Case Study No. 4

CHALLENGE CONDITIONS: *B. subtilis* spores added to 70% for use to sanitize hands in the filling room. Final concentration ~10^4/ml. Alcohol was liberally used during both fills.

FILL CONDITIONS: Two media fills performed during the first week of the course on March 29, 2001.

MEDIA FILL--**GROUP 1**
Lot No. **. 032901-1 (Filled in morning)**
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	121 (10 not capped)	1
2	207 (1 not capped)	0
3	63 (6 not capped)	0
4	23 (8 not capped)	1
5	201 (6 not capped)	1
Total	**615**	**3***

Incubation Conditions: 35° ± 2° C, 21 days, inverted.
*All isolates were large Gram + rods with some spores. All positive vials were from capped vials.
Bulk samples and priming samples were all negative.

MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's) 1	2	Avg	MF Test Samples Growth (+)/No Growth (-) 1	2	3	4	Gram Stain
B. subtilis	97	85	91	+	+	+	+	G + rods
S. aureus	34	25	30	+	+	+	+	G + cocci
P. aeruginosa	28	21	25	+	+	+	+	G - rods
C. albicans	81	97	89	+	+	+	+	Yeast
A. niger	91	94	93	+	+	+	+	Mold

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

SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	25[2]	G (+) rods-spores
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	1	G (+) rods-spores
5S	Entry Door Handle	24[2]	G (+) rods-spores
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher	TNTC[1, 2]	G (+) rods-spores
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	Capper 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	5[2]	G (+) rods-spores
23	Floor--in front of Filler	0	
24	Floor--in front of stop-per bowl	0	
25	Floor—in front of exit door	TNTC[2]	G (+) rods-spores

1 = TNTC = Too Numerous to Count
2= Exceeds Action Limit

MEDIA FILL--**GROUP 1**
Lot No. **032901-02 (Filled in afternoon)**
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	199	0
2	199	1*
3	160	1*
Total	**558**	**2***

Incubation Conditions: *35° ± 2° C, 21 days, upright, stoppered but no caps.*
** Large Gram + rods, few spores.*

Bulk samples and priming samples were all negative.

MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's) 1	2	Avg	MF Test Samples Growth (+)/No Growth (-) 1	2	3	4	Gram Stain
B. subtilis	97	85	91	+	+	+	+	G + rods
S. aureus	34	25	30	+	+	+	+	G + cocci
P. aeruginosa	28	21	25	+	+	+	+	G - rods
C. albicans	81	97	89	+	+	+	+	Yeast
A. niger	91	94	93	+	+	+	+	Mold

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

SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	TNTC[2]	G (+) rods-spores
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	0	
5S	Entry Door Handle	TNTC[1, 2]	G (+) rods-spores
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher	TNTC[2]	G (+) rods-spores
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	TNTC[2]	G (+) rods-spores
15	Control Panel	0	
16	Capper 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	0	
22	Conveyor	5[2]	G (+) rods-spores
23	Floor--in front of Filler	0	
24	Floor--in front of stop-per bowl	0	
25	Floor—in front of exit door	TNTC[2]	G (+) rods-spores
26	Vial Slider (bottom)	0	
27	RCS+ body	TNTC[2]	G (+) rods-spores

1 = TNTC = Too Numerous to Count
2= 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.
In-feed Turn Table	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Filler Deck	Settling Plate	Approx. 1 hr.	2*	NA

** Media spilled in settling plate. Recovered organisms = Gram + rods.*

End of 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.
Supply Table	RCS+	1000 liters	0	<0.07/cu. ft.
Filler Deck	Settling Plate	Approx. 2 ½ hrs.	3*	NA

** Recovered organisms = Gram + rods.*

PERSONNEL MONITORING

Initials	FIPs Rt	FIPs Lf	Sleeves Rt	Sleeves Lf	Chest
JP	1	0	1	0	0
DJM	0	0	NS	NS	NS
BVDD	0	0	0	0	3
DJM	5[1]	0	NS	NS	NS
DAS	0	0	0	0	1
KJen	0	0	NS	NS	NS
DJM	0	0	NS	NS	NS
DJM	0	0	0	0	0
CKK	0	0	NS	NS	NS
CM	0	0	NS	NS	NS
TF	3[1]	0	0	0	0
CKK	0	1	NS	NS	NS
KJen	0	1	0	0	0
CAM	0	0	3	17[1]	0
CB	0	0	NS	NS	NS
CKK	0	0	0	0	0
JL	0	0	NS	NS	NS
KM	2[2]	0	0	0	0
CB	0	0	0	0	0
TF	0	0	0	0	0
JL	0	0	0	0	0

1= Exceed Action Limit
2= Exceeds Alert Level

IDENTIFICATION OF PERSONNEL ISOLATES

JP: Rt. FIP = G(+) rods—spores; Rt. Sleeve = G(+) cocci
BVDD: Chest = G(+) cocci
DJM: Rt. FIP = G(+) rods—spores
DAS: Chest = G(+) rods—spores
TF: Rt. FIP = G(+) rods--spores
CKK: Lft. FIP = G(+) rods—spores
Kjen: Lft. FIP = G(+) rods—spores
CAM: Rt. Sleeve = G(+) cocci & G(+) rods—spores; Lft. Sleeve = G(+) cocci
KM: Rt. FIP = G(+) rods--spores

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.
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.

** Media spilled in settling plate. Recovered organisms = Gram + rods.*

End of 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.
Supply Table	RCS+	1000 liters	0	<0.07/cu.ft.
Supply Table	RCS+	1000 liters	6**	0.17/cu.ft.

** Recovered organisms = Gram + rods.*
*** Exceeds Action Level*

PERSONNEL MONITORING

Initials	FIPS Right	FIPS Left	Sleeves Right	Sleeves Left	Chest
MC	0	0	0	0	0
SS	1	0	0	0	1
CC	0	0	NS	NS	NS
MG	0	0	0	0	0
MJG	0	0	NS	NS	NS
CC	0	2[2]	NS	NS	NS
MJG	2[2]	1	NS	NS	NS
CC	0	0	NS	NS	NS
MJG	0	0	0	0	1
MG	0	0	NS	NS	NS
KV	16[1]	25[1]	0	0	0
CC	0	0	0	0	0
JL	0	0	0	0	0
DN	1	1	0	0	0
GF	0	0	0	0	1

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

IDENTIFICATION OF PERSONNEL ISOLATES

SS: Rt. FIP = G(+) rods—spores; Chest = G(+) coccobacillus in tetrads
CC: Lft. FIP = G(+) rods—spores
MJG: Rt. & Lft. FIP = G(+) rods—spores; Chest = G(+) rods, no spores (Diphtheroids)
KV: Rt. & Lft. FIP = G(+) rods—spores
DN: Lft. FIP = G(+) rods—spores; Rt. FIP = G(+) rods, no spores (Diphtheroids)
GF: Chest = G(+) cocci

Follow-up to Case Study No. 4

CHALLENGE CONDITIONS: None

FILL CONDITIONS: One product fill and one media fill performed during the second week of the course April 26, 2001--same students as March 29 session.

PRODUCT FILL--**GROUP 1**
Lot No. . 0426001-αMGZ69 (Filled in morning)
STERILITY TEST RESULTS

Tray Number	Number Evaluated	Number Positive
1	8	
2	8	
3	8	
Total	**24**	**No Growth**

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

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.

End of 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.
Supply Table	RCS+	1000 liters	0	<0.07/cu. ft.
Gowning Bench	R2S	60 cu. ft.	0	< 0.017/cu. ft.
Gowning Bench	R2S	60 cu. ft.	0	< 0.017/cu. ft.
Gowning Bench	R2S	60 cu. ft.	3	0.05/ cu. ft.
Gowning Bench	R2S	60 cu. ft.	5	0.08/cu. ft.
Gowning Bench	R2S	60 cu. ft.	0	< 0.017/cu. ft.

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	0	
14S	Plexiglas Door Handle	0	
15	Control Panel	0	
16	Capper 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 stop-per bowl	0	
25	Floor—in front of exit door	0	
26	Forceps-unknown set	0	
27	Forceps-turntable	0	
28	Forceps-stopper bowl	0	
29	Forceps-filler	0	

PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
KM	0	0	NS	NS	NS
JP	0	0	NS	NS	NS
CKK	0	0	NS	NS	NS
DJM	0	0	0	0	0
CM	0	0	NS	NS	NS
KM	0	0	NS	NS	NS
KJEN	0	0	NS	NS	NS
CM	0	0	NS	NS	NS
CM	0	0	NS	NS	NS
KJEN	0	0	NS	NS	NS
CM	0	0	NS	NS	NS
CM	0	0	NS	NS	NS
KACM	0	0	NS	NS	NS
KJEN	0	0	NS	NS	NS
JL	0	0	0	0	0
TF	0	0	0	0	0
KJEN	0	0	0	0	0
CM	0	0	0	0	0
TF	0	0	0	0	0
DS	0	0	0	0	0
KH	0	0	0	0	0

NS = Not Sampled

IDENTIFICATION OF PERSONNEL ISOLATES

No Isolates!

MEDIA FILL--**GROUP 2**
Lot No. 0426001-MF (Filled in afternoon)
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	213	0
2	208	0
3	155	0
4	207	0
Total	**783**	**0**

Incubation Conditions: *$35^{o} \pm 2^{o}$ C, 14 days, inverted.*

Bulk samples and priming samples were all negative.

MEDIA GROWTH SUPPORT TEST

Control Counts				MF Test Samples				
Organism	**(CFU's)**			**Growth (+)/No Growth (-)**				**Gram Stain**
	1	**2**	**Avg**	**1**	**2**	**3**	**4**	
B. subtilis	97	85	91	+	+	+	+	G + rods
S. aureus	34	25	30	+	+	+	+	G + cocci
P. aeruginosa	28	21	25	+	+	+	+	G - rods
C. albicans	81	97	89	+	+	+	+	Yeast
A. niger	91	94	93	+	+	+	+	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	0	
14S	Plexiglas Door Handle	0	
15	Control Panel	0	
16	Capper 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	0	
25	Floor—in front of exit door	0	
JL	Under Turret	0	
Forceps	Out-feed	0	
	Stopper bowl	0	
	Aseptic Connection	0	
	Turntable	0	

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.

End of 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.
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.
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+	400L	0	< 0.07/cu. ft.
Staging rack	RCS+	400L	0	< 0.07/cu. ft.
Lyophilizer 2	RCS+	400L	0	< 0.07/cu. ft.
Pass through	RCS+	400L	0	< 0.07/cu. ft.
Staging rack	RCS+	400L	0	< 0.07/cu. ft.
Lyophilizer 2	RCS+	400L	0	< 0.07/cu. ft.

PERSONNEL MONITORING

Initials	FIPS		Sleeves		Chest
	Right	Left	Right	Left	
JL	0	0	NS	NS	NS
KV	0	0	0	0	3
MG	0	0	NS	NS	NS
MC	0	0	0	0	0
GF	0	0	0	0	0
CC	0		NS	NS	NS
TM	0	0	0	0	0
SS	0	0	0	0	0
MJG	0	0	NS	NS	NS
DN	0	0	0	0	0
MG	0	0	0	0	0
MJG	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
MG	0	1[1]	0	0	0
MJG	0	0	0	0	0
JL	0	0	NS	NS	NS
MJG	0	0	NS	NS	NS
SS	0	0	0	0	0
MJG	0	0	NS	NS	NS
CC	0	0	NS	NS	NS
MJG	0	0	NS	NS	NS
CC	0	0	0	0	0
MJG	0	0	0	0	0
JL	0	0	0	0	0

NS= Not Sampled—sampled gloves only

1 = Gram (+) cocci

Case Study No. 5

CHALLENGE CONDITIONS: *B. subtilis* spores added to 70% for use to sanitize hands in the filling room. Final concentration ~10^4/ml. Alcohol was liberally used during both fills.

FILL CONDITIONS: Two media fills performed during the first week of the course on May 17, 2001.

MEDIA FILL--GROUP 1
Lot No. **051701-1**
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	207	24
2	204	15
3	205	7
4	38	4
Total	**654**	**50***

Incubation Conditions: *35° ± 2° C, 28 days, inverted, stoppered but not capped for 24 hours. There were several leaking vials observed when inspected after 24 hours. All vials were then capped, inverted and incubated .*
** Gram positive rods with spores—B. subtilis; Gram Positive cocci. All vials with Gram (+) cocci observed at final reading had significantly less media than other vials, suggesting that they had been vials which had leaked during the first 24 hours of incubation. Bulk samples and priming samples were all negative. Pre-filtration bioburden sample was positive—Gram positive rods with spores. Several vials leaked within 24 hours of filling.*

MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples Growth (+)/No Growth (-)				Gram Stain
	1	2	Avg	1	2	3	4	
B. subtilis	97	85	91	+	+	+	+	G + rods
S. aureus	34	25	30	+	+	+	+	G + cocci
P. aeruginosa	28	21	25	+	+	+	+	G - rods
C. albicans	81	97	89	+	+	+	+	Yeast
A. niger	91	94	93	+	+	+	+	Mold

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

SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Staging Shelves	TNTC[1, 2]	*B. subtilis*
2	Freeze Dryer Door-1	1[3]	*B. subtilis*
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	0	
5S	Entry Door Handle	TNTC[1, 2]	*B. subtilis*
6	In-feed Turn Table	0	
7	In-feed Tray Loader	0	
8	Vial Pusher, handle	TNTC[1, 2]	*B. subtilis*
9S	Fill Nozzle Support	0	
10S	Fill Nozzle	0	
11	Stopper Arm	0	
12	Vial Star-wheel	8[2]	*B. subtilis*
13	Left Plexiglas Door	0	
14S	Plexiglas Door Handle	3[3]	*B. subtilis*
15	Control Panel, center bottom	1	*B. subtilis*
16	Capper 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	1	*B. subtilis*
22	Conveyor	1[3]	*B. subtilis*
23	Floor--in front of Filler	1	*B. subtilis*
24	Floor--in front of stop-per bowl	1	*B. subtilis*
25	Floor—in front of exit door	5[3]	*B. subtilis*
26	Forceps	0	

1 = TNTC = Too Numerous to Count
2 = Exceeds Action Limit
3 = Exceeds Alert Level

IDENTIFICATION OF PERSONNEL ISOLATES

MM: Left sleeve & chest—G (+) cocci
MIM: Left sleeve & chest—G (+) cocci
NG: Chest—G (+) cocci
All other Bacillus, including all TNTC counts from FIP samples—speciated B. subtilis

VIABLE AIR MONITORING

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.
In-feed Turn Table	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Trayer	SMA	60 cu. ft.	0	<0.017/ cu. ft.
Between Stopper and Cap-per bowls	SMA	60 cu. ft.	1*	0.01/ cu. ft.

** Recovered organisms = Gram + rods, with spores (B. subtilis).*

PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
IA	TNTC [1, 2]	TNTC[2]	NS	NS	NS
MM	1	0	NS	NS	NS
CE	0	4[2]	NS	NS	NS
MIM	1	10[2]	2	5[2]	11[2]
IA	TNTC[2]	TNTC[2]	NS	NS	NS
NG	TNTC[2]	TNTC[2]	0	4[3]	7[2]
MM	17[2]	10[2]	1	1	10[2]
CE	20[2]	21[2]	0	0	1
JL	8[2]	6[2]	0	0	0
IA	0	4[2]	0	TNTC[2]	1

1 TNTC = Too Numerous to Count
2 = Exceed Action Limit
3 = Exceeds Alert Level

MEDIA FILL--GROUP 2
Lot No. 051701-02
Date Filled: May 17, 2001
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	203	1*
2	184	4*
3	206	2*
Total	**593**	**7***

Incubation Conditions: 35° ± 2° C, 28 days, inverted.
* Large Gram + rods, few spores—*B. subtilis*

Bulk samples and priming samples were all negative. Pre-filtration bioburden sample was positive—Gram positive rods with spores.

MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples Growth (+)/No Growth (-)				Gram Stain
	1	2	Avg	1	2	3	4	
B. subtilis	97	85	91	+	+	+	+	G + rods
S. aureus	34	25	30	+	+	+	+	G + cocci
P. aeruginosa	28	21	25	+	+	+	+	G - rods
C. albicans	81	97	89	+	+	+	+	Yeast
A. niger	91	94	93	+	+	+	+	Mold

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

SURFACE MONITORING

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1S	Staging Shelves	7[2]	*B. subtilis*
2	Freeze Dryer Door-1	3[2]	*B. subtilis*
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	TNTC[1, 2]	*B. subtilis*
5S	Entry Door Handle	2	*B. subtilis*
6	In-feed Turn Table	0	
7	In-feed Tray Loader	3[2]	*B. subtilis*
8	Vial Pusher	18[2]	*B. subtilis*
9S	Fill Nozzle Support	0	
10S	Fill Nozzle	0	
11	Stopper Arm	0	
12	Vial Star-wheel	1[3]	*B. subtilis*
13	Left Plexiglas Door (Class 10,000 side)	2	*B. subtilis*
14S	Plexiglas Door Handle	3[3]	*B. subtilis*
15	Control Panel	1	*B. subtilis*
16	Capper Bowl-Outside	0	
17	Stopper Bowl-Outside	0	
18	Stopper Bowl-Inside	Not Sampled	Not Sampled
19	Plexiglas by Stopper Bowl	0	
20	Inside Curtain (left)	0	
21	Set-up Cart	3[3]	*B. subtilis*
22	Conveyor	0	
23	Floor--in front of Filler	3	*B. subtilis*
24	Floor--in front of stop-per bowl	3	*B. subtilis*
25	Floor—in front of exit door	3	*B. subtilis*

1 = TNTC = Too Numerous to Count
2= Exceeds Action Limit
3 = Exceeds Alert Level

VIABLE AIR MONITORING

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.	No Sample	
In-feed Turn Table	SMA	60 cu. ft.	1*	0.01/ cu. ft.
Trayer	SMA	60 cu. ft.	1*	0.01/ cu. ft.
Stopper Bowl	SMA	60 cu. ft.	0	<0.017/ cu. ft.

*Recovered organisms = Gram + rods with spores (*B. subtilis).*

PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
GC	TNTC[1, 2]	TNTC[2]	NS[1]	NS	NS
MM	1	0	NS	NS	NS
DB	5[2]	0	NS	NS	NS
MM	TNTC[2]	TNTC[2]	NS	NS	NS
JC	TNTC[2]	TNTC[2]	NS	NS	NS
MW	TNTC[2]	TNTC[2]	2	10[2]	2
MM	17[2]	16[2]	NS	NS	NS
JC	0	0	NS	NS	NS
DB	TNTC[2]	TNTC[2]	2	0	5[2]
MM	4[2]	2[3]	8[2]	0	7[2]
JC	0	0	0	0	2
WH	0	0	NS	NS	NS
JL	17[2]	21[2]	3	2	1
WH	TNTC[2]	TNTC[2]	TNTC[2]	18[2]	1
CS	TNTC[2]	TNTC[2]	4	0	6[2]
DB	3[2]	2[3]	8[2]	3	1

1 = TNTC =Too Numerous to Count; NS= Not Sampled—sampled gloves only
2 = Exceeds Action Limit
3 = Exceeds Alert Level

IDENTIFICATION OF PERSONNEL ISOLATES

GC (12:46): Right FIP—*B. subtilis*
MW (1:45): Right FIP & left sleeve—*B. subtilis*; chest—G (+) cocci
DB (2:10): Chest—*B. subtilis* & G (+) cocci; Left sleeve—*B. subtilis*
MM (2:11): Left sleeve—*B. subtilis*; chest—G (+) cocci
JC (2:12): Chest—Yellow G (+) cocci
JL (3:48): Right and left sleeve, chest—*B. subtilis*; chest & Rt. sleeve—G (+) cocci
WH (4:17): Chest, left & right sleeve—*B. subtilis*
CS (4:20): Chest & left sleeve—*B. subtilis*
DB (4:21): Left & Right sleeve—*B. subtilis*; chest—G (+) cocci

NOTE: All TNTC counts from FIP samples were *B. subtilis.*

Aseptic Solutions Inc.

Follow-up to Case Study No. 5

CHALLENGE CONDITIONS: Inoculated fill nozzle block and stopper bowl with ~10^4 *B. subtilis* spores.

FILL CONDITIONS: One product fill and one media fill performed during the second week of the course June 21, 2001--same students as May 17 session.

PRODUCT FILL--**GROUP 1**

Lot No. 0621001-αMGZ69 (Filled in the morning)
STERILITY TEST RESULTS (Data obtained from UMBC)

Tray Number	Number Evaluated	Number Positive
1	8	
2	8	
3	8	
Total	**24**	**No Growth**

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

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.

End of 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.
Gowning room	R2S	60 cu. ft.	0	< 0.017/cu.ft.
Gowning room	R2S	60 cu. ft.	0	< 0.017/cu.ft.
Filler Deck	Settling plate	60 min.	0	--
Filler Deck	Settling plate	19 min.	0	--

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	1	G(+) rods-spores
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	Capper Bowl-Outside	0	
17	Stopper Bowl-Outside	0	
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 stop-per bowl	0	
25	Floor—in front of exit door	0	
26	Pass through deck	0	
27S	Pass through entry door handle	0	
28S	Forceps-filler	0	
30	Rt. Plexiglas door, clean side	0	
31	Wall, left side of pass through	0	
32	Gowning area bench	0	
33	Alcohol bottle	0	
34	Control panel-FD 1	0	
35	Vial tray	0	
36S	Lyo ring	0	

1 = TNTC = Too Numerous to Count
2= Exceeds Action Limit
S = Swab sample

PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
MIM	0	0	NS	NS	NS
EW	0	0	NS	NS	NS
MIM	0	0	NS	NS	NS
MIM	0	0	NS	NS	NS
EW	0	0	0	0	0
KR	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
EW	0	0	NS	NS	NS
MIM	0	0	NS	NS	NS
KR	0	0	0	0	0
NG	0	0	NS	NS	NS
MIM	0	0	12	15	0
NG	0	0	0	0	0
JL	0	0	0	0	0
MM	0	0	0	0	0
EW	0	0	0	0	0

1= Exceed Action Limit
2= Exceeds Alert Level
NS = Not Sampled

IDENTIFICATION OF PERSONNEL ISOLATES

MIM: Left sleeve = G(+) cocci; Right sleeve = G(+) cocci & rods.

MEDIA FILL--**GROUP 2**

Lot No. 621001-MF (Filled in the afternoon)
MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	171	0
2	67	0
3	143	0
4	207	0
5	206	0
6	207	0
7	176	0
8	39	0
Total	**783**	**0**

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

Bulk samples and priming samples were all negative.

MEDIA GROWTH SUPPORT TEST

Control Counts				MF Test Samples				
Organism	**(CFU's)**			**Growth (+)/No Growth (-)**				**Gram Stain**
	1	**2**	**Avg**	**1**	**2**	**3**	**4**	
B. subtilis	97	85	91	+	+	+	+	G + rods
S. aureus	34	25	30	+	+	+	+	G + cocci
P. aeruginosa	28	21	25	+	+	+	+	G - rods
C. albicans	81	97	89	+	+	+	+	Yeast
A. niger	91	94	93	+	+	+	+	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	0	
14S	Plexiglas Door Handle	0	
15	Control Panel	0	
16	Capper Bowl-Outside	0	
17	Stopper Bowl-Outside	0	
18	Stopper Bowl-Inside	6*	
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 stop-per bowl	0	
25	Floor—in front of exit door	0	
26	Pass through deck	0	
27	Pass through entry door handle	0	
30	Right Plexiglas door, clean side	0	
31	Pump set-up Plexiglas door, clean side	0	
32	Gowning bench	0	
33	Wall, left side of pass through	0	
34	Alcohol bottle	0	
Forceps	Out-feed	0	
	Filler cabinet	0	
	Stopper bowl	0	
	Aseptic Connection	0	
	Turntable	0	

** Gram (+) rods with spores*

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.	TNTC*	A lot/cu.ft.

End of 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.	TNTC*	A lot/cu. ft.
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Stopper Bowl	SMA	60 cu. ft.	TNTC*	A lot/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.
Lyophilizer 2	RCS+	400L	0	< 0.07/cu. ft.
Pass through	RCS+	400L	0	< 0.07/cu. ft.
Staging rack	RCS+	400L	0	< 0.07/cu. ft.
Lyophilizer 2	RCS+	400L	0	< 0.07/cu. ft.

TNTC = Too Numerous To Count*; appeared to be operator's finger on the agar.*

PERSONNEL MONITORING

Initials	FIPs		Sleeves		Chest
	Rt	Lf	Rt	Lf	
MH	0	0	NS	NS	NS
MH	0	0	NS	NS	NS
CS	0	0	NS	NS	NS
CS	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
MH	0	0	0	0	1
CS	0	0	NS	NS	NS
DB	0	0	NS	NS	NS
DB	0	0	NS	NS	NS
WH	0	0	NS	NS	NS
CS	0	1[1]	NS	NS	NS
CS	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
CS	0	0	NS	NS	NS
DB	0	0	0	0	0
CS	0	0	0	1	0
MM	0	0	NS	NS	NS
MM	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
JC	0	0	NS	NS	NS
JC	0	0	NS	NS	NS
MM	0	0	NS	NS	NS
MM	0	0	1	0	0
JC	0	0	0	0	0
JL	0	0	0	0	0
WH	0	0	0	0	0

NS= Not Sampled—sampled gloves only

Aseptic Solutions Inc.