

# MicroBiologics

## Guideline for Microbiology Quantitative Fod Process Controls

### “QNT-FPC”

#### Assayed Control Microorganism Challenges for Plate and MPN Count Methods

This document is designed to serve as an example or guideline in developing a quality assurance program for microbiology Plate Count and MPN count methods. **The guideline must be adapted to the specific demands imposed on each individual laboratory.** Several important considerations must be raised.

#### EZ-FPC™ (Food Process Control) Microorganisms

The EZ-FPC™ Microorganisms are lyophilized and assayed control microorganism preparations. The process design for these preparations offers a quality control challenge to measure and provide documentation that a quantitative test method performs within an anticipated range of tolerance.

The production and process design for these lyophilized microorganism preparations results in a mean assay value that fall within a range of **1,000 TO 9,900** (1.0E+03 – 9.9E+03) **CFU per pellet** for Quantitative (Enumeration) Process Controls.

Quality control documentation includes, but is not limited to, a Certificate of Assay stating:

- a) The identity and traceability of the microorganism preparation to a reference culture
- b) The number of passages the microorganism preparation has been removed from the reference culture
- c) The mean assay value for the microorganism preparation.

#### Microorganism Challenges

Each quantitative food process control (QNT-FPC) consists of a reference stock culture that is derived from and traceable to the American Type Culture Collection (ATCC®) or other authentic reference culture collection. Each lyophilized QNT-FPC preparation contains a target microorganism at a known concentration. Examples of QNT-FPC include, but are not limited to:

*Aspergillus niger*  
*Bacillus cereus*  
*Clostridium perfringens*  
*Escherichia coli*  
*Lactobacillus fermentum*  
*Saccharomyces cerevisiae*  
*Staphylococcus aureus*

#### Objective for QNT-FPC

Each day that a Plate or MPN Count is run, the specific QNT-FPC must be performed. This regular use of QNT-FPC provides evidence and leads to documentation that each Plate Count or MPN Count Method continues to meet the range of tolerance for which the method was validated.

### Food Sample Matrix

A review of the actual procedure will reveal that a sterile solution of non-fat dry milk (NFDM) is used as the sample matrix. The ideal situation would suggest that a QNT-FPC be run with each type of food sample analyzed in the laboratory. The real situation suggests that this practice is cost-prohibitive AND it may be difficult or impossible to achieve a uniform distribution of the QNT-FPC microorganism population throughout some food types.

### QNT-FPC Cocktails

It is not necessary to prepare, process, and run each challenge microorganism separately. Several challenge microorganisms can be combined into a single QNT-FPC preparation or 'cocktail'. Examples might include:

QNT-FPC Preparation A includes *E. coli*, *S. aureus*, and mold (*A. niger*).

QNT-FPC Preparation B includes the remaining quantitative microorganisms.

### QNT-FPC DIRECTIONS FOR USE

#### A. Preparation of 100 mL Vials of 1:10 NFDM

1. Weigh out 10.0 grams of Non-Fat Dry Milk (NFDM).
2. Place into a screw cap vial container.
3. Add 90.0 mL of pH 7.2 Phosphate Buffer.
4. Mix thoroughly.
5. Sterilize the 1:10 NFDM vials at 121°C for 15 minutes.
6. Store the sterile 1:10 NFDM at 4°C to 8°C.

#### B. QNT-FPC Processing

1. Prewarm the vial of 1:10 NFDM for a minimum of one (1) hour at 35°C to 37°C.
2. Remove the appropriate EZ-FPC™ Microorganism vial(s) from refrigerated storage and allow the unopened vial(s) to equilibrate to room temperature.  
**Vials containing lyophilized microorganism pellets should be allowed to equilibrate to room temperature before opening. If cold vials are opened, condensation can collect in the vial. The combination of moisture and oxygen can produce toxic free radicals that can reduce the recovery of lyophilized microorganisms.**
3. Place a single pellet of the lyophilized assayed control microorganism into a prewarmed vial of 1:10 NFDM.  
**More than one (1) pellet can be used to seed or can be placed into a single 1:10 NFDM.**  
**Pellets of *E. coli*, *S. aureus*, and mold (*A. niger*) can be placed into a single 1:10 NFDM and labeled "QNT-FPC Preparation A"**  
**Pellets of *B. cereus*, *L. fermentum*, and yeast (*S. cerevisiae*) can be placed into a second vial of 1:10 NFDM and labeled QNT-FPC Preparation B.**
4. IMMEDIATELY, replace the rubber plug, reseal the vial, and return the desiccant-containing vial of the remaining EZ-FPC™ Microorganism pellets to refrigerated storage.

- Mix each QNT-FPC preparation thoroughly and incubate at 35°C to 37°C for thirty (30) minutes.  
**Gelatin is an integral component of the lyophilized pellet and provides its structure. This gelatin dissolves above 30°C. Prewarming the 1:10 NFDM and incubating each QNT-FPC sample at 35°C to 37°C for 30 minutes is ESSENTIAL to assure a homogenous suspension of the assayed control microorganisms.**
- Following incubation and hydration, the seeded QNT-FPC must be used within thirty (30) minutes.

### C. QNT-FPC Analysis

- Analyze each QNT-FPC according to the appropriate Plate Count or MPN Count Method.  
**Following the incubation, the seeded QNT-FPC samples must be used as a QA challenge within thirty (30) minutes. Following hydration, a lyophilized microorganism will begin resuscitation and metabolic activity. In the absence of adequate nutrients, appropriate temperature, and supporting environmental conditions, the microorganism population will begin to decline. The completion of challenges within 30 minutes assures a stable microorganism population.**
- Record the test results.  
**Each QNT-FPC is considered to be a 1:100 dilution of the pellet. A 1.0 mL volume plated from the QNT-FPC is considered to be a  $10^{-2}$  dilution for the purpose of calculation and analysis.**
- Pellet CFU. The stated performance and specifications for a quantitative EZ-FPC™ Microorganism is a pellet concentration of 1,000 to 9,900 (1.0E+03 – 9.9E+03) CFU per pellet.
- Mean Assay Value. A Certificate of Assay is attached to each EZ-FPC™ Microorganism Unit-Of-Sale. The stated Mean Assay Value provides statistical documentation that the product's performance and specifications have been met.
- QNT-FPC CFU. In theory and excluding method selectivity and detection limit considerations, a 1:100 dilution of each quantitative EZ-FPC™ Microorganism is a QNT-FPC concentration of 10 to 99 (1.0E+01 – 9.9E+01) CFU.
- Each laboratory must select the statistical analysis method most appropriate to the sample and develop a reporting system. The chart provided is just one way to portray Quantitative Food Process Control QA Results.

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