

RELATIVE CLEANABILITY OF VARIOUS STAINLESS  
STEEL FINISHES AFTER SOILING WITH  
INOCULATED MILK SOLIDS<sup>1</sup>

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SUMMARY

A semiautomatic spray-washing device, which provided controlled rinsing and washing without brushing, was used to determine the relative cleanability of Type 302 stainless steel panels having a No. 2B, 3, 4, and 7 finish after soiling with skim milk inoculated with *Bacillus globigii* spores.

Using the Direct Surface Agar Plate test at the 1 and 5% level, no significant difference was observed among these finishes after the test areas were rinsed (T-2), rinsed and washed with alkaline detergent (T-3), and rinsed, washed, and sanitized (T-4). This study indicates that the less highly polished finishes can be cleaned bacteriologically to the same degree as the highly polished No. 7 surface when the same cleaning cycle is used.

No significant difference in bacterial cleanability was observed among finishes at the 5% level with the Standard Swab Contact Test after T-3 and after T-4.

With the Direct Agar Contact Plate, a significant difference was observed among No. 2B, 3, 4, and 7 finishes after T-2, T-3, and T-4. The recovery of bacterial cells by this test was less than that obtained in the DSAP and Swab Contact Tests and indicates the unreliability of this test.

In all trials, the bacteria counts decreased markedly as the washing procedure was changed from a minimum rinse to a complete cycle of rinse-wash and sanitize.

The relationship between the finish of Type 302 stainless steel and the ease with which bacteria may be removed from the surface has been of interest to public health officials, sanitarians, and dairy equipment manufacturers for some time. Numerous studies have been made on the cleanability of Type 302 steel having a No. 4 finish, but little information is available on the comparative cleanability of this steel type having a No. 2B, 3, 4, or 7 finish.

This study was undertaken to determine the relative cleanability of Type 302 stainless steel panels having a No. 2B, 3, 4, and 7 finish when these surfaces are compared under identical conditions of soiling and spray cleaning and tested using the Direct Surface Agar Plate procedure (2), which detects the viable bacteria actually remaining on the surface, in contrast to other procedures which detect the bacteria removed. In this study, a deliberate attempt was made to minimize the cleaning procedures so some bacteria would remain on all surfaces after rinsing, rinsing and washing, and rinsing, washing, and sanitizing. The term relative cleanability is used because the object of the study was to compare the relative numbers of bacteria remaining on each finish after each cleaning treatment.

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Hays *et al.* (4) studied the removal of air-dried films of whole milk, contaminated with *Escherichia coli*, from 18-8 stainless steel panels having 2B, 7 mill, 80, 100, and 120 grit surfaces after scrubbing for 15 sec. with an alkaline cleaner at room temperature; with the No. 7 mill finish, 100% removal was noted. Somewhat similar results were obtained after washing with an anionic, nonionic, or acid cleaner at room temperature.

Ridenour *et al.* (7), using a saline suspension of *Micrococcus aureus* as a soiling material, obtained 49% removal, based on radioactive tracer methods, after rinsing stainless steel with water at 60° F. for 1 min.; 97% of the total removal was observed using a detergent wash at 160° F. for 3.5 min. in a commercial jet-spray dishwasher. The specific finish of the steel surface was not identified. In a milk soil, these workers removed more than 99% of the total soil applied.

Masurovsky *et al.* (6) used radioactive *E. coli* and *Micrococcus pyogenes* var. *aureus* cells in homogenized whole milk as a test soil. Cleaning was accomplished by mechanical brushing in alkaline detergent solution at 120° F. for 1.125 min., followed by rinsing for a similar time. On the basis of Duncan's Multiple Range Test (3), which considers basically the extreme values of the data, no significant difference was observed between stainless steels having a No. 7, 4, or 3 finish. Under the same test conditions, a No. 2B finish was significantly different from the No. 3, 4, and 7 finish. In another series of experiments with no brushing these workers found no significant difference between No. 4 and 7 finishes when *M. pyogenes* var. *aureus* suspension in 0.85% saline was used as the test soil; no significant difference was observed between No. 3 and 4 finishes when these two finishes were compared. Under the same test conditions, the cleanability of the No. 2B finish was significantly different than that obtained with the No. 3, 4, and 7 finishes.

#### MATERIALS AND METHODS

*Selection of panels.* To secure samples representative of the steel industry's production, 8- by 8-in. panels of Type 302 stainless steel having 2B (bright cold-rolled), 3 (80-100 grit), 4 (120-150 grit), and 7 (325 grit plus buffing) finishes were obtained from six of the largest steel companies representing most of the stainless steel production. Where possible, 5-mill samples of each finish were obtained. In all, 30 panels of each finish except 7 were received; only 25 panels of this finish were received. The roughness of all panels was determined, using a Brush Surface Analyzer. The panels of each finish were arranged in order of roughness and 12 panels of each finish were randomly selected to provide the actual specimens used in this study.

An invisible soil on the new panels, possibly from the adhesive used on the protective paper covering or oil film, made it impossible to spread the soil evenly. To remove this film the panels were brushed with tetrachlorethane, washed with alkaline detergent at twice the minimum recommended level for very hard water, brushed with a recommended organic acid cleaner at twice the recommended concentration, and rinsed in distilled water.<sup>2</sup> This treatment was used only initially to remove the invisible soil. Following this preliminary cleaning, the

panels were brush-cleaned after each trial with a common chlorinated alkaline dairy detergent at twice the suggested minimum level for very hard water. Brush cleaning between trials was necessary to remove a stain left by the agar and swab solution. All plates were brushed with an organic acid cleaner after each experimental trial. Since the objective of this study was to investigate the cleanability of the stainless steel with different finishes, a thorough cleaning was undertaken between each trial to eliminate soil build-up.

*Soil suspension and soiling.* Skimmilk was selected as the soiling medium, since it appeared to adhere more tenaciously to the surface than whole milk and represents actual field soiling conditions more nearly than do other soils, such as saline solutions or synthetic charcoal egg mixtures.

Approximately 10 g. of a concentrated paste of *Bacillus globigii* spores were suspended in 100 ml. of sterile distilled water, filtered to remove debris, washed and centrifuged five times to clean the spores, heat-shocked at 80° C. for 10 min., resuspended to a total volume of 400 ml., and stored at 5° C. Immediately before use, 5 ml. of the stock suspension was heat-shocked again and diluted in sterile skimmilk to obtain a spore level of about 500,000 per milliliter.

To minimize complications due to air and dust contamination, the steel plates were flamed with alcohol before each soil application. One-tenth milliliter of the spore-skimmilk suspension was carefully pipetted onto and spread evenly by means of a sterile rubber spatula over the 4 sq. in. test area. The test area was well-defined by means of a rubber mask placed on the plate. Standard plate counts were made to determine the initial level of inoculum. Initial counts on the test areas ranged from 34,000 to 73,000 per 4 sq. in.; for purposes of analysis all results were corrected to a base level of 50,000 cells. The spore-skimmilk film was fixed by drying for 20 min. at 37° C. To eliminate area variables and to randomize the influence of the "lay of the grit lines," the steel panels were rotated 90° after each trial; in this way, it was possible to test four different areas with each of the three tests used. To maintain constant washing conditions in all trials, it was necessary to locate the test area on the outer edges of the test panel.

*Cleaning device and procedures.* An automatic washing device (Figure 1) was used to eliminate the variation associated with any hand-washing procedure. It consisted of a power-driven feed rack which carried the steel panels under a series of four spray arms. Each panel was placed on the rack at the top position and was carried down the rack under the spray arms, which could be controlled individually. The arms were 8 in. long and were located 5 in. above the surface of the test panels. The evenly dispersed, constant-pressure spray was created by a double row of staggered holes  $\frac{1}{16}$  of an inch in diameter and  $\frac{1}{4}$  of an inch apart. The arms were 12 in. apart. The hardness of the water used in this study was approximately 80 p.p.m.

The first spray arm provided sufficient water to merely wet the panel; the flow rate was 2 liters per minute evenly distributed over the length of the arm.

\*Tetrachlorethane is poisonous and should not be used in routine practice; since the initiation of this study, a special commercial cleaner is available which can be obtained from various commercial detergent supply houses.

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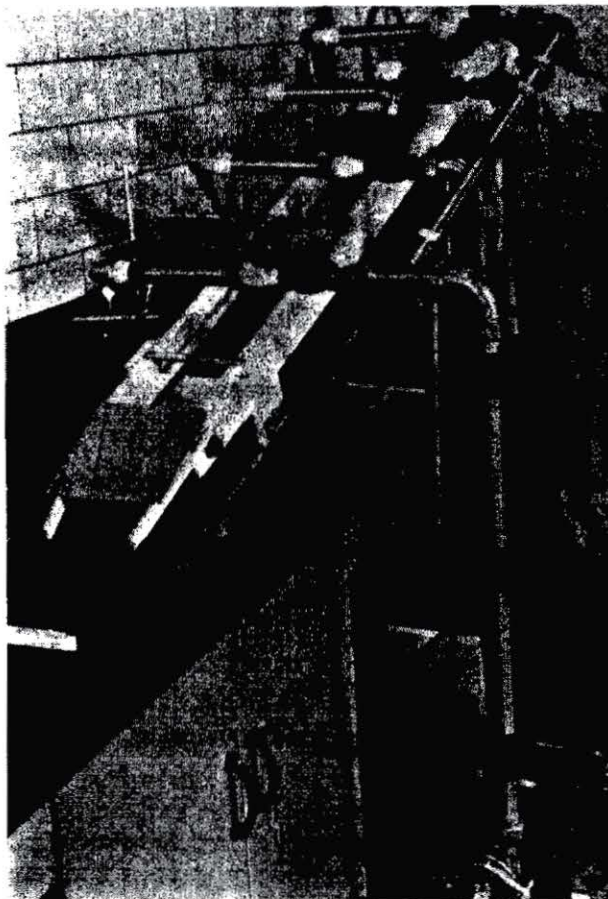


Fig. 1.

Each 4 sq. in. test area was exposed for 2 sec. to the actual spray; this provided about 4 ml. of prerinse water at 45° C. per square inch. This prerinse can be characterized as a running-drip.

A second spray arm similar in design to the above provided a mild spray designed to simulate a fair rinse. The same water supply was utilized, but the flow rate was four times as great. The total time that each panel was wet with flowing water in the prerinse and rinse phase was 18 sec. The rinsing conditions were held to a minimum to prevent removal of all the bacteria.

The third spray arm provided the detergent wash. The hardness of the wash water was 14 p.p.m. A proprietary chlorinated alkali was used at a level of 1 oz. per 5 gal. of water, as recommended for soft water. The water temperature was 70-74° C. The flow rate was 7.1 liters per minute over the entire spray arm; this provided approximately 15 ml. of wash water per square inch of test area. In this operation, the entire panel was wet with detergent solution for 12 sec., but the test area received actual spray treatment for only the standard 2-sec. period.

The fourth spray arm provided a final rinse to flush away the detergent solution. The same water was used as in the initial rinse. The flow rate was 2.4 liters per minute; this provided 5 ml. of rinse water per square inch. The total time the soiled area was exposed to the actual spray and flushing action was 5 sec. The panels were drained to remove excess water prior to sanitization. Drainage time ranged from 5 to 20 sec. Minimum washing conditions were employed to avoid flushing too many organisms from the surface, making a statistical analysis impossible.

Sanitization was accomplished by immersing each panel for 1 min. in 100 p.p.m. chlorine solution. Two 10-sec. rinses in fresh water served to remove the sanitizer from the surface. Drying the panel in a dust-free chamber at 35° C. for 20 min. constituted the final step prior to testing, regardless of the cleaning treatment employed.

Since cleaning operation, as it is usually carried out in practice, is broken down into three major cycles; rinsing, washing, and sanitizing, the effect of each of these treatments on the cleanability of the various surfaces was investigated. The rinse operation (T2) consisted of the prerinse and rinse from spray arms No. 1 and No. 2, respectively. By removing the plates after this treatment, it was possible to study the efficiency of this operation per se. Following this treatment, the entire soiled area remained faintly visible on all surfaces upon drying. By removing the panel after passing under the prerinse, rinse, and detergent-rinse spray nozzles, it was possible to study the effect of a rinse followed by a detergent wash (T3) on the cleanability of the surface. In (T4) the plates were rinsed, washed with detergent, and sanitized. This sequence represented a cleaning operation approximating spray washing.

*Direct Surface Agar Plate.* The Direct Surface Agar Plating (DSAP) technique, as described by Angelotti and Foter (2), was used to determine the bacteria remaining on each surface after the various cleaning treatments. This test differs considerably from the two other tests, in that it indicates the number of viable bacteria remaining on the surface under investigation. The 4 sq. in. test area examined with this procedure was obtained by means of a circle  $2\frac{3}{16}$  in. in diameter. To avoid marking the surface of the plate with wax pencil, rubber jar rings were placed into a previously spotted position on the mask (Figure 2). This ring provided a dam to retain the molten agar on the test area until it hardened. Sterilizing these jar rings eliminated contamination from this source. After aseptically placing the agar-retaining ring into position, the surface was flooded with 10 ml. of plate count agar at 48–52° C. A sterile Petri dish cover containing moistened sterile filter paper was placed over the test area, to aid in maintaining the desired humidity and to eliminate atmospheric contamination. Each panel was placed in a rack and the entire group of panels was incubated over water in a chamber which provided 93% R.H. at  $35 \pm 1^\circ$  C. After 16–18 hr. of incubation, the agar overlay was flooded with 0.5% aqueous solution of 2,3,5-triphenyl 2H-tetrazolium chloride. The colonies became red after this treatment and simplified the counting procedure.



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FIG. 2. DSAP technique.

In this, as well as in all other testing procedures, three panels of each finish were used to give three replications of each test under each cleaning treatment. Fifteen complete experiments were carried out; this provided 45 replications of each test procedure for each cleaning treatment on each finish examined.

*Swab Contact Method.* In using this test, the procedure described in *Standard Methods for the Examination of Dairy Products* (1) was followed, except that 4 rather than 40 sq. in. were tested. The total area examined comprised a 4 sq. in. area (2 by 2 in.). The test area was clearly defined by the film of skimmilk solids which remained after rinsing, but it was not visible after detergent washing. To delineate the limits of the test area, a sterile template was placed on the plate.

The effective contact length of the cotton swab per se was 0.5 in. The 2 by 2 in. area was completely swabbed with four passes of the swab. Each 0.5-by-2-in. band was swabbed by passing the moistened swab over the area three times while continually rotating the swab stick. After swabbing two bands (2 sq. in.), the swab was whip-rinsed in 8 ml. of phosphate buffer in 3-in. screw-cap vials and pressed out prior to swabbing the remaining half of the area. The swab was removed from the stick by breaking it in buffer solution. Plate count agar was used to prepare duplicate 1.0- and 0.1-ml. plates. Counts were made after 48 hr. of incubation at  $35 \pm 1^\circ$  C. The number of colonies per 4 sq. in. area was calculated from these data.

*Direct Agar Contact Plate.* A Direct Agar Contact plate was also used to determine the number of bacteria removed from the various surfaces under the test conditions. The hypodermic syringe technique of Litsky (5) was modified to provide a flat sterile agar surface equivalent to 4 sq. in. of area. The exterior barrel of the modified syringe consisted of an 8-in. piece of glass tubing having an I.D. of  $2\frac{1}{2}$  in. The interior plunger consisted of an 8-in. section of glass tubing having an O.D. of  $2\frac{1}{8}$  in. and an I.D. of  $1\frac{15}{16}$  in. A rubber stopper was fabricated to fit into the end of the interior tube to make the plunger. Using this syringe, it was possible to make a contact impression of the entire 4 sq. in. test area.

The glass syringe was assembled, wrapped in paper, and sterilized by autoclaving. Immediately before use the syringe was unwrapped, clamped around the barrel in a vertical position with the plunger drawn back, and filled with tempered sterile plate count agar. A sterile Petri plate was used to cover the open end of the plunger while the agar hardened and while the unit was not in use. The area designated for the direct agar contact test was located by means of the mask previously described. Two contact impressions each of 5-sec. duration were made on each site; the syringe was rotated  $180^\circ$  between impressions to eliminate dead spots due to entrapped air. After making the contact, the plunger was adjusted to expel  $\frac{3}{8}$  of an inch of solidified agar; this was smoothly sliced from the agar column, using a sharp sterile knife. The agar slab was allowed to slide into a sterile Petri dish with the contact surface up. Bacterial colonies on the contact agar slabs were counted after incubation at  $35 \pm 1^\circ$  C. for 48 hr. The number of colonies per 4 sq. in. of test area was determined.

#### RESULTS AND DISCUSSION

*Direct Surface Agar Plate.* The data given in Tables 1, 2, and 3 for Treatments T-2, T-3, and T-4 represent the average obtained from three replications of each test, using 12 steel panels of each finish. The initial level of the inoculum applied to each test area was determined, using the Standard Plate Count (SPC) procedure. In view of the daily variation observed in the initial level of inoculum (34,000 to 73,000), the counts obtained were corrected to a base level of 50,000 for statistical analysis. In the statistical analysis the corrected actual individual counts were used rather than the average value. The analysis of variance design was set up to answer the question: "Is there a difference in cleanability of Type 302 stainless steel panels having a No. 2B, 3, 4, and 7 finish when these surfaces are compared under comparable conditions of soiling, cleaning, and testing?" The change in bacterial number due to the cleaning technique was used to indicate the degree of cleanliness of the test surface. A summary of the analysis of variances findings is given in Table 4.

The results of the statistical analysis indicate no difference at the 5 or 1% significance level in the cleanability of a No. 2B, 3, 4, or 7 finish after Treatments T-2 T-3, and T-4, when the Direct Surface Agar Plate procedure is used. It is important to note that this test is more critical than either the Swab or Agar Contact Test, since it is not dependent upon the removal of the bacterial cell prior to detection but detects the cell in situ. The fact that the plating and incu-

TABLE 1  
Bacterial counts per 4 square inch of surface after various cleaning treatments using the direct surface agar plate test

Expt. No.	SPC <sup>a</sup>	Finish No. 2B Cleaning treatment				Finish No. 3 Cleaning treatment				Finish No. 4 Cleaning treatment				Finish No. 7 Cleaning treatment			
		T-1 <sup>b</sup>	T-2 <sup>c</sup>	T-3 <sup>c</sup>	T-4 <sup>c</sup>	T-1	T-2	T-3	T-4	T-1	T-2	T-3	T-4	T-1	T-2	T-3	T-4
	(× 1,000)	(× 1,000)			(× 1,000)					(× 1,000)				(× 1,000)			
1	60	63	255 <sup>d</sup>	33 <sup>d</sup>	46 <sup>d</sup>	67 <sup>d</sup>	195 <sup>d</sup>	32 <sup>d</sup>	19 <sup>d</sup>	61 <sup>d</sup>	214 <sup>d</sup>	77 <sup>d</sup>	19 <sup>d</sup>	59 <sup>d</sup>	154 <sup>d</sup>	75 <sup>d</sup>	35 <sup>d</sup>
2	43	62	204	34	14	65	125	20	7	44	98	26	31	53	101	20	6
3	68	78	254	30	7	64	179	24	5	81	224	8	15	79	211	27	2
4	58	66	123	14	17	57	56	23	5	65	28	18	1	51	28	13	3
5	34	52	50	17	2	42	69	23	3	92	96	16	5	38	117	18	5
6	63	67	68	19	4	68	54	15	6	56	52	17	15	55	32	27	25
7	50	52	59	10	4	60	53	9	10	43	70	6	4	52	64	7	5
8	37	42	112	17	6	48	78	25	9	32	62	19	8	30	77	11	6
9	60	44	40	17	10	36	76	17	4	45	132	17	12	37	106	13	17
10	63	52	113	20	13	51	154	20	12	56	137	46	8	50	153	35	14
11	45	45	67	20	3	46	66	12	8	49	83	10	7	47	82	12	8
12	67	76	65	10	9	72	58	11	7	67	76	14	7	68	61	21	9
13	73	75	114	30	18	65	83	22	14	71	92	21	12	72	91	14	15
14	46	71	86	46	15	74	63	32	9	84	81	41	13	79	82	45	15
15	55	49	66	28	11	51	71	23	12	46	85	21	14	49	91	25	24
Av.	55	60	112	23	12	58	92	21	9	59	102	24	11	55	97	24	13

<sup>a</sup> Inoculum level as determined by the SPC test. Statistical analysis is based on these data.

<sup>b</sup> T-1 inoculum level is determined by the Direct Surface Agar Plate Test.

<sup>c</sup> (T-2 rinse treatment) (T-3 wash treatment) (T-4 sanitize treatment).

<sup>d</sup> These numbers are the average of three replications.



TABLE 2

Bacterial counts per 4 square inch of surface after various cleaning treatments using the swab contact test

Expt. No.	SPC <sup>a</sup>	Finish No. 2B Cleaning treatment				Finish No. 3 Cleaning treatment				Finish No. 4 Cleaning treatment				Finish No. 7 Cleaning treatment			
		T-1 <sup>b</sup>	T-2 <sup>c</sup>	T-3 <sup>c</sup>	T-4 <sup>c</sup>	T-1	T-2	T-3	T-4	T-1	T-2	T-3	T-4	T-1	T-2	T-3	T-4
	(× 1,000)	(× 1,000)				(× 1,000)				(× 1,000)				(× 1,000)			
1	60	48	352 <sup>d</sup>	7 <sup>d</sup>	7 <sup>d</sup>	16	235	15	11	24	263	6	11	20	301	10	17
2	43	64	83	7	3	32	.....	22	2	32	40	7	1	48	44	5	5
3	68	64	227	10	2	72	243	4	5	44	144	2	3	44	240	5	....
4	58	64	41	11	5	44	31	15	5	24	21	17	3	12	13	6	1
5	34	48	51	17	0	44	29	6	5	40	53	15	3	36	39	11	4
6	63	36	71	21	9	56	41	33	10	88	136	25	5	44	107	15	6
7	50	16	23	5	1	32	36	3	5	32	16	3	1	24	52	5	2
8	37	28	26	6	2	24	21	2	4	28	33	13	5	32	47	6	6
9	60	16	23	9	4	22	63	9	9	18	98	17	7	46	122	11	8
10	63	36	72	11	4	20	139	15	5	36	160	21	9	22	155	21	18
11	45	22	28	14	2	46	25	6	4	18	35	7	2	22	59	4	3
12	67	86	21	7	5	28	24	5	1	62	72	8	5	42	65	5	7
13	73	60	43	8	12	40	11	11	8	38	50	7	5	48	107	7	5
14	46	56	57	21	7	54	25	13	5	74	30	13	3	46	150	19	8
15	55	54	51	7	4	56	89	8	9	28	42	11	8	32	34	7	9
Av.	55	47	78	11	4	39	72	11	6	39	80	11	5	34	102	9	7

<sup>a</sup> Inoculum level as determined by the SPC test. Statistical analysis is based on these data.<sup>b</sup> T-1 inoculum level as determined by the Swab Contact Test.<sup>c</sup> (T-2 rinse treatment) (T-3 wash treatment) (T-4 sanitize treatment).<sup>d</sup> These numbers are the average of three replications.

TABLE 3

Bacterial counts per 4 square inch of surface after various cleaning treatments using the direct agar contact test

Expt. No.	Finish No. 2B Cleaning treatment				Finish No. 3 Cleaning treatment				Finish No. 4 Cleaning treatment				Finish No. 7 Cleaning treatment				
	SPC <sup>a</sup>	T-1 <sup>b</sup>	T-2 <sup>c</sup>	T-3 <sup>c</sup>	T-4 <sup>c</sup>	T-1	T-2	T-3	T-4	T-1	T-2	T-3	T-4	T-1	T-2	T-3	T-4
	(× 1,000)	(× 1,000)				(× 1,000)				(× 1,000)				(× 1,000)			
1	60	21	67 <sup>d</sup>	5 <sup>d</sup>	4 <sup>d</sup>	27	29	3	7	32	37	11	6	15	62	23	9
2	43	36	30	2	0	29	21	3	1	16	27	7	2	31	43	4	5
3	68	39	136	2	1	32	98	11	1	41	93	1	3	21	133	6	0
4	58	26	28	5	2	21	28	4	0	29	21	5	1	23	20	4	3
5	34	20	13	0	0	9	25	5	1	20	22	1	2	24	22	9	1
6	63	29	19	5	0	45	23	2	1	46	38	10	5	27	41	10	2
7	50	22	23	1	1	32	25	2	1	30	24	3	0	20	51	2	1
8	37	24	23	4	2	16	22	6	3	28	25	7	3	11	28	3	3
9	60	23	27	2	3	19	47	13	1	16	64	7	1	23	47	8	3
10	63	13	68	7	3	26	97	6	2	40	94	19	3	19	81	14	8
11	45	26	25	5	1	18	20	3	1	19	57	6	1	17	41	4	0
12	67	35	13	2	1	40	29	7	2	51	20	10	1	34	37	16	7
13	73	31	43	5	5	35	30	9	4	36	47	3	4	33	70	3	12
14	46	47	31	14	6	48	22	12	3	31	52	23	2	32	52	20	9
15	55	37	53	7	5	28	62	8	5	19	62	6	10	23	75	16	15
Av.	55	29	40	4	2	28	39	6	2	30	46	8	3	24	54	9	5

<sup>a</sup> Inoculum level as determined by the SPC test. Statistical analysis is based on these data.<sup>b</sup> T-1 inoculum level as determined by the Direct Agar Contact Test.<sup>c</sup> (T-2 rinse treatment) (T-3 wash treatment) (T-4 sanitize treatment).<sup>d</sup> These numbers are the average of three replications.

TABLE 4  
Summary of results of analysis of variances at 5% significance level

Cleaning treatment	Direct Surface Agar Plate Test
T-2 <sup>a</sup>	No significant difference in cleanability of 2B, 3, 4, and 7 finishes
T-3	No significant difference in cleanability of 2B, 3, 4, and 7 finishes
T-4	No significant difference in cleanability of 2B, 3, 4, and 7 finishes
	Swab Contact Test
T-2 <sup>b</sup>	A significant difference in cleanability of 2B, 3, 4, and 7 finishes
T-3	No significant difference in cleanability of 2B, 3, 4, and 7 finishes
T-4	No significant difference in cleanability of 2B, 3, 4, and 7 finishes
	Direct Agar Contact Test
T-2	A significant difference in cleanability of 2B, 3, 4, and 7 finishes
T-3	A significant difference in cleanability of 2B, 3, 4, and 7 finishes
T-4	A significant difference in cleanability of 2B, 3, 4, and 7 finishes

<sup>a</sup> (T-2 = Pre-rinse + rinse), (T-3 = T-2 + det. wash and rinse), (T-4 = T-3 and Cl. sanitization).

<sup>b</sup> No significant difference at the 1% level.

bating conditions differ in the DSAP from the Standard Plate Counting procedure as recommended by *Standard Methods* (1) does not materially influence the growth and detection of the organism employed, as an analysis using Fisher's "t" test to compare these tests indicated no significant difference using the control data given in Treatment T-1.

Although no significant difference was observed among the four finishes, after exposure to any specific cleaning treatment with the DSAP test, it is important to note that the more nearly the experimental cleaning treatment approached the recommended practice of washing and sanitizing, the better the degree of cleanliness as indicated by the decrease in bacterial population (Table 5). This is clearly indicated in the DSAP test by the decrease in count on a 2B finish from 112 to 23 to 12 per sq. in. (gr. av.), after T-2, T-3, and T-4, respectively. On a No. 3, 4, and 7 finish the counts decreased following the improved cleaning cycle as follows: 92-21-9, 102-24-11, and 97-24-13, respectively. On a percentage basis, using the grand average values, detergent washing (T-3) reduced the count from that obtained on rinsing by 80%; washing followed by sanitizing reduced the count obtained on rinsing by 89%. Although this represents only a 9% decrease based on the number remaining after rinsing, sanitization actually decreased the number remaining after washing by about 50%.

The data in Table 5 indicate that compliance with the minimum recommended standard (50/4 sq. in.) was obtained in only 16% of the trials on the 2B finish after the mildest cleaning treatment; under the same conditions, the No. 3, 4, and 7 finishes complied 14, 13, and 16% of the time, respectively. It is apparent from this that in the great majority of the trials all finishes were improperly cleaned using experimental mild pre-rinse, rinse treatment. Detergent washing reduced the bacterial load considerably from that observed after rinsing. Compliance with the standards was observed in 100, 91, 96, and 93% of the trials on the

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TABLE 5  
Summary table showing average bacteria counts and the per cent compliance with recommended maximum standards

	Direct Surface Agar Plate				Swab Contact				Direct Agar Contact			
	Finish No.				Finish No.				Finish No.			
	2B	3	4	7	2B	3	4	7	2B	3	4	7
	Prerinse and rinse (T-2)											
% Accept <sup>a</sup>	16	14	13	16	56	63	53	38	67	73	64	49
High <sup>b</sup>	255	195	224	211	352	243	263	301	136	97	64	133
Low	40	53	28	28	21	11	21	13	13	21	20	20
Average	112	92	102	97	78	72	80	102	40	39	46	54
	Rinse and wash (T-3)											
% Accept	100	91	96	93	100	100	100	100	100	100	100	98
High	46	32	77	75	21	33	25	21	14	13	23	23
Low	10	9	6	7	5	2	2	4	0	2	1	2
Average	23	21	24	24	11	11	11	9	4	6	8	9
	Rinse, wash, and sanitize (T-4)											
% Accept	100	100	100	98	100	100	100	100	100	100	100	100
High	46	19	31	35	12	11	11	18	6	7	10	15
Low	2	3	1	2	0	1	1	1	0	0	0	0
Average	12	9	11	13	4	6	5	7	2	2	3	5

<sup>a</sup> Per cent less than the maximum recommended level of 50 per 4 sq. in.; based on 45 analyses in most cases.

<sup>b</sup> From top to bottom these figures represent the highest count per 4 sq. in. (average), the lowest count (average), and the grand average count taken on 15 experimental trials, using three replications per trial.

2B, 3, 4, and 7 finishes, respectively. Although satisfactory compliance was observed in the majority of instances after rinsing and washing without sanitization, it is important to note that chlorine sanitization provided the additional kill to make it possible to obtain 100% compliance on the 2B, 3, and 4 finishes and 98% compliance on the No. 7 finish. On the basis of the statistical analysis employed, no significant difference can be attached to any of the actual or percentage figures within the same cleaning treatment. The high rate of compliance for all finishes after a complete cleaning cycle, as contrasted with the low rate of compliance for all surfaces after only a poor rinsing, indicates the desirability and need for adherence to the recommended cycle to insure the desired end point. Since the experimental cleaning techniques approximated CIP spray-cleaning principles in a minimum manner, and since the data showed excellent compliance (average count of 4 per 4 sq. in.) after a complete cleaning cycle, the feasibility of successful CIP operations using 2B, 3, 4, or 7 finishes is indicated.

*Swab Contact Test.* Using the Swab Contact Method as a technique to measure cleanability, as expressed by the number of bacteria which can be removed by swabbing, a difference at the 5% level was observed among the four finishes examined after T-2, but no difference at the 5% level was observed after T-3 and T-4. The criterion was 2.69 and the F value was 2.848 in the T-2 series; these values are in very close agreement, considering the biological nature of the study, and indicate that the postulated difference may be very slight. The distribution of the grand averages, as given in Table 2 for T-2 (78-72-80-102), are suggestive

of this close agreement with regard to cleanability of the four finishes. A closer inspection of the individual data indicates some wide variations which are considered in the analysis of variance pattern and which contribute to the finding of the significant difference observed in T-2. When T-2 is tested by an analysis of variance at the 1% level, no difference among finishes is noted. An attempt to determine the exact locus of this difference in the group was undertaken, using the Multiple Range Test of Duncan (3). This test showed a difference between the No. 7 and the other finishes after T-2 with the Swab Test. The highest count was on No. 7.

The actual average counts obtained using the Swab Contact Test are given in Table 5. Where bacterial levels are high (greater than 250/4 sq. in.) the swab test tends to give higher values than either of the other tests. This may be due to crowding, making accurate counting difficult with the DSAP when the bacterial level is greater than about 100. In almost all other instances, the DSAP count was higher than the swab count; this is evidence of the more critical nature of the DSAP test. At low population levels (less than 30/4 sq. in.), the swab test was invariably lower than the DSAP, indicating the failure of the former to release bacteria from the swab or to remove them from the surface, when present in low concentrations. It is for this latter reason that a high percentage of acceptable counts was obtained after T-2 (56-63-53-38%). The inability of the swab test to detect low levels of bacterial contamination also explains the 100% acceptability observed after T-3 in all cases. As was evident in the DSAP data, washing with detergent removed the greatest majority (86%) of the bacteria which remained on rinsing; sanitization reduced the level to 95% on the same basis. Sanitizing per se, however, reduced the contamination level about 64% in the case of the 2B, 3, and 4 finish. This compares favorably with the results obtained using the DSAP test.

*Direct Agar Contact.* The results obtained with the Direct Agar Contact Test are shown in Table 3. A significant difference at the 5% level was found between the 2B, 3, 4, and 7 finishes after T-2, T-3, and T-4. The inefficiency of the Direct Agar Contact method in establishing close contact between the panel and agar surface is, in part, responsible for the different findings obtained with this test, as compared to the DSAP and swab tests. Visible air bubbles were occasionally entrapped between the steel surface and the agar; this prevented the intimate contact on which this test is dependent. The failure to establish contact with the bacteria on these areas would result in inefficient cell recovery. The two 5-sec. contact periods also were inadequate to effect bacterial removal. The inefficiency of bacterial removal is clearly indicated by the data in Tables 3 and 5; in every instance the counts are less than those obtained with the DSAP test. Because of these limitations, the results obtained with this test should not be interpreted with respect to relative cleanability of the test finishes.

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