Preservative Efficacy Testing Through the Growth Direct® System

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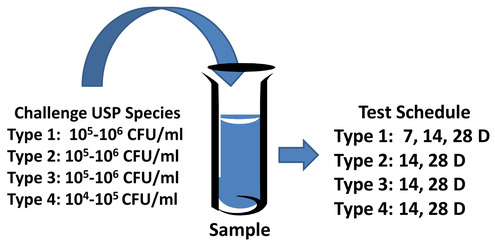
Introduction

Preservative efficacy testing (PET) confirms that added antimicrobial agents retain their activity over time. USP <51> describes the methodology used to test for four classes of materials for efficacy: 1) sterile aqueous injectable and topical products, 2) non-sterile topical samples, 3) oral aqueous products, and 4) antacids. To test, each product is challenged with a concentration of the five USP <51> organisms and the level of bioburden tracked over 28 days. Such testing is labor and resource intensive due to the numerous manual manipulations required to obtain an accurate estimate of the bioburden of the test samples. The effectiveness of the Growth Direct® System (GD) is examined for PET, specifically in resource and labor savings during analysis.

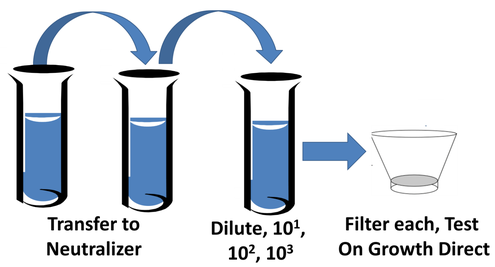
Current Test Methods for PET



- Serial dilution to 10^5 fold and filter to get accurate estimate of bioburden laborious and time consuming.
- Incubate 3-5 Days.
- Prone to human error, such as in dilution series of each organism, mis-aliquots, visual counts.

Comparing Growth Direct® vs. PET by Standard Method

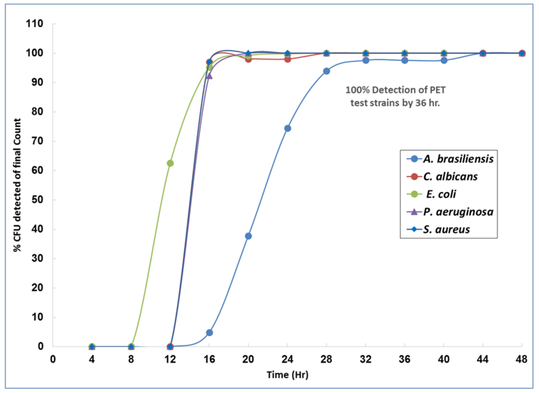
Same Sampling Regime



GD Higher Dynamic Range = less dilution

Strain	Growth Direct	Control (PP)
A. brasiliensis	~500	50-80
C. albicans	~1000	100-200
E. coli	~1000	100-200
P. aeruginosa	~1000	100-200
S. aureus	~1000	100-200

At least 50% faster time to result vs standard method for test PET strains



Bacteria\Yeast in 24 hrs, *A. brasiliensis* in 36 hrs.

Comparability Test of Growth Direct® and the Standard Method

Test Method

- Challenge organisms: *E. coli, S. aureus, A. brasiliensis, P. aeruginosa, C. albicans*
- Spike into each sample at ~1 x 10^6 CFU/ml, incubate RT.
- Sampled at each specified time-point.
- Sample neutralized and diluted in modified letheen broth (MLB).
  - Diluted 10^3 for GD, 10^5 for control.
- Running samples:
  - GD Filter and run on Growth Direct® System: 72 hours @ 32.5C
  - Standard method: filter, transfer to PP, and incubate 3 days @ 32.5C

Test Samples
Type 1: Saline Nasal Spray
Type 2: Contact Lens wash
Type 3: Cough Syrup
Type 4: Liquid Antacid

Acceptance Criteria

Nasal Spray	Bacteria: >1 log reduction from initial CFU Yeast\m\Mold: no increase from initial CFU
Contact Solution	Bacteria: > 2 log reduction from initial CFU Yeast\Mold: No increase from initial CFU
Cough Syrup	Bacteria: >1 log reduction from initial CFU Yeast\Mold: no increase from initial CFU
Antacid	Bacteria:\Yeast\Mold: no increase from initial CFU

Growth Direct® Results are Comparable to the Standard Method Type 1

	A. brasiliensis	*C. albicans*	*E. coli*	*P. aeruginosa*	*S. aureus*
Starting	1x 10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml
7 day results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
14 day results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
28 day results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
Log reduction	10^6 log reduction	10^6 log reduction	10^6 log reduction	10^6 log reduction	10^6 log reduction
Control					
7 day results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
14 day results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
28 day results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml

Comparable Performance with Types 2, 3 and 4

Strain	*A. brasiliensis*	*C. albicans*	*E. coli*	*P. aeruginosa*	*S. aureus*
Starting CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml
Results	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml	<1 CFU/ml
Log Reduction	10^6 log reduction	10^6 log reduction	10^6 log reduction	10^6 log reduction	10^6 log reduction

Summary

For PET testing, the Growth Direct® System provided comparable results to the standard test method, while reducing the time to result for testing by approximately 50%. Additionally, the higher dynamic range of up to 10^3 CFU reduced the number of dilutions required in the assay reducing the labor required for the test. PET thus this another of the many testing applications that are completed more quickly and easily using the Growth Direct® System.