PROGRESS REPORT
NSG 643

THE BACTERIOLOGY OF "CLEAN ROOMS"

G. S. Michaelsen
Project Director
School of Public Health
and University Health Service
University of Minnesota
Minneapolis, Minnesota 55455

October 1, 1964 - March 31, 1965

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During the six month period covered by this report (10-1-64 to 3-31-65), the major accomplishment was the completion of comparative routine sampling procedures in the four previously selected "clean-rooms" of varying expected performance.

Characteristics of Clean Rooms

These rooms were chosen because they represent a wide range of differences in environmental controls and personnel practices which might influence the levels of particulate and microbiological contamination in the air and on surfaces. A general description of individual room characteristics will indicate the features of interest for each area.

Univac Room E (Defense Aerospace Printed Circuit Area) This room is an ordinary factory area with an open bar joist ceiling and about 1,000 sq. ft. of floor space. No special precautions for contamination control are instituted in Room E.

The air supply for this room is furnished from a general, office type air conditioner with standard, 2 inch fiberglass, throw-away filters. Rate of air flow is about ten changes per hour.

Personnel wear ordinary street clothing, and from six to nine individuals are actively engaged in production work in this room. Ordinary janitorial service, primarily floor mopping, are performed routinely, with intermittant dusting of bench tops.

Univac Room D (Memory Unit Manufacturing) Certain special practices have been instituted in this area to reduce contamination by particulates. Between 25 to 28 workers are assembling components in Room D which occupies approximately

2,000 sq. ft. of floor space.

The room is under positive pressure and air is supplied by a conditioning system which controls the temperature at 75° F ± 5° and the relative humidity between levels of 50 to 30 per cent. Before entering the room, all air is passed through a Cambridge Aerosolve #95 mechanical filter. An "Airson" false ceiling system delivers forced air into the area at a rate of approximately 15 changes per hour.

All personnel entering this area are required to wear head covers and a smock over street clothing. An air lock dressing area is provided. Although a shoe cleaner vacuum is available, it appears to be used only casually and infrequently. Shoe covers are not required and there are no special hand washing facilities in the dressing area.

Janitorial services include a once daily vacuuming and wet mopping of floors with detergent designated for clean room use. Bench tops and legs are also vacuumed daily and are washed at one to three month intervals. Occasionally, at the discretion of the workers, they may clean a bench top in their immediate area if "it looks dirty".

Honeywell Room C (Component Preparation and Cleaning Area) This 750 sq. ft. area has a number of features which suggest that it might be somewhat more efficient for prevention of particulate contamination than Room D. Work in this area is usually carried on by five to seven people and the total personnel load never exceeds ten persons.

The air supply for Room C passes through a fiberglass roughing filter, an electrostatic precipitator, an air conditioner and, ultimately, through Cambridge absolute filters. Air enters the room through ceiling louvers and is exhausted through wall vents located near the floor. Temperature is maintained at 72° F ± 5°, relative humidity is controlled to remain below 50 per

cent and the room is under positive pressure. Flow rate of the system is approximately 24 changes per hour, with approximately 60 per cent make-up air. Standards for this room require that dust counts be no higher than 400 particles > 5μ per cubic foot.

Strict control is exercised over personnel entering the area. Except for workers assigned there, registration is required of all people. All persons must use the shoe cleaner and wash their hands before dressing in the outer air lock. Standard dress requirements include smocks or suits, head covers and shoe covers.

Janitorial services are carried out on a rigidly maintained schedule and rigid inspections ensure compliance. During the day shift, all floors are vacuumed twice and outside passages are wet mopped. A second shift crew is responsible for another daily vacuuming of walls, fixtures and horizontal surfaces, except bench tops and shelves. The same shift also again wet mops all floors. Assembly workers are required to cleanse bench tops each morning by swabbing with isopropyl alcohol saturated sponges.

Honeywell Room A (Component Assembly Area) Honeywell Clean Room A appears to be the most sophisticated of the areas studied. Floor space and worker load are almost identical to those described for Room C. Operating characteristics are also similar to those for Room C, except for the following important additional features:

1. An air shower is required for all personnel before admittance to the dressing room air lock.

2. The air handling system is basically that of Room C, but is modified in several aspects. Among these is the addition of an Aerosolve pre-filter stage to the air stream cleansing unit. Furthermore, the flow rate for this room is increased to 30 changes per hour and the supply receives 33 per cent make-up air.

3. Particulate contamination standards are set at 200 particles > 5μ per cu. ft. and 25,000 particles > 0.5μ per cu. ft.

4. All materials entering this room have been cleaned previously in another area of comparable atmosphere. Parts and other materials are transferred into Room A through air lock facilities. Particulate shedding materials such as pencils, paper, etc. are excluded from the room.

Methods

The routine sampling procedures, which were carried out on 8 occasions in each of the four rooms, can be summarized as follows:

1. Ambient air sampling. On each sampling visit a Reyniers slit sampler was set up at bench top level near the center of the room's activities. A series of 5 consecutive one hour samples at one cu. ft. per minute was collected, making a total sampling volume of 300 cu. ft. per day. Samples were collected on TSA agar and incubated 43½ hours at 32° C before counting.

2. Bench top surface sampling. All surface sampling was done with Rodac agar contact plates. Preliminary sampling data were used to calculate the number of randomly chosen bench top sampling sites necessary to measure accurately the bench top contamination in each room. In Rooms A, D, and E, 90 sites were sampled during the a.m. and 90 different sites during the p.m. for a total of 180 Rodac samples per sampling day. An unexplained greater variability in Room C required 150 sampling sites per series or 300 samples per sampling day. Again, TSA agar was used and plates were incubated 43½ hours at 32° C before counting.

3. Stainless steel strip contamination. Stainless steel strips were exposed for evaluation of fall-out contamination in Rooms 'A' and 'D'. Each series consisted of 42 strips which were placed in several bench top locations within each room. Six strips from each series were evaluated at 3 week

intervals for a total of 21 weeks. The analysis consisted of placing each strip in a bottle with 25 cc of phosphate buffer plus 0.02% Tween 20. The bottles were shaken for 5 minutes in a gyrorotatory mechanical shaker; followed by plating of 5 ml aliquots in each of the following ways:

a. Five ml plated in TSA agar and incubated aerobically for 72 hours at 32° C.

b. Five ml plated in TSA agar and incubated anaerobically (in Nitrogen gas) for 72 hours at 32° C.

c. Five ml heat shocked (80° C for 20 minutes), then plated in TSA agar and incubated aerobically for 72 hours at 32° C.

d. Five ml heat shocked (80° C for 20 minutes), then plated in TSA agar and incubated anaerobically for 72 hours at 32° C.

4. Qualitative determinations. From the routine air and bench top samples, a varying number of plates were randomly selected each week for characterization of colonies. The characterizations included a morphological study of all colonies on the plate by smears made on 15 place milk slides. The smears were Gram stained, examined microscopically, and results recorded.

From the stainless steel strips, only those colonies developing from heat shocked aliquots were selected. These are being subjected to detailed identification procedures.

Results

The quantitative data from air and bench top sampling in all four rooms are summarized in Table I. The microbial counts of air from these rooms reflect the control differences in a very predictable manner. For example, the room with greatest control (Honeywell A) yielded a mean of 0.22 col. per cu. ft.,

while in the room with least control (Univac E) mean colony counts reached 4.20 col. per cu. ft. The mean air counts of less than one col. per cu. ft. reported for Rooms A and C represent extremely low bacterial counts for occupied areas.

The data for bench tops is not quite as predictable. The extremely odd distribution of contamination in Room C is apparently responsible for the discrepancy as this room had a mean level of 21.0 col. per Rodac plate but a median level of only 4.0 colonies per plate. If only the medians are considered, the bench top levels demonstrate a relationship to degree of contamination control which is similar to that observed for air counts. These data are now undergoing additional statistical analyses and it may be that the median levels will prove to be the significant estimates of contamination.

Table II summarizes the relative microbial levels detected on stainless steel strips during the 21 week series in Rooms A and D. These data provide evidence that there is no marked contamination build-up on stainless steel strips over a prolonged period of time, even for heat resistant organisms.

Indeed, in Clean Room A, when the room was totally unoccupied for a period of 10 days preceding the last strip analysis, the contamination levels dropped virtually to zero. This information seems to confirm previous data from experiments conducted by the Army Biological Laboratory at Fort Detrick. Another point of interest is the apparently negligible difference in contamination levels on strips between the clean room with rigid controls (Room A) and a clean room with relatively little control (Room D).

Tables III and IV illustrate the qualitative breakdown of microbial contaminants from air and bench top samples in all four rooms. It appears from these data that some differences do occur among rooms in the same building and also among buildings. For instance, the high percentage of

yeasts on bench tops in Room C were not apparent in the air of Room C or in Room A. Also, the very high percentages of diphtheroids in Rooms D and E did not appear in Room A and C. However, it would appear that the great majority of contaminants in all rooms were of human origin (Gram + cocci and diphtheroids) as opposed to typically soil and dust microbes. It also appears that the percentage of "spore forming" bacteria (those which gave visual evidence of spores on Gram stains) remained relatively constant among all four rooms (0.5 - 4.1%).

Discussion

The data reported herein illustrate that ambient air and bench top microbial contamination levels do, in general, reflect the degree of control associated with spacecraft component production areas, at least in the Minnesota area during the winter months. However, the contamination difference associated with stainless steel strips exposed up to 21 weeks in relatively well-controlled vs. relatively poorly-controlled rooms appears to be very small and of little practical significance. It also appears that microbial contamination levels do not build up over a prolonged exposure time, but reflect deposition of contamination only during a short time preceding the analysis. This is well illustrated by the analysis of strips from Room A examined at the 21 week interval after the room had been totally unoccupied for 10 days, showing counts on all except the anaerobic pre-heat plates dropping to zero.

It would also appear, that even after prolonged exposure the percentages of heat resistant microbes remain fairly constant at 10-15% of total contaminants on strips, while in ambient air and on bench tops, spore formers make up less than 5% of all contaminants.

Future Work

During the remaining months of the project several areas remain to be investigated. An effort will be made to identify the heat resistant microbes which were isolated from the strips during the 21 week series. When these are identified they will be subjected to thermal death time experiments in an attempt to pin down this relationship for actual clean room component contaminants. An effort will also be made to determine the time and temperature necessary to actually sterilize strips of various types subjected to natural contamination from fall out and from human handling.

Work will also be initiated to determine the feasibility of assembling various simulated components using aseptic techniques.

In addition, vertical laminar flow "clean rooms" will be studied to determine microbial contamination levels associated with this type of facility.

TABLE I

QUANTITATIVE DATA SUMMARY--HONEYWELL AND UNIVAC CLEAN ROOMS

Sampling Day	HONEYWELL				UNIVAC			
	Room A		Room C		Room D		Room E	
	Air Col/Cu.Ft.	Bench Top Col/Rodac	Air Col/Cu.Ft.	Bench Top Col/Rodac	Air Col/Cu.Ft.	Bench Top Col/Rodac	Air Col/Cu.Ft.	Bench Top Col/Rodac
1	0.38	a.m. 6.8 p.m. 10.8	0.41	a.m. 20.2 p.m. 6.5	0.82	a.m. 10.1 p.m. 14.5	2.71	a.m. 33.2 p.m. 28.1
2	0.33	a.m. 4.1 p.m. 6.1	0.30	a.m. 8.4 p.m. 24.7	1.43	a.m. 16.6 p.m. 22.1	4.54	a.m. 36.3 p.m. 44.3
3	0.16	a.m. 5.7 p.m. 6.5	0.13	a.m. 21.6 p.m. 4.8	1.56	a.m. 15.1 p.m. 17.6	5.86	a.m. 34.6 p.m. 44.2
4	0.30	a.m. 3.9 p.m. 12.1	0.26	a.m. 13.4 p.m. 12.4	1.80	a.m. 22.0 p.m. 17.6	4.66	a.m. 33.6 p.m. 39.3
5	0.19	a.m. 7.7 p.m. 5.6	0.46	a.m. 8.1 p.m. 11.3	1.41	a.m. 11.6 p.m. 11.2	3.21	a.m. 27.6 p.m. 34.2
6	0.11	a.m. 7.9 p.m. 8.9	0.26	a.m. 17.3 p.m. 27.1	2.05	a.m. 17.7 p.m. 19.0	5.69	a.m. 41.6 p.m. 44.1
7	0.11	a.m. 4.7 p.m. 4.1	0.23	a.m. 54.8 p.m. 43.1	1.55	a.m. 12.3 p.m. 15.4	4.40	a.m. 38.1 p.m. 42.0
8	0.17	a.m. 6.1 p.m. 12.7	0.16	a.m. 41.7 p.m. 19.8	0.81	a.m. 17.1 p.m. 15.6	2.54	a.m. 25.6 p.m. 47.0
Mean	0.22	a.m. 5.9 p.m. 8.4 Total 7.2	0.28	a.m. 23.2 p.m. 18.7 Total 21.0	1.43	a.m. 15.3 p.m. 16.6 Total 16.0	4.20	a.m. 33.8 p.m. 40.4 Total 37.6
Median	-	3.2	-	4.0	-	11.3	-	29.5

TABLE II

STAINLESS STEEL STRIP SUMMARIES (COLONIES/1" x 2" STRIP)

Week	HONEYWELL (ROOM 'A')				UNIVAC (ROOM 'D')			
	Aerobic		Anaerobic		Aerobic		Anaerobic	
	Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat
3	43.3	0	-	-	46.7	0	0	0
6	19.2	0.8	2.5	0.8	6.7	1.7	0	0
9	16.7	3.3	12.5	0	9.2	3.3	2.5	0
12	38.3	3.3	0	0	12.5	1.7	0.8	0
15	4.2	1.7	4.2	3.3	15.0	3.3	3.3	2.5
18	16.7	4.2	10.0	0.7	23.3	2.5	29.2	1.7
21	0*	0*	3.3*	0*	36.7	9.0	16.7	2.5
Mean	19.8	1.9	4.6	0.7	21.4	3.1	7.5	1.0

*Room unoccupied for 10 days prior to strip analysis.

TABLE III

HONEYWELL SUMMARY--AIR & SURFACE

RELATIVE OCCURRENCE OF MICROBIAL TYPES

	ROOM A Air	Bench Top	ROOM C Air	Bench Top
Gram + cocci	77.3%	74.4%	74.8%	69.6%
Gram - cocci or coccobacilli	0.5	0.4	0.2	0.1
Gram + bacilli (no spores)	12.2	20.6	14.9	11.8
Gram + bacilli (spores)	2.8	1.0	4.1	0.5
Gram - bacilli	6.1	1.5	0.6	2.0
Diphtheroids	0.8	1.5	5.0	3.6
Actinomycetes	-	0.1	-	0.1
Yeasts	0.3	0.5	0.2	12.1
Molds	-	-	0.2	0.1
Number of colonies examined	362	1,428	463	2,125

*Based on 8 sampling days (12-2-64 to 1-26-65)

TABLE IV

UNIVAC SUMMARY--AIR & BENCH TOP*

RELATIVE OCCURRENCE OF MICROBIAL TYPES

	ROOM D		ROOM E	
	Air	Bench Top	Air	Bench Top
Gram + cocci	68.5	53.7	49.6	38.1
Gram-cocci + coccobacilli	0.1	0.3	0.3	0.1
Gram + bacilli (no spores)	2.5	4.9	6.1	5.4
Gram + bacilli (with spores)	3.2	2.6	2.3	3.2
Gram - bacilli	0.1	0.2	1.3	0.4
Diphtheroids	25.6	37.6	37.1	47.0
Actinomycetes	-	0.1	0.6	0.5
Yeasts	-	0.4	0.2	0.8
Molds	-	0.2	2.5	4.5
Number of colonies examined	786	2,596	1,927	4,535

*Based on 8 sampling days (2-2-65 to 3-23-65)