

## Case Study No. 7

**CHALLENGE CONDITIONS:** *B. subtilis* spores were added to 70% IPA to be used during the fills for glove sanitization. The alcohol was liberally sprayed of operator's gloves during the fills. The concentration of spores in the alcohol after the fills was  $3.8 \times 10^5$  per ml.

**FILL CONDITIONS:** Two media fills performed during the first week of the course on October 4, 2001.

### MEDIA FILL--GROUP 2

Lot No. **100301a** (Filled in the morning)  
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	193	4
2	209	3
3	142	7
4	129*	1**
<b>Total</b>	<b>673</b>	<b>15</b>

**Incubation Conditions:** 35° ± 2° C, 28 days, inverted.

\* All vials manually crimped.

\*\* Loose seal.

Gram stain = Gram (+) rods. Organism identified as *B. subtilis*. Bulk samples and priming samples were all negative. Pre-filtration bioburden sample was negative.

#### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples Growth (+)/No Growth (-)				Gram Stain
	1	2	Avg	1	2	3	4	
<i>B. subtilis</i>	48	50	49	+	+	+	+	G + rods
<i>S. aureus</i>	31	47	39	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	24	25	25	+	+	+	+	G - rods
<i>C. albicans</i>	40	32	36	+	+	+	+	Yeast
<i>A. niger</i>	18	25	21	+	+	+	+	Mold

Final Result: **Valid Media Fill; Media Fill Fails**

### SURFACE MONITORING

Product Contact: Action Level = 1 CFU; Alert Level = None  
Class 100 Critical: Action Level = 3CFU; Alert Level = 1CFU  
Class 100, Non-critical: Action Level = 3CFU; Alert Level = 2CFU  
Class 10,000 (Floors): Action Level = 5 (10); Alert Level = 3 (5)

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Staging Shelves	TNTC <sup>1</sup>	<i>B. subtilis</i>
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	TNTC	<i>B. subtilis</i>
5S	Entry Door Handle	>15	<i>B. subtilis</i>
6	In-feed Turn Table	3	<i>B. subtilis</i>
7	In-feed Tray Loader	0	
8	Vial Pusher, handle	TNTC	<i>B. subtilis</i>
9S	Fill Nozzle Support	TNTC	<i>B. subtilis</i>
10S	Fill Nozzle	No Sample	Technician error
11	Stopper Arm	0	
12	Vial Star-wheel	TNTC	<i>B. subtilis</i>
13	Left Plexiglas Door	12	<i>B. subtilis</i>
14S	Plexiglas Door Handle	>30	<i>B. subtilis</i>
15	Control Panel, center bottom	TNTC	<i>B. subtilis</i>
16	Copper Bowl-Outside	No sample	No capping performed
17	Stopper Bowl-Outside	TNTC	<i>B. subtilis</i>
18	Stopper Bowl-Inside	No sample	
19	Plexiglas by Stopper Bowl	0	
20	Inside Curtain (left)	0	
21	Set-up Cart	7	<i>B. subtilis</i>
22	Conveyor	0	
23	Floor—in front of Filler	>14	<i>B. subtilis</i>
24	Floor—in front of stopper bowl	TNTC	<i>B. subtilis</i> plus other Bacillus species
25	Floor—in front of exit door	>20	<i>B. subtilis</i> plus other Bacillus species
26	Forceps-Trayer	0	
	Cappers	0	
	Turntable	0	
	Back of Turntable	0	
	Filler	3	<i>B. subtilis</i>

<sup>1</sup> = TNTC = Too Numerous to Count

### MEDIA FILL--GROUP 1

Lot No. **100301b** (Filled in afternoon)  
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	205	7
2	126	2, 1 bad crimp
3	207	2
4	155	3
5	79	1
<b>Total</b>	<b>772</b>	<b>15</b>

**Incubation Conditions:** 35° ± 2° C, 28 days, inverted.

\*Gram stain = Gram (+) rods. Identified as *B. subtilis*.

Bulk samples and priming samples were all negative. Pre-filtration bioburden samples were negative.

#### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples Growth (+)/No Growth (-)				Gram Stain
	1	2	Avg	1	2	3	4	
<i>B. subtilis</i>	48	50	49	+	+	+	+	G + rods
<i>S. aureus</i>	31	47	39	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	24	25	25	+	+	+	+	G - rods
<i>C. albicans</i>	40	32	36	+	+	+	+	Yeast
<i>A. niger</i>	18	25	21	+	+	+	+	Mold

Final Result: **Valid Media Fill; Media Fill Fails**

### SURFACE MONITORING

Product Contact: Action Level = 1 CFU; Alert Level = None  
Class 100 Critical: Action Level = 3CFU; Alert Level = 1CFU  
Class 100, Non-critical: Action Level = 3CFU; Alert Level = 2CFU  
Class 10,000 (Floors): Action Level = 5 (10); Alert Level = 3 (5)

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	47	<i>B. subtilis</i>
2	Freeze Dryer Door-1	1	<i>B. subtilis</i>
3	Freeze Dryer Door-2	1	<i>B. subtilis</i>
4	Pass-Thru Door	0	
5S	Entry Door Handle	4	<i>B. subtilis</i>
6	In-feed Turn Table	1	Gram (+) cocci
7	In-feed Tray Loader	8	<i>B. subtilis</i>
8	Vial Pusher	12	<i>B. subtilis</i>
9S	Fill Nozzle Support	0	
10S	Fill Nozzle	0	
11	Stopper Arm	0	
12	Vial Star-wheel	TNTC <sup>1</sup>	Mold ?
13	Left Plexiglas Door (Class 10,000 side)	0	
14S	Plexiglas Door Handle	TNTC	<i>B. subtilis</i>
15	Control Panel	1	
16	Copper Bowl-Outside	0	
17	Stopper Bowl-Outside	3	Mold?
18	Stopper Bowl-Inside	1	<i>B. subtilis</i>
19	Plexiglas by Stopper Bowl	14	<i>B. subtilis</i>
20	Inside Curtain (left)	1	
21	Set-up Cart	1	
22	Conveyor	4	<i>B. subtilis</i>
23	Floor—in front of Filler	21	<i>B. subtilis</i>
24	Floor—in front of stopper bowl	5	
25	Floor—in front of exit door	1	

<sup>1</sup> = TNTC = Too Numerous to Count

### VIABLE AIR MONITORING

Class 100: Action Level = 0.1/ cu. ft.; Alert Level = 0.05/ cu. ft.  
Class 10,000: Action Level = 0.5/ cu. ft.; Alert Level = 0.25/ cu. ft.

Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (by bag set-up) (10:10)	SMA	60 cu. ft.	0	< 0.017/ cu. ft.
In-feed Turn Table (11:25)	SMA	60 cu. ft.	2*	0.03/ cu. ft.
Trayer (11:25)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Between Stopper and Cap-per bowls (11:25)	SMA	60 cu. ft.	12*	0.2/ cu. ft.
Pass-through (11:09)	RCS	400L	1*	0.07/ cu. ft.
Lyo 1 (10:32)	RCS	400L	0	< 0.07/ cu. ft.
Staging Rack (10:41)	RCS	400L	0	< 0.07/ cu. ft.
In-feed Turn Table (12:30)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Trayer (12:30)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Between Stopper and Cap-per bowls (12:30)	SMA	60 cu. ft.	1*	0.017/ cu. ft.
Pass-through (12:45)	RCS	400L	2*	0.14/ cu. ft.
Lyo 1 (12:35)	RCS	400L	12*	0.84/ cu. ft.

\* Isolates all *B. subtilis*

### PERSONNEL MONITORING

FIPs: Action Level = 3; Alert Level = 2  
Apparel: Action Level (bold)= 5; Alert Level = 4  
Total Count > 10 CFU is an Action Level

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
TG-10:03	27	42	NS <sup>2</sup>	NS	NS
KR-10:35	0	0	NS	NS	NS
KR-10:45	0	0	1	0	0
JS-10:57	TNTC <sup>1</sup>	TNTC	0	0	0
JS-11:05	0	0	NS	NS	NS
CH-11:13	0	0	NS	NS	NS
TG-11:40	5	6	NS	NS	NS
JS-11:43	TNTC	TNTC	4	TNTC	TNTC
JL-11:45	23	>26	NS	NS	NS
LS-11:56	0	0	NS	NS	NS
JL-11:59	>39	>30	NS	NS	NS
LS-12:02	0	0	NS	NS	NS
PS-12:20	TNTC	TNTC	>10	>60	>10
CH-12:30	TNTC	TNTC	>100	>65	TNTC
TG-12:37	TNTC	TNTC	6	18	18
JM-12:44	1	4	NS	NS	NS
DH-12:57	>30	25	NS	NS	NS
LS-13:01	TNTC	TNTC	8	>38	>10
JL-13:10	20	30	1	1	1
JM-13:36	6	1	31	22	4
DH-13:39	14	18	TNTC	7	0

<sup>1</sup> TNTC = Too Numerous to Count

<sup>2</sup> NS = Not Sampled

#### IDENTIFICATION OF PERSONNEL ISOLATES

All action level excursions were *B. subtilis*

### VIABLE AIR MONITORING

Class 100: Action Level = 0.1/ cu. ft.; Alert Level = 0.05/ cu. ft.  
Class 10,000: Action Level = 0.5/ cu. ft.; Alert Level = 0.25/ cu. ft.

Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (by bag set-up) (Time?)	SMA	47 cu. ft.	0	<0.017/ cu. ft.
In-feed Turn Table (?)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Trayer (?)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Stopper Bowl (?)	SMA	60 cu. ft.	5	0.09/ cu. ft.
Pass-through (?)	RCS	400 L	28	1.96/ cu. ft.
Staging Rack (?)	RCS	400 L	1	0.07/ cu. ft.
Lyophilizer 1 (?)	RCS	400 L	55	3.85/ cu. ft.
In-feed Turn Table (?)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Trayer (?)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Stopper Bowl (?)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Pass-through (?)	RCS	400 L	4	0.28/ cu. ft.
Staging Rack (?)	RCS	400 L	31	2.17/ cu. ft.

All recovered isolates were *B. subtilis*.

### PERSONNEL MONITORING

FIPs: Action Level = 3; Alert Level = 2  
Apparel: Action Level (bold)= 5; Alert Level = 4  
Total Count > 10 CFU is an Action Level

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
JF-15:21	>70	>120	NS <sup>1</sup>	NS	NS
JF-15:30	0	0	NS	NS	NS
ST-15:41	TNTC <sup>2</sup>	TNTC	1	1	5
JF-15:48	1	9	0	0	0
MS-16:18	TNTC	TNTC	NS	NS	NS
TR-16:20	0	0	NS	NS	NS
MS-16:24	0	1	NS	NS	NS
KAF-16:58	0	1	0	0	0
MS-17:35	TNTC	TNTC	44	TNTC	TNTC
TR-17:36	TNTC	TNTC	17	3	1
JL-17:38	12	18	1	1	0
KS-18:06	25	25	TNTC	2	0
AA-18:07	15	3	18	3	78

<sup>1</sup> = NS = Not Sampled—sampled gloves only

<sup>2</sup> = TNTC = Too Numerous to Count

#### IDENTIFICATION OF PERSONNEL ISOLATES

All Action Limit excursions were *B. subtilis*.

## Follow-up to Case Study No. 7

**CHALLENGE CONDITIONS:** None.

**FILL CONDITIONS:** One product fill and one media fill performed during the second week of the course November 8, 2001--same students as October 4 session.

### PRODUCT FILL--GROUP 1

Lot No. . 051101-aMGZ69 (Filled in the morning)  
STERILITY TEST RESULTS (Data obtained from UMBC)

Tray Number	Number Evaluated	Number Positive
1	8	
2	8	
3	8	
<b>Total</b>	<b>24</b>	<b>No Growth</b>

**Test Conditions:** All vials reconstituted, pooled and filtered through a Steri-Test System into two canisters. One canister filled with Fluid Thioglycollate Medium, the other with Tryptic Soy Broth (TSB).

**Incubation Conditions:** Thio incubated at 32 $\pm$  2 $^{\circ}$  C, 14 days; TSB incubated at 22 $\pm$  2 $^{\circ}$  C, 14 days.

Bulk sterility tests on bulk samples were negative.

### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts			MF Test Samples				Gram Stain
	1	2	Avg	1	2	3	4	
<i>Cl. sporogenes</i> (Thio)	30	36	33	+	+	+	+	G + rods
<i>S. aureus</i> (Thio)	45	54	50	+	+	+	+	G + cocci
<i>P. aeruginosa</i> (Thio)	36	51	43	+	+	+	+	G - rods
<i>B. subtilis</i> (TSB)	78	75	77	+	+	+	+	G + rods
<i>C. albicans</i> (TSB)	40	52	46	+	+	+	+	Yeast
<i>A. niger</i> (TSB)	51	63	57	+	+	+	+	Mold

**Final Result:** Valid Sterility Test; Product Sterility Test Passes

### SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	0	
2	Freeze Dryer Door-1	2	Gram (+) rods-spores
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	0	
5S	Entry Door Handle	1	Gram (+) rods-spores
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher	0	
9S	Fill Nozzle Support	0	
10S	Fill Nozzle	0	
11	Stopper Arm	0	
12	Vial Star-wheel	0	
13	Left Plexiglas Door	0	
14S	Plexiglas Door Handle	0	
15	Control Panel	0	
16	Copper Bowl-Outside	0	
17	Stopper Bowl-Outside	Not sampled	
18	Stopper Bowl-Inside	Not sampled	
19	Plexiglas by Stopper Bowl	0	
20	Inside Curtain (left)	0	
21	Set-up Cart	0	
22	Conveyor	0	
23	Floor--in front of Filler	0	
24	Floor--in front of stopper bowl	0	
25	Floor--in front of exit door	0	
26	Interior Rt. door to fill cabinet	0	
27	Right Plexiglas door, Class 100 side	0	
28	Forceps-turntable front	0	
29	Forceps-turntable back	0	
30	Forceps-Air location 4	0	
31	Forceps-stopper bowl	0	
32	Forceps-trayer	0	
33	Forceps-fill cabinet	Not sampled	
34	Gowning Bench	0	Gram (-) Rod
35	RCS key pad	0	
36	Alcohol bottle	0	
37S	Exit door handle	0	
38	Screw driver blade	0	
39	Screw driver handle	0	

### MEDIA FILL--GROUP 2

Lot No. 110401-MF (Filled in afternoon)  
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	207	0
2	205 (+ 2 empty vials)	0
3	207	0
4	207	0
5	207	0
6	208	0
7	135	0
<b>Total</b>	<b>1376</b>	<b>0</b>

**Incubation Conditions:** 35 $\pm$  2 $^{\circ}$  C, 14 days, inverted.  
Bulk samples and priming samples were all negative.

### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts			MF Test Samples				Gram Stain
	1	2	Avg	1	2	3	4	
<i>B. subtilis</i>	21	14	17	+	+	+	+	G + rods
<i>S. aureus</i>	32	40	36	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	48	52	50	+	+	+	+	G - rods
<i>C. albicans</i>	35	37	36	+	+	+	+	Yeast
<i>A. niger</i>	30	26	28	+	+	+	+	Mold

**Final Result:** Valid Media Fill; Media Fill Passes

### SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	0	
5S	Entry Door Handle	0	
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher	0	
9S	Fill Nozzle Support	0	
10S	Fill Nozzle	0	
11	Stopper Arm	0	
12	Vial Star-wheel	0	
13	Left Plexiglas Door (Class 10,000)	1	Mold
14S	Plexiglas Door Handle	0	
15	Control Panel	0	
16	Copper Bowl-Outside	0	
17	Stopper Bowl-Outside	0	
18	Stopper Bowl-Inside	0	
19	Plexiglas by Stopper Bowl	0	
20	Inside Curtain (left)	0	
21	Set-up Cart	0	
22	Conveyor	0	
23	Floor--in front of Filler	0	
24	Floor--in front of stopper bowl	1	Gram (+) cocci
25	Floor--in front of exit door	0	
26	Interior Rt. door to fill cabinet	0	
27	Right Plexiglas door, Class 100 side	0	
28S	Forceps-turntable front	0	
29	Forceps-turntable back	0	
30	Forceps-Air location 4	0	
31	Forceps-stopper bowl	0	
32	Forceps-trayer	0	
33	Forceps-fill cabinet	0	
34	Gowning Bench	0	
35	RCS key pad	0	
36	Alcohol bottle	0	
37S	Exit door handle	0	
38	Screw driver blade	0	
39	Screw driver handle	0	

### VIABLE AIR MONITORING

Set-up of Fill Samples				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (by bag set-up)	SMA	60 cu. ft.	0	< 0.017/cu. ft.

During the Fill Samples				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
In-feed Turn Table	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Pass through	RCS+	1000 liters	0	< 0.07/cu. ft.
Staging rack	RCS+	1000 liters	0	< 0.07/cu. ft.
Lyophilizer 2	RCS+	1000 liters	0	< 0.07/cu. ft.
Gowning room	R2S	60 cu. ft.	0	< 0.017/cu. ft.
Filler Deck	Setting plate	60 min.	0	--

### PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
JL	0	0	NS	NS	NS
JM	0	0	NS	NS	NS
JM	0	0	0	0	0
DH	0	0	NS	NS	NS
DH	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
DS	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
JM	0	0	0	0	1*
DS	0	0	0	0	1*
DH	0	0	NS	NS	NS
JL	0	0	0	0	0
PS	0	0	0	0	0
JS	0	0	0	0	2*

NS = Not Sampled  
\* = All Gram (+) cocci

### VIABLE AIR MONITORING

Set-up and Beginning of Fill Samples				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (by bag set-up)	SMA	60 cu. ft.	0	< 0.017/cu. ft.

During the Fill Samples				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
In-feed Turn Table	SMA	60 cu. ft.	0	< 0.017/cu. ft.
In-feed Turn Table	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Pass through	RCS+	400L	0	< 0.07/cu. ft.
Staging rack	RCS+	400L	0	< 0.07/cu. ft.
Staging rack	RCS+	400L	0	< 0.07/cu. ft.
Lyophilizer 1	RCS+	400L	0	< 0.07/cu. ft.
Lyophilizer 1	RCS+	400L	0	< 0.07/cu. ft.
Pass through	RCS+	400L	0	< 0.07/cu. ft.
Filler Deck	Setting Plate	1 hour (13:55)	0	NA*
Filler Deck	Setting Plate	1 hour (14:55)	0	NA*
Filler Deck	Setting Plate	1 hour (16:00)	0	NA*

\*NA = Not Applicable

### PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Right	Left	Right	Left	
ST	0	0	NS	NS	NS
TR	0	0	NS	NS	NS
MS	0	0	NS	NS	NS
TR	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
ST	0	0	NS	NS	NS
MS	0	0	NS	NS	NS
TR	0	0	NS	NS	NS
TR	0	0	NS	NS	NS
ST	0	0	NS	NS	NS
AA	1	0	0	0	0
MS	0	0	0	0	0
TR	0	0	0	1 (mold)	0
JL	0	0	0	0	0
DW	0	0	3 (mold)	0	1 (Gram + cocci)
CM	0	1	0	0	0

NS = Not Sampled--sampled gloves only

## Case Study No. 8

**CHALLENGE CONDITIONS:** *B. subtilis* spores were added to 70% IPA to be used during the fills for glove sanitization. The alcohol was liberally sprayed of operator's gloves during the fills. The concentration of spores in the alcohol after the fills was  $2.3 \times 10^2$  per ml.

**FILL CONDITIONS:** Two media fills performed during the first week of the course on January 17, 2002.

### MEDIA FILL--GROUP 2 Lot No. 010702-2 (Filled in the morning) MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	214	0
2	211	0
3	149	0
4	208	0
5	82	0
BG	24	0
<b>Total</b>	<b>888</b>	<b>0</b>

Incubation Conditions: 35° ± 2° C, 28 days, inverted.  
Bulk samples and priming samples were all negative. Pre-filtration bioburden sample was negative.

#### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	Growth (+)/No Growth (-)				
<i>B. subtilis</i>	22	32	27	+	+	+	+	G + rods
<i>S. aureus</i>	38	35	36.5	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	8	9	8.5	+	+	+	+	G - rods
<i>C. albicans</i>	94	92	93	+	+	+	+	Yeast
<i>A. niger</i>	65	61	63	+	+	+	+	Mold

Final Result: **Valid Media Fill; Media Fill Passes**

### SURFACE MONITORING

Product Contact: Action Level = 1 CFU; Alert Level = None  
Class 100 Critical: Action Level = 3CFU; Alert Level = 1CFU  
Class 100, Non-critical: Action Level = 3CFU; Alert Level = 2CFU  
Class 10,000 (Floors): Action Level = 5 (10); Alert Level = 3 (5)

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Staging Shelves	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	0	
5S	Entry Door Handle	4	Mold**
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher, handle	0	
9S	Fill Nozzle Support	TNTC*	Mold**
10S	Fill Nozzle	2	Mold**
11S	Stopper Arm	TNTC	Mold**
12	Vial Star-wheel	0	
13	Left Plexiglas Door	0	
14S	Plexiglas Door Handle	TNTC	Mold**
15	Control Panel, center bottom	0	
16	Copper Bowl-Outside	0	
17	Stopper Bowl-Outside	0	
18	Stopper Bowl-Inside	NS	
19	Plexiglas by Stopper Bowl	0	
20	Inside Curtain (left)	0	
21	Set-up Cart	1	
22	Conveyor	0	
23	Floor--in front of Filler	0	
24	Floor--in front of stopper bowl	0	
25	Floor--in front of exit door	0	
26	Forceps--vial loading	0	
27	Forceps--filler	0	
28	Forceps--stopper bowl	0	
29	Forceps--tray-loader	0	

\* TNTC = Too Numerous to Count  
\*\* Mold--suspected laboratory (incubator) contamination

### MEDIA FILL--GROUP 1 Lot No. 010702-1 MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	206	0
2	218	0
3	192	0
4	206	0
5	139	0
<b>Total</b>	<b>961</b>	<b>0</b>

Incubation Conditions: 35° ± 2° C, 28 days, inverted.

Bulk samples and priming samples were all negative. Pre-filtration bioburden samples were negative.

#### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	Growth (+)/No Growth (-)				
<i>B. subtilis</i>	22	32	27	+	+	+	+	G + rods
<i>S. aureus</i>	38	35	36.5	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	8	9	8.5	+	+	+	+	G - rods
<i>C. albicans</i>	94	92	93	+	+	+	+	Yeast
<i>A. niger</i>	65	61	63	+	+	+	+	Mold

Final Result: **Valid Media Fill; Media Fill Passes**

### SURFACE MONITORING

Product Contact: Action Level = 1 CFU; Alert Level = None  
Class 100 Critical: Action Level = 3CFU; Alert Level = 1CFU  
Class 100, Non-critical: Action Level = 3CFU; Alert Level = 2CFU  
Class 10,000 (Floors): Action Level = 5 (10); Alert Level = 3 (5)

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	0	
5S	Entry Door Handle	0	
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher	0	
9S	Fill Nozzle Support	0	
10S	Fill Nozzle	0	
11	Stopper Arm	0	
12	Vial Star-wheel	0	
13	Left Plexiglas Door (Class 10,000 side)	0	
14S	Plexiglas Door Handle	0	
15	Control Panel	0	
16	Copper Bowl-Outside	0	
17	Stopper Bowl-Outside	0	
18	Stopper Bowl-Inside	0	
19	Plexiglas by Stopper Bowl	0	
20	Inside Curtain (left)	0	
21	Set-up Cart	0	
22	Conveyor	0	
23	Floor--in front of Filler	23*	
24	Floor--in front of stopper bowl	0	
25	Floor--in front of exit door	0	
26	Procedural control	0	
27	Control	0	
28	Trayer forceps	0	
29	Filler forceps	0	
30	Vial tray	10*	Intentionally inoculated
31	Stopper Forceps	0	
32	Turntable forceps	0	
33	Set-up forceps	0	
34S	Filler forceps	0	
35S	Procedural Control	0	

\* *B. subtilis*

### VIABLE AIR MONITORING

Class 100: Action Level = 0.1/ cu. ft.; Alert Level = 0.05/ cu. ft.  
Class 10,000: Action Level = 0.5/ cu. ft.; Alert Level = 0.25/ cu. ft.

Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (by bag set-up) (9:41)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
In-feed Turn Table (9:47)	SMA	60 cu. ft.	0	0.03/ cu. ft.
Trayer (9:47)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Between Stopper and Capper bowls (9:47)	SMA	60 cu. ft.	0	0.2/ cu. ft.
Filler	MAS	2 m <sup>3</sup>	0	
Pass-through	RCS	400L	0	0.07/ cu. ft.
Lyo 1	RCS	400L	0	< 0.07/ cu. ft.
Staging Rack	RCS	400L	0	0.28/ cu. ft.
Outside door	R <sub>2</sub> S	60 cu. ft.	5	0.083 / cu. ft.
Outside door	R <sub>2</sub> S	60 cu. ft.	0	<0.017/ cu. ft.
Gowning Area	R <sub>2</sub> S	60 cu. ft.	0	<0.017/ cu. ft.

<sup>1</sup> TNTC = Too Numerous to Count  
<sup>2</sup> = Exceeds Action Limit  
<sup>3</sup> = Exceeds Alert Level

### PERSONNEL MONITORING

FIPs: Action Level = 3; Alert Level = 2  
Apparel: Action Level = 5; Alert Level = 4  
Total Count > 10 CFU is an Action Level

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
JC (10:15)	0	0	1*	0	1*
PJ (10:25)	0	0	NS	NS	NS
JL (11:10)	0	0	NS	NS	NS
LW (11:20)	0	0	1*	4	TNTC*
SK (11:45)	0	0	NS	NS	NS
JL (12:00)	0	0	NS	NS	NS
SK (12:07)	1**	2**	NS	NS	NS
CL (12:10)	0	0	NS	NS	NS
LH (12:17)	3**	2**	0	0	0
PJ (12:18)	0	0	0	0	0
SK (12:20)	0	0	0	0	9
JL (12:45)	0	0	2	0	0
CL (12:55)	0	0	0	0	26
MJ (12:58)	0	0	0	0	0

Additional Samples: JC (10:15)—Chest under head—8 cfu's; JL (12:45)—Above eyes—17 cfu's, Stomach—8 cfu's, L Leg—10 cfu's, R Leg—4 cfu's

NS = Not Sampled  
\* Mold--suspected laboratory (incubator) contamination  
\*\* *B. subtilis*

### VIABLE AIR MONITORING

Class 100: Action Level = 0.1/ cu. ft.; Alert Level = 0.05/ cu. ft.  
Class 10,000: Action Level = 0.5/ cu. ft.; Alert Level = 0.25/ cu. ft.

Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (by bag set-up) (14:32)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
In-feed Turn Table (14:41)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Trayer (14:43)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Stopper Bowl (14:45)	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Pass-through (15:15)	RCS	400 L	0	<0.07/ cu. ft.
Staging Rack (15:30)	RCS	400 L	0	<0.07/ cu. ft.

### PERSONNEL MONITORING

FIPs: Action Level = 3; Alert Level = 2  
Apparel: Action Level = 5; Alert Level = 4  
Total Count > 10 CFU is an Action Level

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
Kinn (2:49)	0	0	NS <sup>1</sup>	NS	NS
Thomas (3:03)	0	0	NS	NS	NS
Kinn (3:34)	0	0	1	1	0
Kinn (3:36)	0	0	0	0	0
Roy (3:56)	6*	3*	0	0	0
Thomas (3:58)	0	0	0	1	3
Anil (4:01)	0	0	NS	NS	NS
John (4:18)	1*	0	0	0	0
Anil (4:20)	0	0	NS	NS	NS
Ilias (4:37)	3*	1	NS	NS	NS
Ilias (4:43)	0	0	0	0	0
Anil (4:55)	0	5*	0	0	0
Ilias (4:55)	0	1	0	1	0
John (4:59)	3*	4*	0	0	0
Steve (5:32)	1	0	0	0	0
Scott (5:33)	0	0	0	0	0

<sup>1</sup> = NS = Not Sampled—sampled gloves only  
\* *B. subtilis*; All other isolates were Gram (+) cocci.



Aseptic Solutions Inc.