

# Evaluation of Commercially Prepared Microbial Cultures for Use in Performing USP <51> Antimicrobial Effectiveness Testing

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## PURPOSE

The purpose of this protocol was to compare the use of the BioBall™ MultiShot 10E8 commercially prepared microbial cultures and microbial cultures freshly prepared from American Type Culture Collection (ATCC) organisms to determine if the BioBall product is suitable for use in performing USP General Chapter <51> Antimicrobial Effectiveness Testing (AET).

The Time Zero population of each culture type was compared to population results after 24 hours, 3 days, 7 days, 14 days and after 28 days inoculated into test products and stored at 20 – 25°C as described in USP <51>.

The BioBall™ MultiShot 10E8 is a commercially prepared microbial culture available for use in antimicrobial effectiveness testing. It was hypothesized that the BioBall™ product could simplify the preparation of the inoculating suspension and could provide a more precise inoculum compared to traditional methods that require the purchase of ATCC freeze-dried organisms, organism resuscitation, growth amplification and harvesting to prepare the inoculating suspension.

This protocol consisted of performing a side by side comparison of the antimicrobial effectiveness test using the two inoculum preparations. Test Products consisted of:

- Fluid A (Simulated Product) containing no preservatives
- Product Number 1 Non-sterile Preserved Saline Spray (Category 2 Product)
- Product Number 2 Sterile Preserved Eye Drops (Category 1 Product)

## CONCLUSIONS

•Based on the results of this study it can be concluded that the BioBall™ product can be utilized to perform USP <51> Antimicrobial Effectiveness testing with no change in the outcome of the test results.

•It can be concluded from this data that in the absence of preservative the BioBall™ organisms as well as the ATCC organisms would survive and grow throughout the incubation period of the test and that the results of the test do truly reflect whether the preservative is working in the product. This is important to note since there is no positive inoculum control required for the AET test as specified in USP.

• It was shown that the BioBall™ product was stable and that the inoculum concentration was achieved within the target range specified in USP in 100% of the tests initiated.

• It was shown that the organism preparation time for the BioBall™ product was approximately 10 minutes total compared to 1-1.5 hours of preparation time for the ATCC cultures. BioBall™ products are ready for use with no lead time compared to the 2 to 13 days of lead time required for ATCC culture preparation and incubation.

• The ATCC cultures were out of the target inoculum range on eighteen occasions, affecting thirty test set ups, resulting in a loss of at least 90 hours due to retests (\$9450) that had to be initiated. In addition to the time lost, supplies for setting up the studies as well as test product were consumed and had to be replaced resulting in additional cost to the study.

## METHOD VALIDATION

PRIOR TO TESTING, THE RECOVERY METHODS FOR ALL PRODUCTS TO BE TESTED WERE VALIDATED ACCORDING TO USP <1227>.

Table 2. Summary of Acceptance Criteria for USP <51> Testing.

Organism Type	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Initial to Day 28
<b>Category I Products</b> Injections, other parenterals including emulsions, otic products, sterile nasal products and ophthalmic products made with aqueous bases or vehicles.			
Yeast and Mold	No increase	No increase	No increase
Organism Type	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
Bacteria	Not less than 1.0 log reduction	Not less than a 3.0 log reduction	No increase
<b>Category II Products</b> Typically used products made with aqueous bases or vehicles, non-sterile nasal products and emulsions, including those applied to mucous membranes.			
Organism Type	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Initial to Day 28
Yeast and Mold	No criteria specified	No increase	No increase
Organism Type	Log Change From Initial to Day 7	Log Change from Initial Count to Day 14	Log Change from Day 14 to Day 28
Bacteria	No criteria specified	Not less than a 2.0 log reduction	No increase
<b>Category III Products</b> Oral products other than antacids, made with aqueous bases or vehicles			
Organism Type	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Initial to Day 28
Yeast and Mold	No criteria specified	No increase	No increase
Organism Type	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
Bacteria	No criteria specified	Not less than a 1.0 log reduction	No increase

No increase defined as NMT 0.5 log<sub>10</sub> units higher than previous value.

### Challenge Organisms Utilized

Organism Name	Culture ID	BTF Part Number
<i>Aspergillus brasiliensis</i>	NCPF 2275; ATCC 16404	410106
<i>Candida albicans</i>	NCPF 3179; ATCC 10231	56145
<i>Escherichia coli</i>	NCTC 12923; ATCC 8739	56146
<i>Pseudomonas aeruginosa</i>	NCTC 12924; ATCC 9027	56147
Organism Name	Culture ID	BTF Part Number
<i>Staphylococcus aureus</i>	NCTC 10788; ATCC 6538	10788

Note: Rehydration Fluid Part Number: 56021

## RESULTS FOR SIMULATED PRODUCT

### FLUID A

Table 3. AET Test Results for Fluid A (Simulated Product).

BioBall Organisms				
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28	Meets USP <51> for Category 1?
AB	-0.4 to 0.2	-0.2 to 0.2	-0.9 to 0.2	Yes
CA	0.8 to 1.0	0.9 to 1.3	0.9 to 1.5	No
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28	Meets USP <51> for Category 1?
EC	1.9 to 3.1	2.7 to 3.2	-0.2 to 0.7	No
PA	2.0 to 2.7	2.4 to 3.5	-0.9 to 1.0	No
SA	1.3 to 3.4	2.4 to 3.3	-0.5 to 0.8	No
ATCC Organisms				
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28	Meets USP <51> for Category 1?
AB	-0.1 to 0.8	-0.2 to 0.6	-0.3 to 0.3	No
CA	0.1 to 1.9	1.0 to 2.5	0.6 to 2.6	No
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28	Meets USP <51> for Category 1?
EC	2.5 to 2.9	2.3 to 2.6	-0.1 to 0.1	No
PA	2.2 to 3.1	2.6 to 3.4	0.1 to 0.9	No
SA	2.5 to 3.6	1.4 to 2.9	-1.0 to 0.5	No

Table 4. AET Test Results for Fluid A (Simulated Product).

BioBall Organisms				
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28	Meets USP <51> for Category 2?
AB	-0.4 to 0.2	-0.2 to 0.2	-0.9 to 0.2	Yes
CA	0.8 to 1.0	0.9 to 1.3	0.9 to 1.5	No
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28	Meets USP <51> for Category 2?
EC	1.9 to 3.1	2.7 to 3.2	-0.2 to 0.7	No
PA	2.0 to 2.7	2.4 to 3.5	-0.9 to 1.0	No
SA	1.3 to 3.4	2.4 to 3.3	-0.5 to 0.8	No
ATCC Organisms				
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28	Meets USP <51> for Category 2?
AB	-0.1 to 0.8	-0.2 to 0.6	-0.3 to 0.3	No
CA	0.1 to 1.9	1.0 to 2.5	0.6 to 2.6	No
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28	Meets USP <51> for Category 2?
EC	2.5 to 2.9	2.3 to 2.6	-0.1 to 0.1	No
PA	2.2 to 3.1	2.6 to 3.4	0.1 to 0.9	No
SA	2.5 to 3.6	1.4 to 2.9	-1.0 to 0.5	No

Tables 3 to 5 evaluate the results determined when inoculating Fluid A as if it were a finished product in order to determine what the growth/death rate would look like without the effect of a preservative. In all three cases the product fails to meet the acceptance criteria for the USP <51> Antimicrobial Effectiveness Testing. These results are consistent with what one would expect since the product does not contain a preservative.

It can be concluded from this data that in the absence of preservative the BioBall organisms as well as the ATCC organisms would survive and grow throughout the incubation period of the test and that the results of the test do truly reflect whether the preservative is working in the product. This is important to note since there is no positive inoculum control required for the AET test as specified in USP.

The second important point to note is that the results are consistent between the BioBall product and the ATCC cultures in that there would be no difference in the overall outcome of the testing if the BioBall cultures were used in place of the ATCC cultures.

Table 5. AET Test Results for Fluid A (Simulated Product).

BioBall Organisms				
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28	Meets USP <51> for Category 3?
AB	-0.4 to 0.2	-0.2 to 0.2	-0.9 to 0.2	Yes
CA	0.8 to 1.0	0.9 to 1.3	0.9 to 1.5	No
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28	Meets USP <51> for Category 3?
EC	1.9 to 3.1	2.7 to 3.2	-0.2 to 0.7	No
PA	2.0 to 2.7	2.4 to 3.5	0.9 to 1.0	No
SA	1.3 to 3.4	2.4 to 3.3	-0.5 to 0.8	No
ATCC Organisms				
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28	Meets USP <51> for Category 3?
AB	-0.1 to 0.8	-0.2 to 0.6	-0.3 to 0.3	No
CA	0.1 to 1.9	1.0 to 2.5	0.6 to 2.6	No
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28	Meets USP <51> for Category 3?
EC	2.5 to 2.9	2.3 to 2.6	-0.1 to 0.1	No
PA	2.2 to 3.1	2.6 to 3.4	0.1 to 0.9	No
SA	2.5 to 3.6	1.4 to 2.9	-1.0 to 0.5	No

Remaining graphs on the reverse of this page.

# RESULTS FOR SIMULATED PRODUCT

## FLUID A CONTINUED

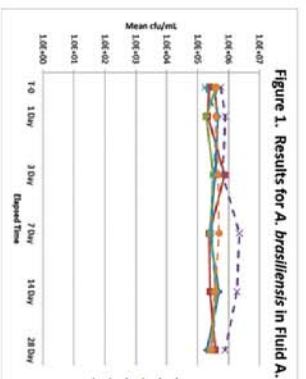


Figure 1. Results for *A. brasiliensis* in Fluid A.

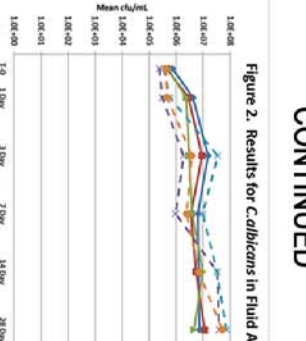


Figure 2. Results for *C. albicans* in Fluid A.

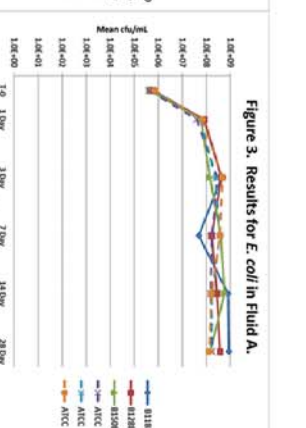


Figure 3. Results for *E. coli* in Fluid A.

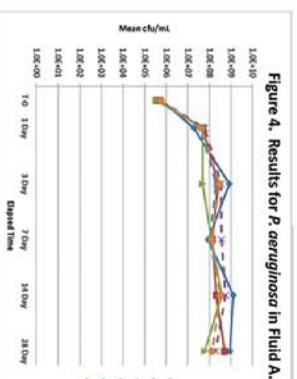


Figure 4. Results for *P. aeruginosa* in Fluid A.

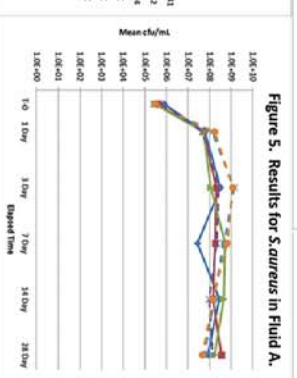


Figure 5. Results for *S. aureus* in Fluid A.

Figures 1 through 5 show the results for the three BioBall lots compared to the results for the ATCC cultures. All results show that all six challenge organisms are capable of surviving and growing in the Fluid A for the duration of the 28 day trial period. Since the Fluid A does not contain preservatives this is not unexpected. These graphs clearly show that there is no significant difference between the results seen for the BioBall cultures when compared to the ATCC cultures. This simulated product fails to meet the acceptance criteria of USP <51> testing for all organisms utilizing either BioBall or ATCC cultures.

## PRODUCT NUMBER 1 NON-STERILE PRESERVED SALINE SPRAY (CATEGORY 2 PRODUCT)

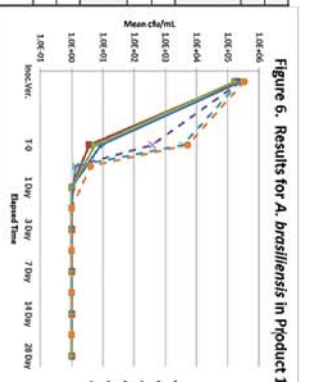


Figure 6. Results for *A. brasiliensis* in Product 1.

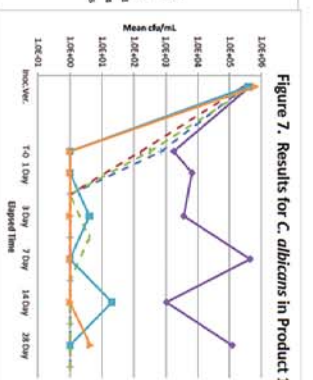


Figure 7. Results for *C. albicans* in Product 1.

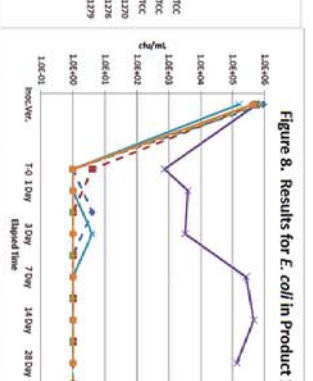


Figure 8. Results for *E. coli* in Product 1.

Comparison of Results to USP <51> Acceptance Criteria for Product 1.			
BioBall Organisms			
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28
AB	-5.5 to -5.4	-5.5 to -5.4	-5.5 to -5.4
CA	-5.8 to 0.4	-5.8 to -2.5	-5.8 to -0.1
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
EC	-5.6 to 0.1	-5.6 to 0.1	-1.1 to 0.0
PA	-5.6 to -5.1	-5.6 to -3.7	0.0 to 0.0
SA	-5.5 to -5.1	-5.5 to -5.1	-2.7 to 0.0
ATCC Organisms			
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28
AB	-5.4 to -5.1	-5.4 to -5.1	-5.4 to -5.1
CA	-5.7 to -5.4	-5.7 to -5.4	-5.7 to -5.4
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
EC	-6.0 to -5.7	-6.0 to -5.7	0.0 to 0.0
PA	15.4 to -5.3	-5.4 to -5.1	0.0 to 0.0
SA	-5.6 to -5.6	-5.6 to -5.6	0.0 to 0.0

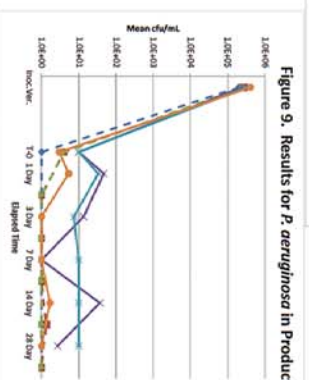


Figure 9. Results for *P. aeruginosa* in Product 1.

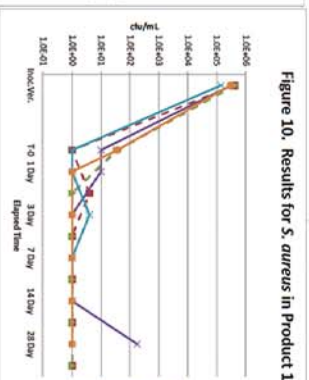


Figure 10. Results for *S. aureus* in Product 1.

Figures 6 through 10 show the results for the three BioBall lots compared to the results for the ATCC cultures when inoculated into Product 1 (non-sterile preserved saline spray). All results demonstrate that the *A. brasiliensis* (Fig. 6) was not capable of surviving in the product. Results for the other organisms in this product were more variable and it appeared that the BioBall organisms were possibly more difficult to kill than the ATCC organisms. The reason for this is not known. It could be that the cultures are harder or it could be that there was variation in the preservative level from lot-to-lot of this product. Although some differences were seen in the rates of die off, the overall outcome of the testing was consistent between the BioBall and ATCC cultures. This product meets the acceptance criteria of USP <51> testing for all organisms utilizing either BioBall or ATCC cultures.

## PRODUCT NUMBER 2 STERILE PRESERVED EYE DROPS (CATEGORY 1 PRODUCT)

Comparison of Results to USP <51> Acceptance Criteria for Product 2.			
BioBall Organisms			
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28
AB	-1.0 to -0.8	-1.0 to -0.7	-1.9 to -0.8
CA	-5.8 to -0.3	-5.6 to -1.3	-5.6 to -4.8
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
EC	-5.8 to -2.2	-5.8 to -5.2	-0.3 to 0.0
PA	-5.6 to -5.4	-5.6 to -4.7	0.0 to 0.0
SA	-5.5 to -5.1	-5.5 to -4.5	0.0 to 0.6
ATCC Organisms			
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28
AB	-2.0 to -1.6	-2.6 to -2.3	-2.7 to -2.3
CA	-5.7 to -4.0	-5.7 to -5.0	-5.7 to -5.0
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
EC	-6.0 to -5.7	-6.0 to -5.7	0.0 to 0.0
PA	-5.4 to -4.3	-5.4 to -5.3	0.0 to 0.0
SA	-5.6 to -5.6	-5.6 to -5.6	0.0 to 0.0

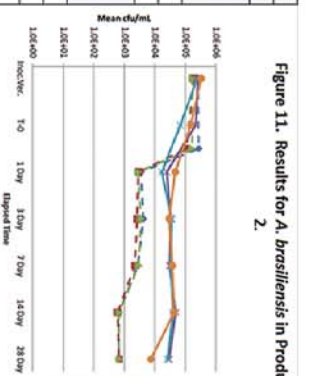


Figure 11. Results for *A. brasiliensis* in Product 2.

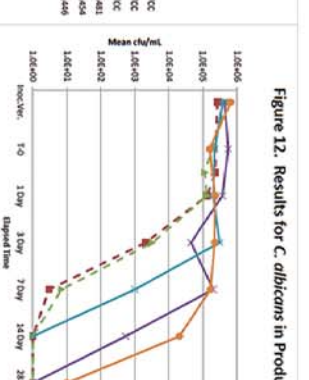


Figure 12. Results for *C. albicans* in Product 2.

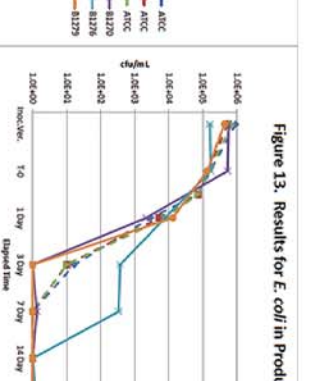


Figure 13. Results for *E. coli* in Product 2.

Comparison of Results to USP <51> Acceptance Criteria for Product 2.			
BioBall Organisms			
Organism	Log Change From Initial Count to Day 7	Log Change from Initial Count to Day 14	Log Change from Initial Count to Day 28
AB	-2.0 to -1.6	-2.6 to -2.3	-2.7 to -2.3
CA	-5.7 to -4.0	-5.7 to -5.0	-5.7 to -5.0
Organism	Log Change From Initial to Day 7	Log Change from Initial to Day 14	Log Change from Day 14 to Day 28
EC	-6.0 to -5.7	-6.0 to -5.7	0.0 to 0.0
PA	-5.4 to -4.3	-5.4 to -5.3	0.0 to 0.0
SA	-5.6 to -5.6	-5.6 to -5.6	0.0 to 0.0



Figure 14. Results for *P. aeruginosa* in Product 2.

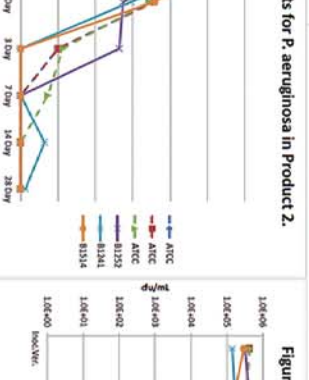


Figure 15. Results for *S. aureus* in Product 2.

Figures 11 through 15 show the results for all BioBall lots compared to the results for the ATCC cultures when inoculated into Product 2 (sterile preserved eye drops). All results show that the organisms are not capable of growing in the product. For many of the organisms there was a drop off within the first 1-3 days and then some organisms survived at very low levels before dying off. Some organisms survived the entire 28 days but never increased. For *C. albicans*, the three BioBall cultures survived in the product at reduced numbers for 14-28 days while the ATCC cultures died off more quickly (by Day 7). The reason for this difference is not known. It is possible that the BioBall *C. albicans* organism is more stable and therefore takes longer to respond to the preservative in this product. Although there were some differences seen in the rate of die off, this product meets the acceptance criteria of USP <51> testing for all organisms utilizing either BioBall or ATCC cultures.