



## Production of MicroBioLogics Reference Stock Cultures

MicroBioLogics microorganism products are designed and manufactured to support microbiology quality control (QC) functions. MicroBioLogics customers offer a wide variety of testing services that are subject to the mandates of many different guidelines and standards. The production of products must be outlined to determine if a specific product has application in a particular QC function. The outline of microorganism production employs some very specific terms. Please review the Technical Information Bulletin “**Glossary of Terms and Definitions [TIB.168]**” for a definition of these important terms.

### Production Outline for Reference Stock and Enumerated Cultures

- A. **Reference Cultures**
  - Traceability
  
- B. **Primary Growth**
  - Traceability
  - Authenticity
  
- C. **Subculture # 1**
  - Traceability
  
- D. **Subculture # 2**
  - Traceability
  
- E. **Serial Strain Status**
  - Traceability
  - Authenticity
  
- F. **Routine Microorganism Production**
  - Traceability
  
- G. **Subculture # 3**
  - Traceability
  
- H. **Subculture # 4**
  - Traceability
  
- I. **Reference Stock Cultures**
  - Traceability
  - Authenticity

**A. Reference Cultures**

- Origin or source of the Reference Stock Culture is an authentic reference culture purchased from a culture type collection.
- Receipt of each reference culture initiates record keeping and documentation.
- Traceability  
The receipt date and lot number of each reference culture is recorded. The reference culture can then be traced through all subsequent documentation.

**B. Primary Growth**

- Processing  
The reference culture is hydrated or thawed according to instructions.
- Inoculation and Incubation  
A portion of the hydrated / thawed reference culture is inoculated to non-selective agar media. The inoculated media is incubated under conditions that optimize the growth of the reference culture.
- Authenticity  
Established growth on the "Primary Growth" medium is selected and subcultured to a selection of nutrient, enriched, selective and differential media. The inoculated media are incubated under different conditions to establish and document essential growth requirements and characteristics. Following incubation, the media are examined for:
  - (a) Growth requirements (e.g., nutrient, enriched, incubation temperature, aerobic, 5% to 10% carbon dioxide dependence, microaerophilic, anaerobic, etc.)
  - (b) Selective and differential characteristic
  - (c) Macroscopic colony features
  - (d) Purity

Isolated and representative colonies are selected to perform additional authenticity testing which may include:

- (a) Microscopic morphology features
- (b) Biochemical activity
- (c) Serological features
- (d) Antibiotic susceptibility determinations
- (e) Other features unique to the microorganism

Test results are recorded to document reference culture authenticity.

**C. Subculture # 1**

Established growth on the "Primary Growth" medium is selected and subcultured to a selection of nutrient, enriched, selective and differential media. The inoculated media are incubated under different conditions to establish and document essential growth requirements and growth characteristics. Following incubation, the Subculture # 1 media are examined for:

- (a) Growth requirements (e.g., nutrient, enriched, incubation temperature, aerobic, 5% to 10% carbon dioxide dependence, microaerophilic, anaerobic, etc.)
- (b) Selective and differential characteristic
- (c) Macroscopic colony features.

Test results are documented.

**D. Subculture # 2**

Subculture # 2 facilitates an important function, namely, "Serial Strain Production".

- **Serial Strain Production**

Established growth on the "Subculture # 1" medium is selected and subcultured to non-selective agar media for harvesting and lyophilization. The inoculated media are incubated under conditions that optimize growth. Following incubation, the growth is harvested and placed in suspension medium. The microorganism suspension is dispensed, frozen, lyophilized, packaged, labeled and stored according to the product's Device Master Record. This lyophilized microorganism preparation is designated as a "Serial Strain."

**E. Serial Strain Status**

Two important considerations must be addressed, "Post-Lyophilization Serial Strain Authenticity Testing" and "Routine Lyophilized Microorganism Production."

- **Post-Lyophilization Serial Strain Authenticity Testing**

Lyophilized Serial Strain preparations are selected and hydrated according to the product's Serial Strain Device Master Record. A portion of the hydrated microorganism suspension is inoculated on media, incubated, and subcultured. The authenticity testing previously described is repeated to document authenticity of the reference culture and to ensure that mutations or aberrations have not occurred during the lyophilization process.

- **Routine Lyophilized Microorganism Production**

The Serial Strain, resulting from processing "Subculture # 2" growth, is the origin for the production of retail microorganism preparations.

**F. Routine Microorganism Production**

- **Processing**

The Serial Strain is hydrated, inoculated to non-selective agar media, and incubated under conditions according to the product's Device Master Record.

**G. Subculture # 3**

Established growth on the "Serial Strain Growth" medium is selected and subcultured to a selection of nutrient, enriched, selective and differential media. The inoculated media is incubated under different conditions to document essential growth requirements and growth characteristics. Following incubation, the Subculture # 3 media are examined for:

- (a) Growth requirements (e.g., nutrient, enriched, incubation temperature, aerobic, 5% to 10% carbon dioxide dependence, microaerophilic, anaerobic, etc.)
- (b) Selective and differential characteristic
- (c) Macroscopic colony features

Test results are documented.

**H. Subculture # 4**

Subculture # 4 facilitates an important function, namely, "Routine Microorganism Production."

- **Routine Microorganism Production**

Established growth on "Subculture # 4 media is selected and subcultured to non-selective agar media for harvesting and lyophilization. The inoculated media is incubated under conditions that optimize growth. Following incubation the growth is harvested and placed in suspension medium. The microorganism suspension is dispensed, frozen, lyophilized, packaged, labeled and stored according to the product's Device Master Record. This lyophilized microorganism preparation is designated as a "Reference Stock Culture."

**I. Reference Stock and Enumerated Cultures**

- Processing  
The reference stock culture is hydrated according to instructions.
- Inoculation and Incubation  
A portion of the hydrated reference stock culture is inoculated to non-selective, selective or differential media. The inoculated media is incubated under different conditions to document essential growth requirements and characteristics.
- Authenticity  
Following incubation, the media are examined for:
  - (a) Growth requirements (e.g., nutrient, enriched, incubation temperature, aerobic, 5% to 10% carbon dioxide dependence, microaerophilic, anaerobic, etc.)
  - (b) Selective and differential characteristic
  - (c) Macroscopic colony features
  - (d) Purity.

Isolated and representative colonies are selected to perform additional authenticity testing, which may include:

- (a) Microscopic morphology features
- (b) Biochemical activity
- (c) Serological features
- (d) Antibiotic susceptibility determinations
- (e) Other features unique to the microorganism.

Test results are recorded in order to document reference culture authenticity.

- Four (4) Passages  
MicroBioLogics Reference Stock Culture products such as LYFO DISK<sup>®</sup>, KWIK-STIK<sup>™</sup>, EZ-COMP<sup>™</sup> Samples, and enumerated products such as EZ-CFU<sup>™</sup>, EZ-CFU<sup>™</sup> One Step, Epower<sup>™</sup>, EZ-PEC<sup>™</sup>, EZ-FPC<sup>™</sup>, and EZ-SPORE<sup>™</sup> Microorganisms are four (4) passages or subcultures removed from Reference Culture status.
- Two (2) Passages  
MicroBioLogics Reference Stock Culture product KWIK-STIK<sup>™</sup> Plus is two (2) passages or subcultures removed from Reference Culture status. This product line is similar to the production of Serial Strains as previously noted.

This outline can be used to define important considerations regarding the production and quality assurance of MicroBioLogics lyophilized microorganism products. The important considerations include:

**Reference Stock and Enumerated Cultures:**

- √ **Production Documentation**
- √ **Traceability Documentation**
- √ **Authenticity Documentation**
- √ **Passage Documentation**