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Effectiveness of Cultivo™ in Preventing Contamination

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Abstract

In order to test the ability of the Baker Cultivo™ CO_2 incubator to prevent contamination of the incubation space, a group of various bacteria were cultivated on a nutrient agar media and placed in a nutrient broth that was swabbed onto various surfaces of the Cultivo interior chamber to mimic a spill or splatter. The capability of the Cultivo to maintain air quality and prevent the further growth of the bacteria (and therefore, the contamination of the unit) was assessed. The bacteria chosen were an array of Gram negative and positive species.

Introduction

The overall method utilized was developed to approximate a media mishap that would typically occur in the laboratory. An unanticipated event that could contaminate the working environment, such as the bumping of a plate or flask, or a dish knocking into a shelf, is a common incident in many labs. The actual instrumental response of the Cultivo™ to such an event is critical to the prevention of contamination of the unit.

The tests outlined below include two phases. In the first phase, a challenge of exponential growth phase bacteria and fungi that would be exposed outside of the container to see if there is a spread of contamination or aspiration into the airstream of material that could cross-contaminate the cultures placed in the environment. A Cultivo Ultra model with ultrasonic humidity delivery was used for this phase. Open plates of media were used to gauge the fallout of the prepared spills and splatter locations. The plates were placed on various shelves to quantify the depth of possible exposure to contamination. In the first three configurations outlined in Phase 1 of the procedure below, the media used was nutrient agar and 5% sheep's blood agar. However, in configurations 4-8, nutrient agar was replaced with trypticase soy agar to be consistent with the use of trypticase soy broth in Phase 2.

In the second phase, a Cultivo base model with evaporative humidity delivery was used. A bacterial suspension was introduced into the water pan to test the level of contamination found there following a 15-minute ultraviolet (UV) treatment.

Definitions

ART	Ambient Room Temperature	**SBA**	Sheep's Blood Agar
BSC	Biological Safety Cabinet	**TSA**	Trypticase Soy Agar
CFUs	Colony Forming Units	**TSB**	Trypticase Soy Broth
NA	Nutrient Agar		

Materials and Equipment

	Materials	Serial / Lot #	Exp. Date	Catalogue #	Supplier
1	*Serratia marcescens* (non-pigmented)	247-30	30 Sep 2014	0247	Microbiologics, Inc. Saint Cloud, MN
2	*Serratia marcescens* (pigmented)	806-43-2	31 Oct 2014	0806	Microbiologics, Inc. Saint Cloud, MN
3	*Citrobacter freundii*	229-23	31 Dec 2014	0229	Microbiologics, Inc. Saint Cloud, MN
4	*Staphylococcus epidermidis*	412-77	31 Mar 2015	0412	Microbiologics, Inc. Saint Cloud, MN
5	*Saccharomyces cerevisiae*	699-64-6	30 Apr 2015	0699L	Microbiologics, Inc. Saint Cloud, MN
6	BD/BBL Nutrient Agar	4046299	11 Jun 2014	297801	Becton-Dickinson Sparks, MD
7	BD/BBL 5% Sheep Blood Agar	4029344	15 Apr 2014	221733	Becton-Dickinson Sparks, MD
8	BD/BBL Nutrient Agar Slants	3305841	01 May 2015	220971	Becton-Dickinson Sparks, MD
9	BD/BBL 5% Sheep Blood Agar Slants	4035336	07 Aug 2014	220830	Becton-Dickinson Sparks, MD
10	BD/BBL Trypticase Soy Agar				
11	BD/BBL Trypticase Soy Broth	3005182	05 Jul 2014	221716	Becton-Dickinson Sparks, MD
12	Puritan Sterile Cotton Swabs	3621 3141	30 Sep 2018 21 May 2017	25-806 1WC 25-806 2WC	Fisher Scientific
13	Fisher Brand Sterile Disposable Inoculating Loops	121221605	n/a	23-363-605	Fischer Scientific
14	Copan Sterile Disposable Inoculating Loops (Calibrated)	4029	31 Jan 2017	COP-S10	Fisher Scientific
15	Cultivo™ CO_2 Incubators	IncPro2 110960 110963	n/a n/a	INCPILOT	The Baker Company Sanford, ME
16	SterilGARD® e3 Class II Type A2 Biological Safety Cabinet	110347	n/a	SG404	The Baker Company Sanford, ME
17	Dometic Biomedical Refrigerator	1231747	n/a	LR 490 G	The Baker Company Sanford, ME
18	Vortex Mixer	VBC010525	n/a	MX-S	Scilogex Rocky Hill, CT
17	95% Ethanol Lab Grade			S25309C	Fisher Science Education
18	10X PBS	134361	31 Jan 2017	BP3 99-500	Fisher Scientific
16	WFI-Quality, Cell Culture Grade H_2O	25055522	30 Apr 2016	25-055	Corning Cellgro MediaTech, Inc. Manassas, VA

Procedure

Phase 1

The five bacterial species were cultivated and maintained in cultures changed every 36 to 48 hours to keep a growing and viable culture available for transfer or expansion. Cultivo™ CO_2 incubator (S/N: 110960) was the maintenance incubator in this test. Cultivo Ultra CO_2 incubator with ultrasonic humidity delivery (ID: IncPro2) was used for the procedures below. In this configuration, the UV light is situated close to the internal water reservoir used by the nebulizer. The bacterial suspension was swabbed in various agreed-upon locations inside the Cultivo, as indicated in Figure 1. The locations were selected to mimic a spill or splatter.

The procedures below were performed on the following eight configurations of contamination prevention technologies. In all cases, the Cultivo was set to 37°C, 95% relative humidity (RH) and 5.0% CO_2.

1. Test of system with no protection.
2. Test of HEPA filter alone.
3. Test of HEPA filter with UV light.
4. Test of UV light alone.
5. Test of Cu+ / Cu+ alloy (>67%) shelves & supports alone.
6. Test of Cu+ / Cu+ alloy (>67%) shelves & supports with HEPA filter.
7. Test of Cu+ / Cu+ alloy (>67%) shelves & supports with UV light.
8. Test of Cu+ / Cu+ alloy (>67%) shelves & supports with HEPA filter and UV light.

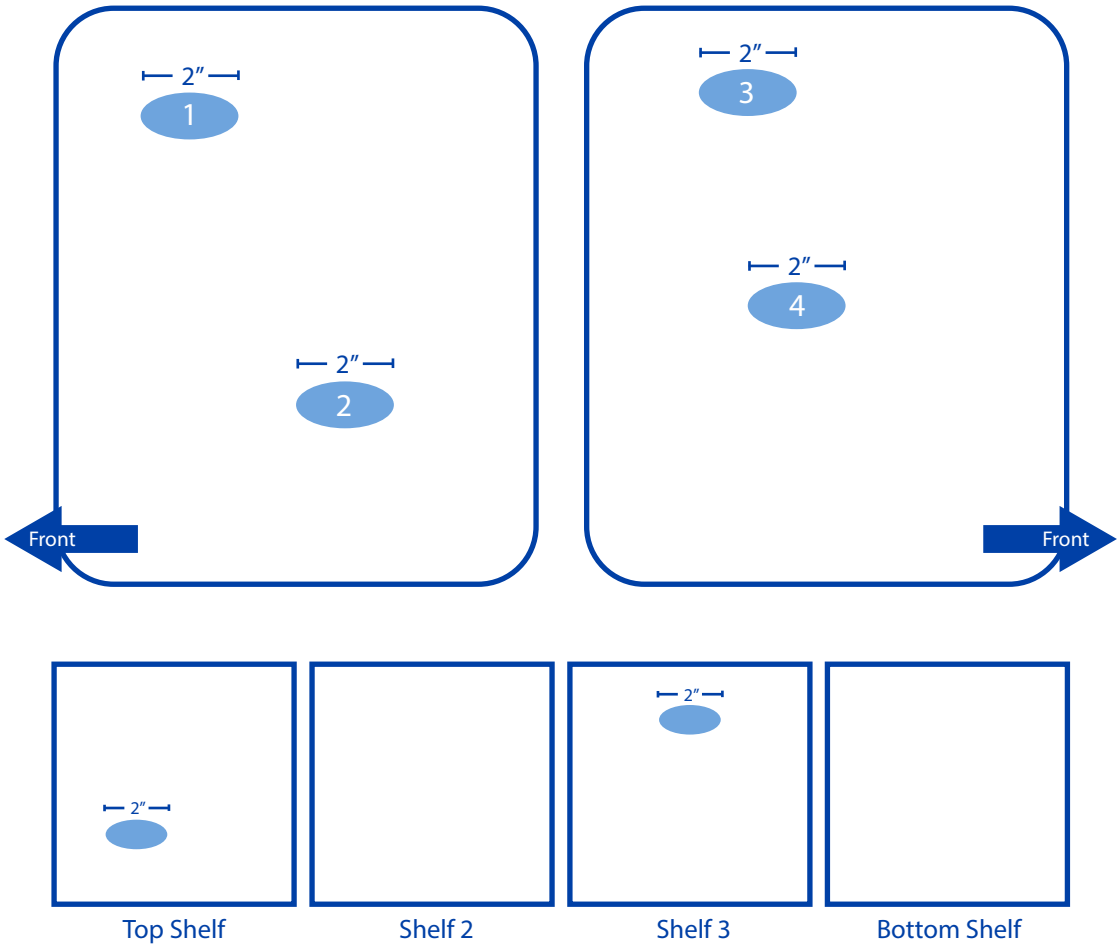

Figure 1: Position of Bacterial Suspension Swabs Inside Cultivo™

The setup for each contamination prevention test contained the following steps:

1. Plate positions within the incubator were agreed upon and marked (Figure 2) for consistency throughout all eight tests, and for comparison of the testing results.
2. The Cultivo™ chamber was allowed to come to set conditions before introduction of the bacterial challenge.
3. The plates, two TSA media and one 5% SBA, were brought to room temperature and kept in the BSC until the test was initiated.
4. The shelves and sides of the unit were cleaned with a 70% EtOH solution prepared as described below:
    a. All preparation is accomplished inside of a BSC to prevent contamination.
    b. 95% ethanol (EtOH) is diluted to 70% with WFI reagent grade water.
    c. The resulting solution is mixed and placed in a spray bottle and marked for identification.
    d. This mixture is given an expiration date of 10 days post creation. For valid use.
5. The mixture of bacteria contained all four species currently being maintained in the M-E Lab. The ¼ of a loop surface of each exponential growth culture was added to a fresh ART TSB tube (5 mL).
6. The mixture was pulse vortexed for approximately 30 seconds to disperse the growth added to the mixture.
7. The predetermined locations (Figure 1) were swabbed and the chamber was allowed to come to the set conditions again.
8. The plates were placed uncovered on the incubator shelves to approximate the area exposed to any recirculated bacteria aspirated into the airflow of the chamber (Figure 1).
9. The plates were placed on the incubator shelves on the marked locations.
10. Uncovered plates were left in the chamber for ~24 hours to approximate an inadvertent spill or splash that may go undetected for a set amount of time. The unit was allowed to run unopened during this time.
11. The plates were covered before removal. (The plates were, of course, less moist then when first introduced.)

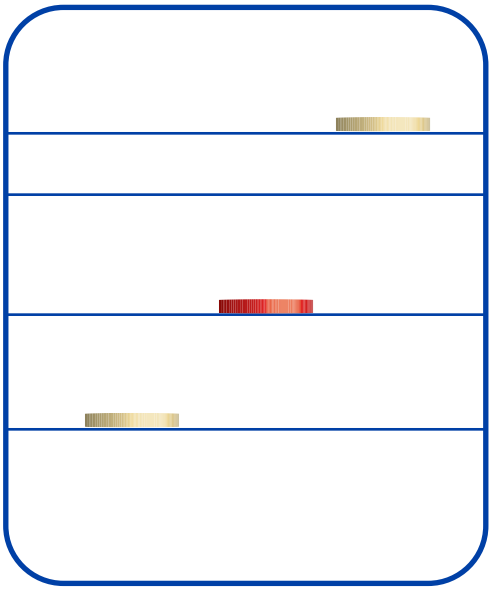

Figure 2: Positioning of Plates Inside Cultivo™

12. The swabbed areas were sampled with a fresh, clean swab moistened in PBS (125 µL).
13. All samples were moved to the BSC and further processed for growth analysis in another Cultivo CO_2 incubator used for growth maintenance.
14. The colony forming units (CFUs) were counted and the size of the colony was noted by three designations.
    a. Small: a colony that was, on average, "pinpoint" in size.
    b. Medium: a colony showing average size for the cultured norms.
    c. Large: an expansive colony spreading across the surface.
15. To test viability of a sample of recorded bacterial CFUs, if present on the dry media plates, the plates were remoistened with ~2 mL of TSB and allowed to rehydrate for a few minutes.
16. The resulting surface was swabbed and re-plated on a fresh plate of the same media to assess viability of the CFUs.
17. The swabs were used to inoculate a quadrant on a labeled plate.
18. Any growth after ~36 hours on these samples was noted and recorded.
19. The chamber was cleaned with 70% EtOH and set up for the next iteration after complete teardown of interior to clean all surfaces.

Phase 2

The Cultivo™ CO_2 incubator with evaporative humidity delivery (ID: 110963) was used for the procedure below. In this configuration, the UV light was situated above a water pan and a HEPA filter was installed.

1. The Cultivo chamber was allowed to come to set conditions before introduction of the bacterial challenge.
2. The five bacterial species were added to an ART TSB tube and vortexed for ~30 seconds by pulsing. The total volume of the mix was 5 mL, and the mix showed turbidity.
3. The 5 mL of bacterial suspension was stirred into the water pan, which contained ~8 L of Lab Grade H_2O.
4. The incubator door was closed and the unit was allowed to sit undisturbed for three hours.
5. The UV light was switched on for 15 minutes.
6. The incubator was allowed to sit undisturbed for an additional 15 minutes.
7. Next, three samples of the water were taken. For each sample, a swab was dipped ~3/4" from the water's surface and dragged through the water pan volume in a sweeping motion.
    a. An initial sample of water of was taken from the water pan after the 15-minute period in step 6.
    b. The incubator was allowed to sit undisturbed for 36 hours following the removal of the initial sample, and then a second sample was taken.
    c. The incubator was allowed to sit undisturbed for 12 hours following the removal of the second sample, and then a third sample was taken.
8. Immediately following sampling, each sample was placed in a sterile TSB tube that had been properly labeled and allowed to come to ART before use. The tube was then sealed and the lid turned ~1/4 turn to allow venting. The tube was placed inside the Cultivo incubator (ID #110960) set at 37°C, 5.0% CO_2 and 95% RH and allowed to incubate for one week. After one week, the broth inside the tube was observed for turbidity.

Results

Phase 1

Figure 3 shows the total number and sizes of CFUs formed on all plates following incubation for each of the eight contamination prevention technology configurations, as well as whether growth occurred in the samples taken from the contaminated areas of sidewalls and shelves.

Configuration	Plated Media CFUs Counted (S / M / L)	Total CFUs Counted	Sidewall / Shelf Sample Growth
No Protection	47 / 4 (M-L) / 5	~56	Growth on all surfaces
UV Light Only	~20 / 1 / 2	~23	Growth on all surfaces
HEPA Filter Only	0 / 0 / 0	0	Growth on all surfaces
HEPA Filter & UV Light	0 / 0 / 0	0	Growth on all surfaces
Cu+ Interior Components Only	>106 / 5 / 1	>112	No growth on any surface
Cu+ Interior Components & HEPA Filter	0 / 0 / 0	0	No growth on any surface
Cu+ Interior Components & UV Light	0 / 0 / 0	0	No growth on any surface
HEPA Filter, UV Light & Cu+ Interior Components	0 / 0 / 0	0	No growth on any surface

Figure 3: Phase 1 Results

Phase 2

No growth was observed in any of the three samples after one week of incubation.

Conclusion

In an incubator with evaporative humidification, an exposure of the water pan to 15 minutes of UV light may prevent the growth of contaminants there for up to 48 hours after the light is switched off, but this appears to be the limit of what a UV light can do. As shown in the incubator with ultrasonic humidification, a UV light alone is not enough to prevent the spread of contaminants on the sidewalls, shelves and media plates.

A HEPA filter is effective at preventing the spread of contaminants aspirated into the airstream, but its effects do not extend to the sidewalls and shelves. Cu+ sidewalls and shelves effectively kill contaminants that come into contact with them, but do not prevent the spread of contaminants aspirated into the airstream.

In an ultrasonic incubator system, although the combination of a HEPA filter and Cu+ interior components appear to provide effective contamination prevention, it is prudent to consider adding a UV light to prevent contamination of the water reservoir. Further testing will be conducted to assess the effects of UV light in that area. In an evaporative incubator system, because of the addition of the water pan and the risk of contaminants there, the best combination for effective protection is the use of a HEPA filter, Cu+ interior components and a UV light. CO_2 incubators that do not make use of all three of these technologies pose a serious risk of contamination to cultures, the incubator and the laboratory environment.

To learn more about Cultivo™, the CO_2 incubator from Baker, please visit bakerco.com/products/cultivo

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