**FOCUS Scientific Services LLC**

# ANALYTICAL REPORT: Comparison of the Microbial Recovery Efficacy of QI Medical EnviroTest Paddles versus a Conventional Contact Plate

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**Comparison of the Microbial Recovery Efficacy of QI Medical EnviroTest Paddles versus a Conventional Contact Plate**

**Abstract:**

The EnviroTest paddle is a convenient tool for recovering microbial contaminants from compounding pharmacy surfaces. The paddle shape offers advantages to conventional round contact plates in its ability to reach corners and follow the curves of door knobs, light switches and equipment controls; these are all places where either frequent personnel contact or accumulation of organic matter is known to be high. The paddle is attached to a screw cap closure which when replaced to the rigid test tube container remedies the problems of condensation migration, closure flipping and container breakage which one encounters when using conventional contact plates. However, the benefits of shape and packaging are lost if the paddle does not recover microbial contaminants from compounding pharmacy surfaces as effectively as conventional contact plates.

A pair of controlled studies were performed at a cGMP laboratory with the purpose of

1. Study #1 - Comparing each device's (EnviroTest and contact plate) ability to lift and grow dried microbes from typical compounding surfaces; and,
2. Study #2 - Evaluating the nutritive properties of each device's media component. This study was designed to stress the ability of the EnviroTest screw-cap package to maintain the nutritive agar by comparing aged EnviroTest to fresh control media.

The data generated from these studies verify that the EnviroTest paddle is as effective as conventional contact plates as an environmental monitoring tool.

| **Equivalency Test** | **Organisms** | **EnviroTest versus Contact Plate** |
|---|---|---|
| Recovery of dried inocula from compounding surface coupons by an exhaustive recovery method | Gram positive cocci | Equivalent |
| | Yeast | Equivalent |
| | Bacterial Spores | Equivalent |
| Recovery of dried spores from compounding surfaces versus a rinsed and plated sample | Bacterial Spores | Equivalent |
| Growth promotion and nutritive properties of media *Aged* EnviroTest and fresh growth media | Gram positive cocci | Equivalent |
| | Gram negative rods | Equivalent |
| | Yeast | Equivalent |
| | Mold | Equivalent |
| | Bacterial spores | Equivalent |

**Testing Summary:**

Test Species:
*Bacillus subtilis* ATCC 6633 NCTC 10400
*Staphylococcus aureus* ATCC 6538 NCTC 10788
*Pseudomonas aeruginosa* ATCC 9027 NCTC 12924
*Candida albicans* ATCC 10231 NCTC 3179
*Aspergillus brasiliensis* ATCC 16404 NCTC 2275

Test species were prepared from BioBall inoculation products, a precise quantitative inoculum patented and manufactured by Biomerieux. The BioBall product uses a flow cytometer to dispense individual cells and count them in each inoculum, resulting in a very precise number of cells. BioBall utilize NCTC cultures which are equivalent to the ATCC Strains listed above.

Devices:

1. EnviroTest Tryptic Soy Agar Paddles: contains tryptose, yeast extract, dextrose, agar, lecithin and polysorbate 80, Lot # 1556804, 1564724, 15790764
2. EnviroTest Malt Extract Agar Paddles: contains malt agar, yeast extract, dextrose, lactic acid, antibiotics, agar, lecithin, polysorbate 80, histidine, and thiosulfate Lot # 1562027
3. EMD Millipore Tryptic Soy Agar Contact Plates: contains tryptose, yeast extract, dextrose, agar, lecithin and polysorbate 80. Lot # 123051, 122852, 125136
4. EMD Millipore Malt Extract Agar Contact Plates: contains maltose, dextrin, glycerol, peptone, agar, lecithin and polysorbate 80. Lot #1331518

Surfaces used in Study #1:
Vinyl Tile Flooring Material
Borosilicate Glass
Stainless Steel

Control Media used in Study #2:

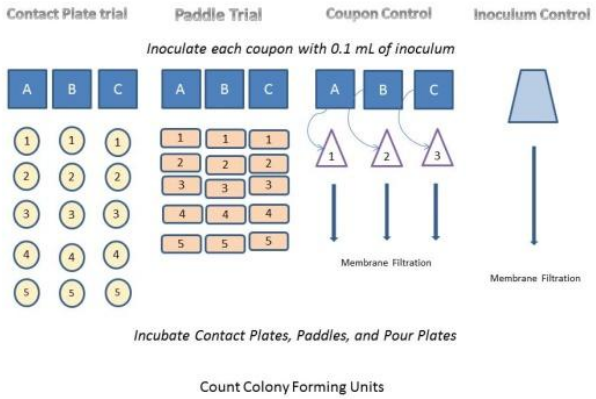
1. Tryptic Soy Agar Plus Neutralizers (TSA+) Control Plates: contains tryptose, yeast extract, dextrose, agar, lecithin, and polysorbate 80. Prepared by the testing laboratory from a commercial blend.
2. Malt Extract Agar Plus Neutralizers (MEA +) Control Plates: contains maltose, dextrin, glycerol, peptone, agar, lecithin and polysorbate 80. Prepared by the testing laboratory from a commercial blend.

## Procedure for Study #1 - Microbial Recovery from Compounding Surfaces:

1. Coupon Preparation:
    a. Materials (vinyl, glass or stainless steel) were cut to 2 x 2 inch coupons
    b. The steel and glass coupons were cleaned by rinsing with DI water and sterilized in an autoclave
    c. The vinyl coupons were cleaned by rinsing with DI water and sterilized in an ETO sterilization cycle. The coupons were allowed to aerate for 4 days prior to testing.
2. Microbial inoculation:
    a. BioBall culture preparations were removed from freezer and allowed to equilibrate at room temperature prior to use.
    b. BioBall was transferred into the rehydration fluid, the cap was replaced, and the inoculum dissolved for 30 seconds.
    c. The resulting mixture was vortexed for 5 seconds or until BioBall was completely dissolved and there was a visibly homogenous distribution.

d. 9 replicates of each coupon type were placed in 9 individual petri plates. 3 plates were labelled for Contact, 3 were labelled for EnviroTest, and 3 were labelled for Control.
e. Each of the replicate coupons was inoculated with 0.1 mL of 1.0 x $10^3$ cfu of prepared microbial suspension. The inoculum was spread evenly across the coupon, leaving approximately ¼ inch on the left and right side of the coupon free of inocula.
f. A 10 mL phosphate buffered saline blank was simultaneously inoculated with 0.1 mL of 1.0 x $10^3$ cfu.
g. Inoculated coupons were dried in a 35 – 39°C incubator with petri plate lids on. Inoculum was allowed to dry completely.

3. Enumeration:
   a. Contact Plates: Surface of coupon was pressed with a contact plate, firmly enough so that the entire surface of the agar was in contact with the coupon. Plate was held in this position for 5 seconds. Contact plate was removed and lid was replaced. Each tile was sampled an additional four times, with four fresh contact plates, so that an initial recovery percentage could be calculated.
   b. EnviroTest: Surface of coupon was pressed with a paddle and the paddle was gently rocked. The paddle was then flipped and the other side was pressed to the other half of the coupon, again slightly rocking the paddle. The paddle was removed from coupon and placed back in the tube. Each tile was sampled an additional four times, with four fresh paddles, so that an initial recovery percentage could be calculated.




   c. Control Tile: Inoculated coupon was placed in whirl pack bag with 10 mL phosphate buffered saline. The coupon was thoroughly massaged through the bag to dislodge all inoculum from coupon. The resulting phosphate buffered saline was passed through a 0.45 micron filter. Filter was placed on TSA+ for bacteria and MEA+ for fungi.
   d. Bacterial plates were incubated at 30° - 35° C for 3 days, fungal plates were incubated at 20 – 25°C for 5 days and colony forming units were counted.

4. Calculations:
   a. Percent Recovery = $\frac{\text{Sample 1 cfu}}{\text{Total cfu of Samples 1 – 5}}$ x 100

   b. Comparative recovery = $\frac{\text{Contact Device cfu}}{\text{Control Tile cfu}}$ x 100

**Study #1 Results:**

**Table 1:** Percent recovery of test species from various test surfaces as determined by exhaustive recovery

| | *Bacillus subtilis* | | *Candida albicans* | | *Staphylococcus aureus* | |
|---|---|---|---|---|---|---|
| | EnviroTest | Contact Plate | EnviroTest | Contact Plate | EnviroTest | Contact Plate |
| Vinyl | 32% | 24% | 33% | 33% | 36% | 22% |
| Glass | 61% | 42% | 94% | 88% | 71% | 43% |
| Steel | 43% | 35% | 84% | 88% | 64% | 52% |

**Table 1. Discussion:**

The exhaustive recovery data clearly demonstrate that the contact plates and EnviroTest paddles are comparative methods for determining microbial populations on surfaces, and only a fraction of the population is actually recovered from any surface using either device on the first pressing. This is an important point to remember when setting Alert and Action Levels for an environmental monitoring program, as the counts achieved are meant to be relative to one another and then trended over time, they do not represent an absolute value of microbes on a surface. The exhaustive recovery efficiency is similar on both devices across material types, with vinyl flooring showing the worse initial recovery, and the smooth surfaces of glass and steel offering better opportunity for high recovery.

**Table 2:** Percent recovery of *B. subtilis* spores from various surfaces as determined by comparison to a control tile.

| | *Bacillus subtilis* | |
|---|---|---|
| | EnviroTest | Contact Plate |
| Vinyl | 27% | 21% |
| Glass | 55% | 31% |
| Steel | 53% | 33% |

**Table 2. Discussion:**

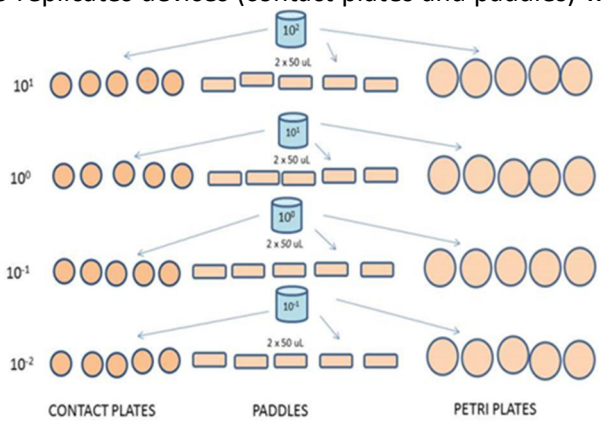
Bacillus spores are the ideal test subjects to compare counts between products: they are ubiquitous, airborne, and desiccant resistant, alleviating concern of test species viability during drying cycles. In fact, comparative counts utilizing plate count methods were attempted with vegetative cells but the results were too variable to make any conclusions. We believe the variability was due to the impact of drying on vegetative culture viability between sampling.

Regardless, when counts of a dried Bacillus inoculum recovered from a surface monitoring device are compared to those recovered by vigorous washing and rinsing, it is clear once again that not all surface contaminants are picked up by the agar press on the first pass by either device. The two devices are equivalent in their ability to recover dried spores from compounding surfaces. Repeating the trend witnessed in the exhaustive recovery trial, recovery efficacy in general is better from steel and glass

surfaces than from vinyl tile, again underscoring the importance of frequent monitoring and trending similar over time since actual recovery is a relative and not absolute number.

**Study #2 - Procedure for Comparing Nutritive Quality of Two Devices:**

1) 5 replicates devices (contact plates and paddles) were labelled for each organism and each dilution: $10^{1}$, $10^{0}$, $10^{-1}$, and $10^{-2}$. Similarly, 5 replicates of fresh TSA+ for bacteria and MEA+ for fungi.




2) Contact Plates: Contact plates were aged prior to initiation of the study; TSA plates were stored at 30°-35°C for 3 days and MEA plates were stored at 26° – 30°C for 5 days Using a sterile micropipette, two (2) 50 µl aliquots of the 1000 cfu/mL standardized culture of a test species were inoculated to the agar surface of the contact plate. Immediately after inoculating, the inoculum was aseptically spread around the entire agar surface. The lid was replaced and the process was repeated for the other 4 plates.

3) EnviroTest: EnviroTest paddles were aged prior to initiation of the study; TSA paddles were stored at 30°-35°C for 3 days and MEA paddles were stored at 26° – 30°C for 5 days.. Using a sterile micropipette, 50 µl of the 1000 cfu/mL standardized culture of a test species was inoculated to one side of the EnviroTest paddle. Immediately after inoculating, the inoculum was aseptically spread around the entire agar surface of that side of the EnviroTest paddle. 50 µl of the 1000 cfu/mL standardized culture was then inoculated to the other side of the paddle and evenly distributed across the surface. The paddle was replaced inside tube and screw cap was closed. This process was repeated for the other four EnviroTest paddles.

4) Control Plates: Using a sterile micropipette, 2 x 50 µl of the 1000 cfu/mL standardized culture was delivered to the agar surface and the inoculum was aseptically spread around the entire surface of the agar. The lid was replaced the process was repeated for the other four plates.

5) Most Probable Number Scoring and Calculation:

   a) Each plate that had growth was scored as positive, regardless of the amount of growth.

   b) Each plate that had no growth was scored as negative.

   c) The number of positive plates was totaled for each dilution.

   d) When calculating MPN values, the intent is to find a 3 dilution sequence to match to an MPN chart. If the lowest dilution resulted all in zeroes, it was discarded. If the highest dilution results all in fives, the next dilution down was used.

   e) Conversion of growth totals to MPN values were derived from FDA BAM Chart[1], reproduced in Appendix 1. MPN values were corrected for the dilution level, understanding that the first 100µl delivery is a $10^{-1}$ dilution of the original sample.

[1] See FDA Bacterial Analytical Manual discussion on the MPN method for more information on the theory of this method http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm

**Study #2 Results:**

**Figure 2: Graphic Representation of Growth Promotion by MPN**

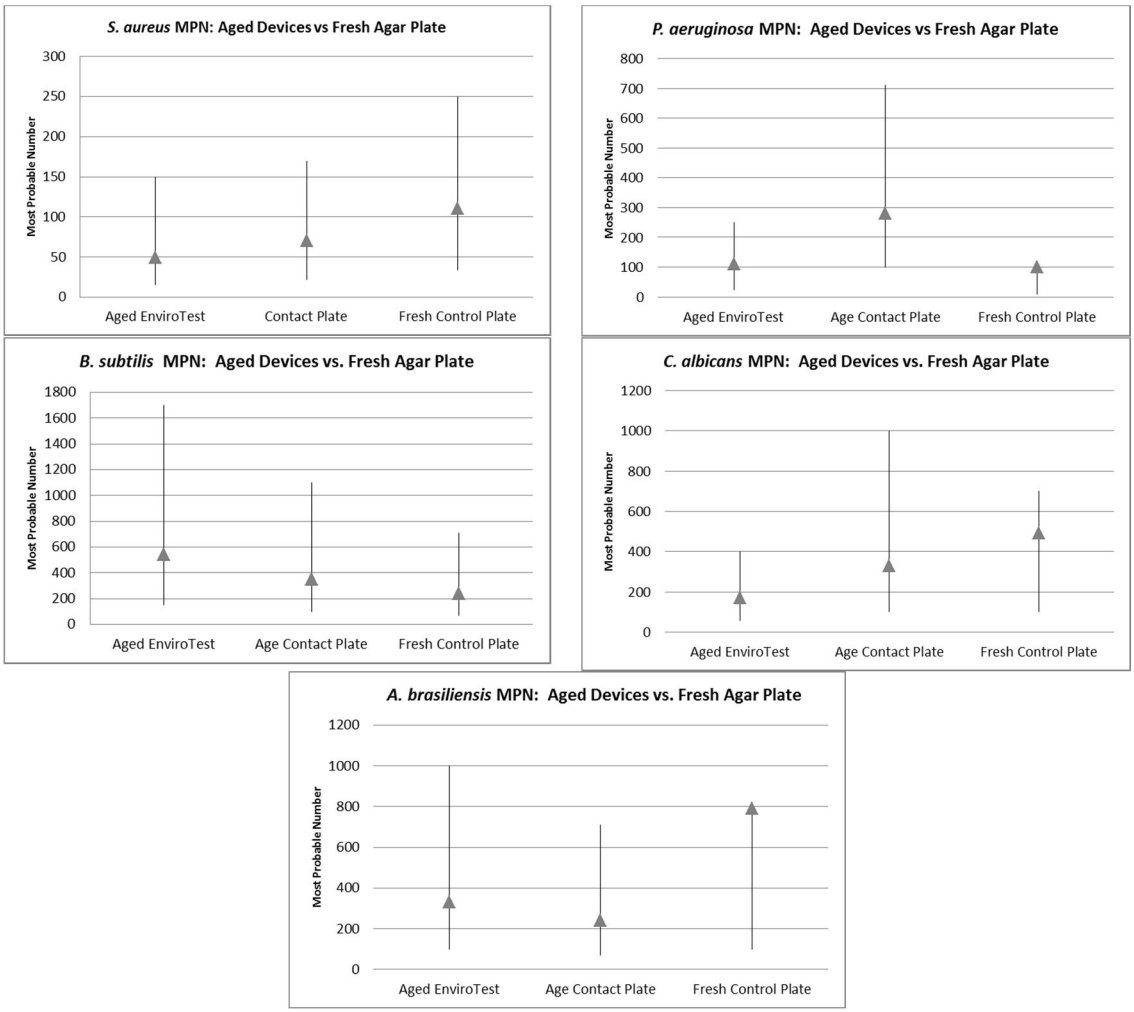



**Figure #2. Growth Promotion Discussion:**

A colony forming unit is not always a discreet unit and may not represent a single microbial cell or spore. Clumping microbes, fungal ultrastructures, microbes caught in reproduction would all present as a single colony forming unit, even though they are in fact several cells. The ability to suspend dilute quantities of single microbes in small volumes makes low level inoculation an inherently variable exercise. MPN analysis allows for a statistical calculation of high and low confidence interval for each count and offers a good alternative to standard plate count methods by giving confidence intervals in the results[1].

In our growth promotion studies, EnviroTest and contact plates MPN confidence levels overlapped each other and a fresh nutrient agar plate. While the absolute number may seem trivially higher in one or the other sampling device these differences are not statistically significant. The aged EnviroTest, Contact Plates and fresh control plates all show the same growth promotion ability.

**OVERALL CONCLUSION:**

Routine environmental monitoring is required to demonstrate that a compounding pharmacy is in a state of control. EnviroTest paddles offer a convenient method of sampling areas in the compounding pharmacy where microbes are likely to linger. The data generated from this study show that EnviroTest paddles are as effective as contact plates at recovering dried microbial test species from various compounding pharmacy surfaces and that its media is as nutritive as a standard contact plate. .

EnviroTest paddles offer convenience and unique sampling advantages for compounding pharmacy surfaces and recover microbes as efficaciously as standard contact plates.