25°C to assure growth.	
<i>Deinococcus sp.</i>	Method 1
<i>Delftia sp.</i>	Method 1
<i>Edwardsiella sp.</i>	Method 1
<i>Eggerthella sp.</i>	Method 4
<i>Eikenella sp.</i>	Method 3
<i>Enterobacter sp.</i>	Method 1
<i>Enterococcus sp.</i>	Method 1
<i>Erysipelothrix sp.</i>	Method 2
<i>Escherichia coli</i>	Method 1
<i>Exiguobacterium sp.</i>	Method 1
<i>Fingoldia sp.</i>	Method 4
<b>Note:</b> Incubate 72 to 96 hours in anaerobic atmosphere.	
<i>Flavobacterium sp.</i>	Method 1
<b>Note:</b> Incubate at 30°C.	
<i>Fluoribacter sp.</i>	Method 8
<i>Fusobacterium sp.</i>	Method 4
<i>Gardnerella sp.</i>	Method 9
<i>Gemella sp.</i>	Method 4
<i>Geobacillus sp.</i>	Method 1
<b>Note:</b> <i>Geobacillus stearothermophilus</i> strains must be incubated at 55°C.	
<i>Geotrichum sp.</i>	Method 5
<i>Haemophilus sp.</i>	Method 3
<i>Hafnia sp.</i>	Method 1
<i>Issatchenkia sp.</i>	Method 5
<i>Kingella sp.</i>	Method 2
<b>Note:</b> Incubate in 5-10% CO <sub>2</sub> .	
<i>Klebsiella sp.</i>	Method 1
<i>Kloeckera sp.</i>	Method 5
<i>Kocuria sp.</i>	Method 1
<i>Lactobacillus sp.</i>	Method 2
<b>Note:</b> <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. delbrueckii</i> and <i>L. gasserii</i> must use Method 11.	
<i>Lactococcus sp.</i>	Method 2
<i>Leclercia sp.</i>	Method 1
<i>Legionella sp.</i>	Method 8
<i>Listeria sp.</i>	Method 1
<i>Macrooccus sp.</i>	Method 1
<i>Mannheimia sp.</i>	Method 1
<i>Methylobacterium sp.</i>	Method 1
<b>Note:</b> Incubate at 25°C. Grows best on Standard Plate Count Agar.	
<i>Microbacterium sp.</i>	Method 1
<b>Note:</b> Incubate at 30°C.	
<i>Micrococcus sp.</i>	Method 1
<i>Micromonas sp.</i>	Method 4
<b>Note:</b> <i>Micromonas micros</i> requires 5 to 7 days of anaerobic incubation.	
<i>Microsporium sp.</i>	Method 5
<i>Moraxella sp.</i>	Method 2
<i>Morganella sp.</i>	Method 1
<i>Mycobacterium sp.</i>	Method 7
<b>Note:</b> <i>Mycobacterium gordonae</i> , <i>terrae</i> and <i>tuberculosis</i> may require up to one month incubation.	

*Mycoplasma sp.*  
**Note:** *Mycoplasma hominis* must use Method 13. *Mycoplasma orale* must use Method 15. *Mycoplasma pneumoniae* must use Method 14.  
*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.  
*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  
*Pasteurella sp.* Method 2  
*Pediococcus sp.* Method 11  
**Note:** *Pediococcus damnosus* must be subcultured to MRS Agar from MRS Broth.  
*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:** 72 to 96 hours of anaerobic incubation is required.  
*Proteus sp.* Method 1  
*Prototheca sp.* Method 5  
*Providencia sp.* Method 1  
*Pseudomonas sp.* Method 1  
**Note:** *Pseudomonas fluorescens* should be incubated at 25° C. *Pseudomonas species* (*MicroBioLogics Catalog #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  
*Sphingobacterium sp.* Method 1  
*Sphingomonas sp.* Method 1  
**Note:** Incubate at 25°C.  
*Staphylococcus sp.* Method 1  
*Stenotrophomonas sp.* Method 1

*Streptococcus sp.* Method 2  
**Note:** *Streptococcus cricetus* must be incubated in a microaerophilic environment. *Streptococcus sp.* (*MicroBioLogics Catalog #0978*) should be grown in CO<sub>2</sub>.  
*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 Catalog #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  
**Note:** *Vibrio alginolyticus* should be grown on Columbia Blood Agar or Tryptic Soy Agar.  
*Virgibacillus sp.* Method 1  
*Yarrowia sp.* Method 5  
*Yersinia sp.* Method 1  
*Zygosaccharomyces sp.* Method 5