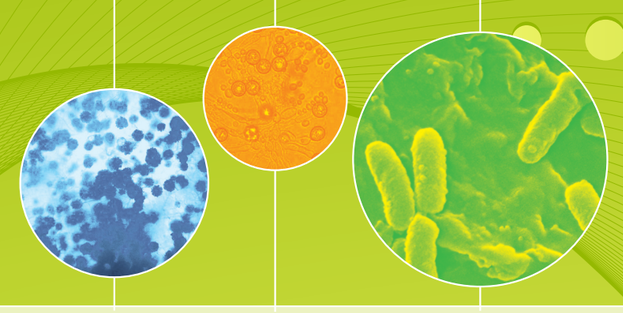
COMPARISON TIME STUDY – GROWTH PROMOTION TESTING:
Using Commercially-Prepared Microorganisms vs. Traditionally-Prepared Microorganisms

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ABSTRACT

The Growth Promotion Test is used to determine the suitability of new batches of media by inoculating the media with 100 colony forming units (CFU) or less. This study compares the time required to deliver 100 CFU or less using traditional spectrophotometric and turbidimetric methods versus methods using commercially prepared microorganism suspensions. Microorganism strains recommended by USP <61> were tested.

ATCC® microorganisms *Aspergillus brasiliensis* 16404™, *Bacillus subtilis* subsp. *spizizenii* 6633™, *Candida albicans* 10231™, *Pseudomonas aeruginosa* 9027™ and *Staphylococcus aureus* 6538™ were quantified spectrophotometrically and turbidometrically, then 10-fold serially diluted to deliver <100 CFU/0.1 ml.

BioMérieux, Inc. BioBall® MultiShot 550, Microbiologics® EZ-Accu Shot™, and Remel Quanti-Cult Plus® prepared microorganisms were hydrated according to directions in order to deliver <100 CFU/0.1 ml. Times for each method were divided into hands-on-time (HOT) and waiting-time (WAT).

The commercially prepared microorganism suspensions, MultiShot 550, EZ-Accu Shot™ and Quanti-Cult Plus®, required significantly less HOT and WAT time than the traditional methods to complete the Growth Promotion Test.

OBJECTIVE

The objective is to demonstrate how to save time while performing the Growth Promotion Test of media by using commercially-prepared microorganisms versus microorganisms prepared using the traditional spectrophotometric and turbidimetric methods. MultiShot 550, EZ-Accu Shot™ and Quanti-Cult Plus® microorganisms require significantly less hands-on-time than microorganisms prepared using traditional methods.

MATERIALS

Microorganisms

The ATCC® microorganisms were purchased directly from ATCC®: *Aspergillus brasiliensis*, ATCC® 16404™, *Bacillus subtilis* subsp. *spizizenii*, ATCC® 6633™, *Candida albicans*, ATCC® 10231™, *Pseudomonas aeruginosa* ATCC® 9027™, and *Staphylococcus aureus*, ATCC® 6538™.

The Microbiologics® EZ-Accu Shot™ and Remel Quanti-Cult Plus® microorganisms were ATCC® derivatives of the same strains. Both Microbiologics® and Remel are part of the ATCC® Licensed Derivative program and required an ATCC® End User Agreement to be completed prior to purchase.

ATCC®, a not-for-profit biological resource center, describes the ATCC® Licensed-Derivative Program as a program in which "companies agree to maintain the integrity of the ATCC® ingredient and allow ATCC® to verify the quality of the ingredient before it reaches end-users."

The bioMérieux BioBall® MultiShot 550 microorganisms were labeled as derivatives of *Aspergillus brasiliensis*, NCPF 2275, *Bacillus subtilis*, NCTC 10400, *Candida albicans*, NCPF 3179, *Pseudomonas aeruginosa* NCTC 12924, and *Staphylococcus aureus*, NCTC 10788. BioMérieux did not require completion of an end user agreement.

Other materials

1. The following other materials were used in the study: a Spectronic™ GENESYS™ 20 spectrophotometer, a HACH® 2100P portable turbidimeter, Tryptic Soy Agar, phosphate buffer pH 7.2, a calibrated timer, a vortex mixer and dally rods.

METHODS

The microorganisms tested in the study were the strains recommended for growth promotion testing in USP <61>, *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests.*[1] The USP instructs laboratories to, "Inoculate portions/plates of Soybean-Casein Digest Broth and Soybean-Casein Digest Agar with a small number (not more than 100 CFU) of the microorganisms." In the study different methods of obtaining 100 CFU or less were tested. Two methods were traditional: they were the spectrophotometric method and the turbidimetric method. Three methods used commercially prepared microorganism preparations.

For the spectrophotometric method, a suspension of a microorganism strain was prepared from a fresh culture using phosphate buffer as the diluent. Turbidity was adjusted to 50%T at 653 nm. A series of ten-fold dilutions were made in order to obtain a concentration of 100 CFU or less per 0.1 ml.

In the turbidimetric method, a suspension of a microorganism strain was prepared from a fresh culture using phosphate buffer as the diluent. Turbidity was adjusted to 194-232 Formazin Nephelometric Units (FNU) and then a series of ten-fold dilutions were made in order to obtain a concentration of 100 CFU or less per 0.1 ml.

The MultiShot 550, EZ-Accu Shot™, and Quanti-Cult Plus® commercially prepared microorganisms were hydrated according to manufacturers directions in order to deliver 100 CFU or less per 0.1 ml.

Each Tryptic Soy Agar plate was inoculated with <100 CFU/0.1 ml of an individual microorganism strain. Only one strain per plate was tested. The spread plate method was used to disperse the microorganisms. Tests were performed in duplicate for each microorganism suspension. After incubation at 30-35°C, the colonies on each plate were counted. Three trials for each method were performed. In each trial, a new microorganism suspension was made. One lot of each strain was tested from each of the four sources (ATCC®, MultiShot 550, EZ-Accu Shot™, and Quanti-Cult Plus®).

Times for each method were divided into hands-on-time (HOT) and waiting-time (WAT). Times included preparation of strains, preparation of inoculum, incubation time and determination of CFU counts. Hands-on-time is defined as time when the laboratory professional is directly involved in the testing. The inoculation of Tryptic Soy Agar plates is an example of hands-on-time. Waiting-time is defined as time when the test was proceeding without the direct involvement of the laboratory professional. Incubation time is an example of waiting-time.

RESULTS

MultiShot 550, EZ-Accu Shot™ and Quanti-Cult Plus® microorganisms required significantly less hands-on-time than microorganisms prepared using traditional methods.

The total HOT time for traditional spectrophotometric and turbidimetric methods was 115 minutes for five microorganisms, whereas the total HOT time for methods using commercially prepared microorganisms was only 30 minutes for five microorganisms.

The total WAT time was significantly longer for traditional spectrophotometric and turbidimetric methods because each microorganism needed to be grown before preparing an inoculum of 100 CFU or less.

Study conducted December 2010 – January 2011

Summary of time required to perform the growth promotion test on five microorganisms using Traditional Methods vs. Methods using Commercial Products:

Method	Preparation of Reference Culture Strains			Preparation of Inocula of 100 CFU or less		Incubation Time	Counting Colony Forming Units	TOTAL HOT[1]	TOTAL WAT[2]	Number of Steps per Method
	WAT[3]	HOT (Reconstitution)	WAT (Incubation Time)	WAT[3,4]	HOT	WAT	HOT			
Spectrophotometric	20 minutes	10 minutes	• 19 hours bacteria • 48 hours *C. albicans* • 5-7 days *A. brasiliensis*		85 minutes	• 19 hours bacteria • 48 hours *C. albicans* and *A. brasiliensis*	20 minutes	115 minutes	• 38 hours bacteria • 96 hours *C. albicans* • 7-9 days *A. brasiliensis*	21
Turbidimetric	20 minutes	10 minutes	• 19 hours bacteria • 48 hours *C. albicans* • 5-7 days *A. brasiliensis*		85 minutes	• 19 hours bacteria • 48 hours *C. albicans* and *A. brasiliensis*	20 minutes	115 minutes	• 38 hours bacteria • 96 hours *C. albicans* • 7-9 days *A. brasiliensis*	21
Microbiologics® EZ-Accu Shot™				20 minutes	10 minutes	• 19 hours bacteria • 48 hours *C. albicans* and *A. brasiliensis*	20 minutes	30 minutes	• 19 hours 20 minutes bacteria • 48 hours 20 minutes *C. albicans* and *A. brasiliensis*	11
Remel Quanti-Cult Plus®				35 minutes	10 minutes	• 19 hours bacteria • 48 hours *C. albicans* and *A. brasiliensis*	20 minutes	30 minutes	• 19 hours 35 minutes bacteria • 48 hours 35 minutes *C. albicans* and *A. brasiliensis*	14
BioMérieux BioBall® MultiShot 550				20 minutes	10 minutes	• 19 hours bacteria • 48 hours *C. albicans* and *A. brasiliensis*	20 minutes	30 minutes	• 19 hours 20 minutes bacteria • 48 hours 20 minutes *C. albicans* and *A. brasiliensis*	11

[1]Hands-On-Time [2]Waiting-Time [3]ATCC®, EZ-Accu Shot® and BioBall® MultiShot 550 organisms require 20 minutes for organisms to equilibriate to room temperature before reconstitution. [4]Quanti-Cult Plus®organisms require 20 minutes to equilibriate to room temperature and 15 minutes to dissolve in pre-warmed hydration fluid.

DISCUSSION

Three trials were performed for each method. The Mean Assay Value for the number of CFU per Tryptic Soy Agar plate was as follows:

Strain	Spectrophotometric Method	Turbidimetric Method	EZ-Accu Shot™	Quanti-Cult Plus®	BioBall® MultiShot 550
Aspergillus brasiliensis	23	21	30	42	31
Bacillus subtilis subsp. *spizizenii*	92	16	27	39	46
Candida albicans	15	21	32	70	49
Pseudomonas aeruginosa	18	39	46	23	48
Staphylococcus aureus	12	32	54	44	46

CONCLUSION

Using commercially prepared microorganisms to prepare inoculum of 100 CFU or less will save time when performing the growth promotion test.

REFERENCES

[1] United States Pharmacopeia-National Formulary, Volume 33 NF28. 2010. <61> *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests.*

Storage and Shipping Conditions

MultiShot 550 shipped on ice and hydration fluid shipped at ambient temperature; microorganisms stored at ≤ -18°C.

EZ-Accu Shot™ and Quanti-Cult Plus® shipped at ambient temperature; microorganisms stored at 2-8°C.

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