

## Case Study No. 1

**CHALLENGE CONDITIONS:** ~10<sup>3</sup> *B. subtilis* spores in 70% IPA inoculated onto the pass-thru door, the entry door handle and the stoppering arm.

**FILL CONDITIONS:** Two media fills performed during the first week of the course on May 18, 2000.

### MEDIA FILL--GROUP 2 Lot No. 051800-02 (Filled in the morning) MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	204	0
2	204	0
3	204	0
<b>Total</b>	<b>612</b>	<b>0</b>

Incubation Conditions: 35° ± 2° C, 14 days, inverted.

#### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	Growth (+)/No Growth (-)				
<i>B. subtilis</i>	35	53	44	+	+	+	+	G + rods
<i>S. aureus</i>	97	94	96	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	70	72	71	+	+	+	+	G - rods
<i>C. albicans</i>	13	14	14	+	+	+	+	Yeast
<i>A. niger</i>	65	53	59	+	+	+	+	Mold

Final Result: Valid Media Fill; Media Fill Passes

### NON-VIABLE AIR MONITORING

Sample Location	Time	Number of Particulates > 0.5 microns per cu. ft.				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Avg. Count
In-feed Trayer	9:15	9	51	6	1	16.8
In-feed Trayer	9:21	63	12	3	3	20.3
Stopper Bowl	9:43	51	54	74	110	61.3
Stopper Bowl	9:48	49	27	36	177*	37.3 (first 3 counts)
Fill Line	8:58	25	33	28	43	32.3
Fill Line	10:15	42	45	32	31	37.5
Fill Line	10:32	47	12	19	34	28
Fill Line	10:50	28	26	21	16	22.8
Fill Line	10:57	31	32	13	32	27
Fill Line	11:14	28	27	21	26	25.5
Fill Line	11:23	31	23	26	16	24

\* Cleaning Conveyor

### SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Table Top	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	18	Bacillus species
5	Entry Door Handle	0	
6S	In-feed Turn Table	0	
7S	In-feed Tray Loader	0	
8S	Conveyor Belt	0	
9	Fill Nozzle Support	0	
10	Fill Nozzle	0	
11	Inside Stopper Bowl (inside panel)	NA	Not sampled due to fill scheduled in afternoon
12	Inside Stopper Bowl (outside panel)	NA	Not sampled due to fill scheduled in afternoon
13	Stopper Arm	0	
14	Vial Star-wheel	13	2-3 colony forms, all Bacillus species
15	Control Panel	0	
16	Tray Vial Pusher	0	
17	Capper Bowl-Outside	0	
18	Plexiglas Door Handle	0	
19	Left Plexiglas Door	0	
20	Inside Curtain (left)	0	
21	Floor--in front of Filler	0	
22	Floor--in front of stopper bowl	0	
23	Floor--in front of exit door	6	Bacillus species
24	Between Curtains	1	Bacillus species

### MEDIA FILL--GROUP 1 Lot No. 051800-01 (Filled in the afternoon) MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
FD-1	204	0
FD-2	204	0
FD-3	204	0
4	204	0
5	204	0
6	204	0
7	110	0
8	110	0
<b>Total</b>	<b>891</b>	<b>0</b>

Incubation Conditions: 35° ± 2° C, 14 days, upright, stoppered but no caps.

#### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	Growth (+)/No Growth (-)				
<i>B. subtilis</i>	35	53	44	+	+	+	+	G + rods
<i>S. aureus</i>	97	94	96	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	70	72	71	+	+	+	+	G - rods
<i>C. albicans</i>	13	14	14	+	+	+	+	Yeast
<i>A. niger</i>	65	53	59	+	+	+	+	Mold

Final Result: Valid Media Fill; Media Fill Passes

### NON-VIABLE AIR MONITORING

Sample Location	Time	Number of Particulates > 0.5 microns per cu. ft.				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Avg. Count
In-feed Trayer	13:35	12	94	62	24	48
Stopper Bowl	14:26	62	40	33	147*	70.5
Top of Out-feed	12:38	9	1	396*	21	10.3 (Avg. of 3 counts)
Capper	12:29	90	152	9	91	85.5
Capper	14:17	454	82	78	26	160
Fill Line	13:28	27	30	37	33	31.8
Fill Line	14:33	24	25	31	22	25.5
Fill Line	14:39	19	27	17	31	23.5
Fill Line	14:44	17	28	22	24	22.8
Fill Line	14:49	26	24	23	23	24
Lyophilizer	15:07	2051*	8514*	6174*	4366*	5276.3
Lyophilizer	15:14	304*	757*	968*	1326*	763.6
Out-feed Trayer	13:49	140*	94	260*	59*	138.3

\* No notes or explanation for the high counts.

### SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Table Top	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	8	Bacillus, Gram + cocci
5	Entry Door Handle	0	
6S	In-feed Turn Table	0	
7S	In-feed Tray Loader	0	
8S	Conveyor Belt	0	
9	Fill Nozzle Support	0	
10	Fill Nozzle	0	
11	Inside Stopper Bowl (inside panel)	0	
12	Inside Stopper Bowl (outside panel)	0	
13	Stopper Arm	3	Bacillus
14	Vial Star-wheel	0	
15	Control Panel	1	
16	Tray Vial Pusher	1	Gram + cocci
17	Capper Bowl-Outside	0	
18	Inside Curtain (left)	2	Gram + cocci
19	Plexiglas Door Handle	0	
20	Left Plexiglas Door	0	
21	Floor--in front of Filler	2	Bacillus
22	Floor--in front of stopper bowl	0	
23	Floor--in front of Exit Door	5	Bacillus

### 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.
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Supply Table	RCS+	200 liters	0	< 0.14/cu. ft.

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.
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Supply Table	RCS+	200 liters	0	< 0.14/cu. ft.

End of Fill Samples				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (other side)	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
In-feed Turn Table (other side)	RCS	200 liters	0	< 0.14/cu. ft.

### PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest		Thigh		Back of Neck		Rt. Elbow	Goggles	Mask
	Rt	Lf	Rt	Lf	Rt	Lf	Rt	Lf	Rt	Lf			
AH	0	0	0	2	4	0	3	NS*	NS	NS	NS	NS	NS
PB	0	0	1	0	12	0	0	0	0	NS	NS	NS	NS
LC	0	0	0	1	0	0	0	NS	NS	NS	NS	NS	NS
JB	0	0	0	0	1	NS	NS	NS	NS	NS	NS	NS	NS
AR	0	0	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS
AH-2	0	0	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS
PB-2	0	0	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS
BB	0	0	0	1	0	0	0	0	0	NS	NS	NS	NS
LA	0	0	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS
JDL	0	0	0	0	3	NS	NS	NS	NS	NS	NS	NS	NS
EL	0	0	0	1	0	NS	NS	NS	NS	NS	NS	NS	NS
JL	0	0	0	0	0	0	0	NS	NS	0, 0, 0	0	0	0

\*NS = Not Sampled

#### IDENTIFICATION OF PERSONNEL ISOLATES

AH: Chest—Gram + cocci, diphtheroids; Thigh—Gram + cocci; Left Sleeve--  
PB: Chest—Gram + cocci, Bacillus; Rt. Sleeve—Gram + cocci  
LC: Left Sleeve—Gram + cocci  
BB: Left Sleeve—Bacillus  
EL: Left Sleeve—Gram + cocci  
JB: Chest—Gram + cocci  
AH: Sleeve—Gram + cocci, Bacillus  
JDL: Chest—Gram + cocci

### VIABLE AIR MONITORING

Samples Taken at 15:00				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Turn Table (bag side)	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	< 0.017/cu. ft.
In-feed Turn Table (other side)	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.

Samples Taken at 16:40 to 17:00				
Sample Location	Sampler Type	Volume of Air Sampled	CFU per Sample	CFU per Volume of Air
In-feed Trayer	SMA	20 cu. ft.	0	< 0.05/cu. ft.
Stopper Bowl	SMA	20 cu. ft.	0	< 0.05/cu. ft.
Out-feed Trayer	SMA	20 cu. ft.	0	< 0.05/cu. ft.

### PERSONNEL MONITORING

Initials	FIPS		Sleeves		Chest	Goggles	Mask
	Right	Left	Right	Left			
1	0	0	2	0	0	NS*	NS
2	0	0	0	0	0	NS	NS
3	0	0	0	0	0	NS	NS
4	0	0	0	0	1	NS	NS
5	0	0	0	0	11	NS	NS
6	0	0	0	0	46	NS	NS
7	1	0	0	0	1	NS	NS
8	0	0	0	1	1	NS	68
9	0	0	1	0	2	NS	0
10	0	0	0	0	0	NS	0
11	0	0	0	0	0	NS	1
12	0	0	0	0	0	0, 0	2

\*NS = Not Sampled

#### IDENTIFICATION OF PERSONNEL ISOLATES

1: Right Sleeve—Gram + cocci  
4: Chest—Gram + cocci  
5: Chest—Gram = cocci, Bacillus  
6: Chest—Gram + cocci, some Bacillus  
7: Right FIP—Bacillus  
8: Chest—1 Bacillus, mask—Gram + cocci  
9: Left Sleeve and Chest—Gram + cocci  
10: Right Sleeve and Chest—Gram + cocci  
11: Mask—Gram + cocci  
12: Mask—Gram + cocci



## Case Study No. 2

**CHALLENGE CONDITIONS:** ~10<sup>4</sup> *B. subtilis* spores in 70% IPA inoculated onto the pass-thru door, the entry door handle and the fill nozzle support and the vial turret.

**FILL CONDITIONS:** Two media fills performed during the first week of the course on August 24, 2000.

### MEDIA FILL--GROUP 1

Lot No. 082400-02 (Filled in the afternoon)  
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	208	0
2	208	0
3	201	0
<b>Total</b>	<b>617</b>	<b>0</b>

Incubation Conditions: 35° ± 2° C, 14 days, inverted.

### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	Growth (+)/No Growth (-)				
<i>B. subtilis</i>	35	53	44	+	+	+	+	G + rods
<i>S. aureus</i>	97	94	96	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	70	72	71	+	+	+	+	G - rods
<i>C. albicans</i>	13	14	14	+	+	+	+	Yeast
<i>A. niger</i>	65	53	59	+	+	+	+	Mold

Final Result: Valid Media Fill; Media Fill Fails

### NON-VIABLE AIR MONITORING

Sample Location	Time	Number of Particulates > 0.5 microns per cu. ft.				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Avg. Count
In-feed Trayer	9:15	24	21	3	5	14
In-feed Trayer	9:21	0	6	0	2	2
Stopper Bowl	9:43	123	254	89	58	131
Stopper Bowl	9:48	56	43	68	72	60
Fill Line	8:58	0	0	0	0	0
Fill Line	10:15	0	12	5	9	7
Fill Line	10:32	0	0	0	0	0
Fill Line	10:50	2	0	0	1	1
Fill Line	10:57	4	0	0	0	1
Fill Line	11:14	16	2	12	6	9
Fill Line	11:23	0	2	0	0	1

### SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Table Top	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	TNTC**	G (+) cocci
5	Entry Door Handle	0	
6S	In-feed Turn Table	0	
7S	In-feed Tray Loader	0	
8S	Conveyor Belt	0	
9	Fill Nozzle Support	TNTC**	G (+) cocci
10	Fill Nozzle	0	
11	Inside Stopper Bowl (inside panel)	0	
12	Inside Stopper Bowl (outside panel)	0	
13	Stopper Arm	0	
14	Vial Star-wheel	4 <sup>1</sup>	2-3 colony forms, G (+) cocci & rods with spores
15	Control Panel	2	G (+) cocci
16	Tray Vial Pusher	0	
17	Capper Bowl-Outside	0	
18	Plexiglas Door Handle	0	
19	Left Plexiglas Door	0	
20	Inside Curtain (left)	6 <sup>1</sup>	G (+) rods-spores
21	Floor—in front of Filler	0	
22	Floor—in front of stopper bowl	0	
23	Floor—in front of exit door	3	G (+) rods-spores

\*\* TNTC = Too Numerous to Count  
1= Exceeds Action Limit

### MEDIA FILL--GROUP 2

Lot No. 082400-01 (Filled in the morning)  
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	205	0
2	207	0
3	238	0
4	260	0
<b>Total</b>	<b>910</b>	<b>0</b>

Incubation Conditions: 35° ± 2° C, 14 days, upright, stoppered but no caps.

### MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	Growth (+)/No Growth (-)				
<i>B. subtilis</i>	35	53	44	+	+	+	+	G + rods
<i>S. aureus</i>	97	94	96	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	70	72	71	+	+	+	+	G - rods
<i>C. albicans</i>	13	14	14	+	+	+	+	Yeast
<i>A. niger</i>	65	53	59	+	+	+	+	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
1	Table Top	0	
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	7	Gram + rods-spores
5	Entry Door Handle	2	Gram + cocci
6S	In-feed Turn Table	0	
7S	In-feed Tray Loader	0	
8S	Conveyor Belt	0	
9	Fill Nozzle Support	TNTC**	Gram + rods-spores
10	Fill Nozzle	0	
11	Inside Stopper Bowl (inside panel)	0	
12	Inside Stopper Bowl (outside panel)	0	
13	Stopper Arm	0	
14	Vial Star-wheel	12 <sup>1</sup>	Gram + rods-spores & Gram + cocci
15	Control Panel	0	
16	Tray Vial Pusher	6 <sup>1</sup>	Gram + rods-spores & Gram + cocci
17	Capper Bowl-Outside	0	
18	Inside Curtain (by tray loader)	0	
19	Plexiglas Door Handle	0	
20	Left Plexiglas Door	0	
21	Floor—in front of Filler	2	Gram + rods-spores
22	Floor—in front of stopper bowl	0	
23	Floor—in front of Exit Door	0	
24	Forceps	0	

\*\* TNTC = Too Numerous to Count  
1= Exceeds Action Limit

### 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.
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.
Supply Table	RCS+	400 liters	0	< 0.07/cu. ft.

End 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.
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.
Supply Table	RCS+	400 liters	0	< 0.07/cu. ft.

\*\* NOTE: All data invalid—see raw data sheets.

### PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
UB	0	0	5 <sup>1</sup>	0	0
PM	0	0	1	0	0
JC	1	0	0	0	0
DB	5 <sup>1</sup>	0	0	4 <sup>2</sup>	2
SP	*	*	3	6 <sup>1</sup>	4 <sup>2</sup>
JC-2	0	0	4 <sup>2</sup>	2	0
FK	0	0	0	17 <sup>1</sup>	2
MC	0	1	0	0	3
DO	7 <sup>1</sup>	0	0	0	0
PH	0	12 <sup>1</sup>	1	0	4 <sup>2</sup>
JL	0	0	0	0	2

\* NOTE: Person removed gloves before monitoring;  
1= Exceeds Action Limit;  
2= Exceeds Alert Level

### IDENTIFICATION OF PERSONNEL ISOLATES

UB: Right Sleeve—G(+) cocci  
DB: Left FIP—Gram (+) cocci, Left Sleeve—Gram + cocci  
SP: Left Sleeve—Gram + cocci & G (+) rods-spores; chest: G (+) rods-spores  
JC2: Right Sleeve—Gram (+) rods-spores  
FK: Left Sleeve—Gram (+) cocci  
DO: Right FIPs—Gram (+) cocci & G (+) rods-spores  
PH: Left FIPs—Gram (+) cocci & G (+) rods-spores

### VIABLE AIR MONITORING

NOTE: Unable to locate plates from incubator.

### NON-VIABLE AIR MONITORING

Sample Location	Time	Number of Particulates > 0.5 microns per cu. ft.				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Avg. Count
In-feed Trayer	13:35	12	94	62	24	48
Stopper Bowl	14:26	62	40	33	147*	70.5
Top of Out-feed	12:38	9	1	396*	21	10.3 (Avg. of 3 counts)
Capper	12:29	90	152	9	91	85.5
Capper	14:17	454	82	78	26	160
Fill Line	13:28	27	30	37	33	31.8
Fill Line	14:33	24	25	31	22	25.5
Fill Line	14:39	19	27	17	31	23.5
Fill Line	14:44	17	28	22	24	22.8
Fill Line	14:49	26	24	23	23	24
Lyophilizer	15:07	2051*	8514*	6174*	4366*	5276.3
Lyophilizer	15:14	304*	757*	988*	1326*	763.6
Out-feed Trayer	13:49	140*	94	260*	59*	138.3

\*No notes or explanation for high counts.

### PERSONNEL MONITORING

Initials	FIPS		Sleeves		Chest
	Right	Left	Right	Left	
Nick	0	0	NA	NA	NA
Fred	0	0	NA	NA	NA
John	0	0	NA	NA	NA
Anna	0	0	0	1	4 <sup>2</sup>
Wendell	0	0	NA	NA	NA
Wendell-2	0	3 <sup>1</sup>	0	12 <sup>1</sup>	6 <sup>1</sup>
Nick-2	7 <sup>1</sup>	0	0	0	3
Fred-2	1	5 <sup>1</sup>	0	1	0
Bill	0	11 <sup>1</sup>	0	0	4 <sup>2</sup>
John	1	0	0	0	1

NA= Not Applicable—sampled gloves only  
1= Exceeds Action Limit  
2= Exceeds Alert Level

### IDENTIFICATION OF PERSONNEL ISOLATES

Wendell-2: Left FIPs—Gram + cocci; Left Sleeve—Gram + rods-spores & G (+) cocci  
Nick-2: Right FIPs—Gram + cocci  
Fred-2: Left FIPs—Gram + cocci & Gram + rods—no spores  
Bill: Left FIPs—Gram + cocci, mixed; Chest—Gram + cocci, Gram + rods-spores