

Case Study No. 3

CHALLENGE CONDITIONS: *B. subtilis* spores added to 70% for use to sanitize hands in the filling room. Final concentration ~10⁵/ml. Alcohol was liberally used during both fills.

FILL CONDITIONS: Two media fills performed during the first week of the course on January 25, 2001.

MEDIA FILL--GROUP 2 Lot No. 012501-02 (Filled in the morning) MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive	# Manual Filled	No Cap	Bad Crimp
1-A	157	0	1	15	0
1-B	75	0	0	20	0
2	202	0	2	30	0
3	204	2	2	13	1
4	66	0	5	21	0
5	204	4	2	8	0
Total	913	6*	12	107	1

Incubation Conditions: 25° ± 2° C, 12 days, upright, then 32° ± 2° C, 17 days inverted.

MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	1	2	3	4	
<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

VIABLE AIR MONITORING (Action Limit Excursions in RED)

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 Shelves	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.	6	0.10/cu. ft.
In-feed Turn Table	SMA	60 cu. ft.	6	0.10/cu. ft.
Cart	RCS+	1000 liters	15	0.429/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	SMA	60 cu. ft.	7	0.117/cu. ft.
Out-feed Trayer	SMA	60 cu. ft.	0	< 0.017/cu. ft.
Cart	RCS	1000 liters	29	0.829/cu. ft.

SURFACE MONITORING (Action Limit Excursions in RED)

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Table Top	TNTC**	Gram + rods with spores
2	Freeze Dryer Door-1	12	Gram + rods with spores
3	Freeze Dryer Door-2	0	
4	Pass-Thru Door	1	Gram + rods with spores
5S	Entry Door Handle	TNTC	Gram + rods with spores
6	In-feed Turn Table	0	
7	In-feed Tray Loader	4	Gram + rods with spores
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	TNTC	Gram + rods with spores
14S	Left Plexiglas door handle	0	
15	Control Panel	TNTC	Gram + rods with spores
16	Capper Bowl-Outside	7	Gram + rods with spores
17	Stopper Bowl-Outside	0	
18	Stopper Bowl-Inside	Not Sampled	
19	Plexiglas Above Stopper Bowl	0	
20	Inside Curtain (left)	1	
21	Stainless Steel set-up Cart	3	
22	Conveyor Belt	13	Gram + rods with spores
23	Floor-in front of Filler	TNTC	Gram + rods with spores
24	Floor-in front of stopper bowl	3	
25	Floor-in front of exit door	7	
26	Outside of 70% IPA Bottle	19	Gram + rods with spores
27	Vial Pusher Handle	0	
28	Tray Loader	0	
29	Stopper Hopper Chute	0	
30	Sharpie	0	

**TNTC = Too Numerous to Count

PERSONNEL MONITORING

Initials	FIPS		Sleeves		Chest	Elbows		Lower Back
	Right	Left	Right	Left		Right	Left	
PL	2	4	NS	NS	NS	NS	NS	NS*
PL-2	0	1	NS	NS	NS	NS	NS	NS
JL	13	18	NS	NS	NS	NS	NS	NS
PL-3	0	0	NS	NS	NS	NS	NS	NS
HL	TNTC	TNTC	NS	NS	NS	NS	NS	NS
PH	TNTC	TNTC	1	1	1	0	6	0
HL-2	26	36	NS	NS	NS	NS	NS	NS
FT	TNTC	TNTC	3	0	37	6	0	0
PL-4	TNTC	TNTC	0	0	1	3	TNTC	0
HL-3	TNTC	TNTC	25	10	73	12	1	2
JN	TNTC	TNTC	2	1	1	51	5	4
MC	TNTC	TNTC	4	2	0	8	6	2
DW	TNTC	TNTC	7	1	3	17	1	0
RG	30	31	2	0	0	8	0	0
JL	32	26	2	1	0	2	0	0
IT	17	6	0	0	0	0	0	0

*NS = Not Sampled

IDENTIFICATION OF PERSONNEL ISOLATES

DW: R. sleeve & chest, HL: chest, PH: R. sleeve, HF: R. sleeve all gram positive cocci. All others gram positive rods with some spores.

MEDIA FILL--GROUP 1 Lot No. 012501-01 (Filled in the afternoon) MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive	# Manual Filled	No Cap	Bad Crimp
1	207	2	3	10	2
2	207	0	4	9	0
3	108	14	8	14	1
4	209	22	1	11	1
5	209	7	3	41	0
Total	1012	45*	19	85	4

Incubation Conditions: 35° ± 2° C, 12 days, upright, then 25° ± 2° C, 17 days inverted.
* All isolates were large Gram (+) rods with spores.

MEDIA GROWTH SUPPORT TEST

Organism	Control Counts (CFU's)			MF Test Samples				Gram Stain
	1	2	Avg	1	2	3	4	
<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

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 (bag side)	SMA	60 cu. ft.	21	0.35/cu.ft.
In-feed Turn Table (other side)	SMA	60 cu.ft.	2	0.03/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	SMA	20 cu. ft.	1	0.01/cu.ft.
Cart	RCS+	1000L	~300	~8.49/cu.ft.

NON-VIABLE AIR MONITORING (Action Limit Excursions in RED)

Sample Location	Time	Number of Particulates > 0.5 microns per cu. ft.				Avg. Count
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	
In-feed Trayer	14:59	11	16	9	2	9.50
In-feed Trayer	16:46	62	40	55	73	57.50
Stopper Bowl	15:17	40	18	29	16	25.75
Stopper Bowl	17:14	66	141	40	75	80.50
Top of Out-feed	15:06	36	0	1	0	9.25
Top of Out-feed	16:54	184	26	11	1	55.50
Top of Out-feed	17:02	148	30	193	350	180.25
Top of Out-feed	17:08	59	71	49	53	58.00
Fill Line	14:36	7	8	12	16	10.75
Fill Line	14:40	8	12	8	7	8.75
Fill Line	14:45	7	17	9	14	11.75
Fill Line	14:51	16	9	13	6	11.00
Fill Line	16:29	9	7	8	6	7.50
Fill Line	16:34	6	4	7	5	5.50
Fill Line	17:53	10	24	10	6	12.50
Fill Line	17:58	2	1	2	3	2
Fill Line	18:27	5	1	2	1	4.25
Fill Line	18:42	4	7	1	9	5.25
Location 4 ?	17:21	236	642	417	645	485

SURFACE MONITORING (Action Limit Excursions in RED)

Location Number (NOTE: S = Swab)	Location Identification	Results (CFU's/25 sq. cm.)	Comments
1	Shelf	31	Gram + rods with spores
2	Freeze Dryer Door-1	0	
3	Freeze Dryer Door-2	4	Gram + rods with spores
4	Pass-Thru Door	24	Gram + rods with spores
5S	Entry Door Handle	TNTC*	Gram + rods with spores
6	In-feed Turn Table	25	Gram + rods with spores
7	In-feed Tray Loader	18	Gram + rods with spores
8	Vial Pusher	38	Gram + rods with spores
9S	Fill Nozzle Support	18	Gram + rods with spores
10S	Fill Nozzle	2	Gram + rods with spores
11	Stopper Arm	8	Gram + rods with spores
12	Vial Star-wheel	49	Gram + rods with spores
13	Left Plexiglas Door	1	
14S	Left Plexiglas Door Handle	85	Gram + rods with spores
15	Control Panel	TNTC	Gram + rods with spores
16	Capper Bowl-Outside	0	
17	Stopper Bowl-Outside	8	Gram + rods with spores
18	Stopper Bowl-Inside	0	
19	Stopper Bowl Plexiglas	14	Gram + rods with spores
20	Tray Loader Curtain	18	Gram + rods with spores
21	Stainless steel Set-up Cart	8	Gram + rods with spores
22	Conveyor Belt	10	Gram + rods with spores
23	Floor-in front of Filler	24	Gram + rods with spores
24	Floor-in front of stopper bowl	39	Gram + rods with spores
25	Floor-in front of Exit Door	11	Gram + rods with spores

*TNTC = Too Numerous to Count

PERSONNEL MONITORING (Action Limit Excursions in RED)

Initials	FIPS		Sleeves		Chest	Forehead	Neck
	Right	Left	Right	Left			
John	NS	NS	0	0	0	0	NS*
Dandan	7	14	0	0	0	0	0
Jeff	5	43	NS	NS	NS	NS	NS
John-2	TNTC**	TNTC	NS	NS	NS	NS	NS
Jeff-2	5	4	NS	NS	NS	NS	NS
Karin	9	25	0	0	1	0	NS
Diane	TNTC	TNTC	0	0	1	5	NS
Michael	16	18	NS	NS	NS	NS	NS
Michael-2	0	0	0	0	0	NS	NS
Jeff-3	TNTC	TNTC	NS	NS	NS	NS	NS
Jeff-4	0	40	NS	NS	NS	NS	NS
Laura	TNTC	TNTC	1	7	9	14	NS
Kristal	8	TNTC	2	5	2	14	NS
Jeff-5	71	65	15	9	14	NS	NS
Joey	23	25	6	4	19	15	NS
Karin-2	35	26	0	3	0	0	NS
Paul	26	25	0	0	0	0	NS
John-3	TNTC	TNTC	2	5	1	14	NS

*NS = Not Sampled

**TNTC = Too Numerous to Count

IDENTIFICATION OF PERSONNEL ISOLATES

John: L-sleeve, Karin: L. sleeve, Joey: L. sleeve, Diane: chest & forehead all Gram (+) cocci. All others Gram (+) rods with some spores.



Aseptic Solutions Inc.

* Clean-up of broken vial
** Manual Stopping
*** Cap Intervention
\$ Setting needles and priming filler
\$\$ Cap Jam

Follow-up to Case Study No. 3

CHALLENGE CONDITIONS: None

FILL CONDITIONS: One product fill and one media fill performed during the second week of the course March 1, 2001--same students as January session.

PRODUCT FILL -- GROUP 1 Lot No. 03012001-aMGZ69 (Filled in morning) STERILITY TEST RESULTS

Tray Number	Number Evaluated	Results
1	8	
2	8	
3	8	
4	8	
5	8	
Total	40	No Growth

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.

PERSONNEL MONITORING

Initials	FIPS		Sleeves		Chest
	Right	Left	Right	Left	
JL	0	0	NS	NS	NS
DL	0	0	NS	NS	NS
MG	0	0	NS	NS	NS
DZ	0	0	0	0	0
KW	0	0	0	0	5
JL	0	0	NS	NS	NS
MG	0	0	NS	NS	NS
MG	0	0	NS	NS	NS
MG	0	0	NS	NS	NS
TL	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
JL	0	0	NS	NS	NS
KA	0	0	NS	NS	NS
MG	0	0	0	0	0
TL	0	1	0	0	0
DL	1	0	0	0	0
KA	0	0	0	0	0
JL	0	0	0	0	0
JR	0	0	0	0	0
PH	0	0	0	0	0
JL	0	0	0	0	0

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

IDENTIFICATION OF PERSONNEL ISOLATES

KW: Chest = G(+) rods-spores

MEDIA FILL--GROUP 2 Lot No. 03012001-MF (Filled in afternoon) MEDIA FILL RESULTS

Tray Number	Number Evaluated	Number Positive
1	134	0
2	205	0
3	207	0
4	208	0
5	107	0
Total	861	0

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

Bulk samples and priming samples were all 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>	97	85	91	+	+	+	+	G + rods
<i>S. aureus</i>	34	25	30	+	+	+	+	G + cocci
<i>P. aeruginosa</i>	28	21	25	+	+	+	+	G - rods
<i>C. albicans</i>	81	97	89	+	+	+	+	Yeast
<i>A. niger</i>	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	1	G(+) cocci
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	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	0	
25	Floor--in front of exit door	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	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	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	

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 (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.

PERSONNEL MONITORING

Initials	FIPS		Sleeves		Chest
	Right	Left	Right	Left	
IT-1	0	0	NS	NS	NS
CR-1	1 ¹	1 ²	NS	NS	NS
RG-1	0	0	NS	NS	NS
FT-1	0	0	NS	NS	NS
CR-2	0	0	0	0	0
FT-2	0	0	NS	NS	NS
RG-2	1 ¹	0	NS	NS	NS
IT-2	0	0	NS	NS	NS
RG-3	0	0	NS	NS	NS
FT-3	0	0	0	0	0
RG-4	0	1 ¹	NS	NS	NS
JL-1	0	0	NS	NS	NS
PH-1	0	0	NS	NS	NS
PH-2	0	1 ¹	0	0	0
RG-5	0	0	0	0	0
JL-2	1 ¹	0	0	0	0
DW	1 ¹	0	0	0	0
VO	0	0	0	0	0
IT-3	0	0	0	0	1 ²

NS= Not Sampled--sampled gloves only
1 = G (+) rods-spores
2 = G (+) cocci



Aseptic Solutions Inc.