This study is concerned with qualitative and quantitative recovery of bacteria from laundered toweling to determine proper laundering of fabrics. A number of issues are investigated, findings presented, and the implications discussed.

CRITICAL ANALYSIS OF THE MICROFLORA OF TOWELING

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Introduction

THERE can be no argument with the concept that the best practices in modern laundering are to be found in commercial and/or large institutional laundries characterized by capable managers, competent and experienced personnel, reasonably efficient equipment, and suitable washing formulae. The expertise and efficiency of such reference standards, when directed to the finished product, should be outside the realm of regulatory coercion, for there is a constant concern by such laundries to provide hygienically safe products—despite the absence of minimal, acceptable, standards or definitions of what constitutes "hygienically clean."

The definitive American sanitary study of commercial laundry practices was that of Arnold.[1] Conclusions based on data from this comprehensive evaluation were the basis for recommended procedures of the American Institute of Laundry[2] as well as for the American Hospital Association.[3] The summary of this study can be paraphrased for the critical issues: (1) High temperature washing formulae for white clothes and fabrics have sufficient temperature and holding time to insure a safe procedure *from a public health standpoint;* (2) the low temperature washing formulae used for other classifications of washable fabric may require more attention; and (3) the extraction and ironing procedures remove or kill the residual adherent bacteria in all instances studied.

A second critical American study was that of Church and Loosli[4] on the role of laundry in the recontamination of washed bedding. These authors, while generally in agreement with the first study, could not corroborate Arnold's data on the sterility of products after extraction and ironing, and warned that ". . . a good laundry process for removing bacteria and other pathogenic agents becomes of little importance if the environment in which it is carried out serves only to recontaminate the bedding with the same organisms."

The data of Church and Loosli apparently have not received the same widespread creditability given the Arnold study—yet each was definitive in its own sphere. The former authors were admittedly concerned with potentials for disease agent transmission and, correctly and very carefully, phrased the published literature on "secondary reservoirs" of infectious agents.

The concept that all washed and ironed laundry or linen is sterile, of course is not true. The concept that all autoclaved fabrics or linens are sterile likewise is not true. The former contention ignores factors of time, in either

chemical or thermal destruction of bacteria (or viruses), as well as those from human handling after washing. The latter opinion ignores human errors in overloading autoclaves with minimum heat-pressure-time operational cycles as well as the vagaries of storage conditions and times.

Nevertheless, and despite modern gravitation to increasingly aseptic environments, the stated goal of laundering still remains the removal of soil loads from fabrics by solvation, emulsification, and dispersion. In less absolute terms, it seems reasonable to accept that a properly laundered fabric should be one free of acquired soils and those contaminants associated with soiling. Ridenour[5] evaluated factors of suitable equipment design of automatic washers by microbiologic techniques, but the concern of this classic report was not to evaluate naturally occurring microbial soil populations.

Factors that influence the validity of recoverable and viable microbial counts (types and numbers) from laundry effluents and finished linens include:

(a) weight ratios (cloth:water:chemicals);
(b) bound water (diffusion and stabilization of chemicals);
(c) temperatures;
(d) wash supply sequences;
(e) flushes and rinses;
(f) bleaching agents;
(g) sours, finishing agents, and adjunct procedures;
(h) extraction and ironing;
(i) final packaging;
(j) environmental and human contamination of product;
(k) suitable methods for sample collection, transport, and processing.

The issues of concern and of interest here are those which affirm or deny that: (1) There may be a kind of indigenous fabric-associated microflora; (2) it is possible under stated conditions to differentiate between exogenous soil microflora and endogenous fabric-associated microflora; (3) bacterial sensitivity or resistance to heat, chemicals, pH, and so on, will vary according to resident microflora; (4) microbial sensitivity to microenvironmental conditions will differ between "naked" populations and "fabric-adherent" populations; and (5) the conflicting reports of published literature may be based on the contention that, the more sophisticated and comprehensive the laboratory evaluation, the greater will be the disparity in qualitative and quantitative reports among laboratory reports.

A logical assumption, not yet clearly defined, is that the microflora of washed linen somehow must correlate mathematically to the microflora of the effluents of a washing cycle. In this paper we will examine this concept to some extent.

Methods and Materials

Diluents

In this study, "saline" refers to 0.85 per cent sodium chloride in distilled water at pH 7.0-7.2; phosphate buffered saline (PBS) refers to 0.85 per cent sodium chloride in distilled water with 20 ml/L Beckman pH 7.0 buffer concentrate; and "LN-diluent" indicates 0.85 per cent sodium chloride, with 5.0 gm sorbitan monooleate (tween 80,)* 0.7 gm asolectin,† 20 ml Beckman 7.0 buffer concentrate, and 5 gm yeast extract per liter. Such diluents are used for collecting wash cycles effluents, for dilution blanks, and for processing cotton swatches in "rinse-extract" procedures.

Growth or Recovery Media

This study is concerned with qualitative (types) and quantitative (numbers) bacterial recovery. Severe limits were

* Emulsion Engineering, Inc., Elk Grove Village, Ill.

† Associated Concentrates, Inc., Woodside, Long Island, N. Y.

imposed by the choices of selective media, for it was essential to obtain quantitative recovery from whatever media proved suitable.

Surface-streaked inocula of 0.1 ml, in duplicate, were performed on the following basic BBL* media: blood agar (trypticase soy agar, and 0.5% yeast extract) (BAP), phenylethanol agar with 6 per cent blood (PEA), Tergitol-7 TTC agar, MacConkey agar, mycophil agar (MYCO), Sabouraud dextrose agar, and Herman and Morelli egg yolk modified Staphylococcus 110 agar.[6] Total viable counts were obtained by "pour plate" techniques (1.0 ml/plate in duplicate) using trypticase soy agar with yeast extract and glucose (TSA/YE), and by TSA/YE reconstituted in LN-diluent (TSA/LN). Occasionally "pour plate" data are reported as "embedded" counts.

Modified "most probable number" (MPN) series for the enumeration of spores were prepared in duplicate 3-tube systems using TSB/YE and fluid thioglycollate medium with glucose and resazurin (BBL No. 11260) (THIO). All MPN assays limited exclusive to heat-shocked samples (80° C/10 min) were gross indicators of spores whether Bacillus spp. or Clostridium spp. Rarely, micrococci survived the process in very low numbers. Incubation temperatures for the MPN spore series were held at or below 35° C and thereby excluded thermophilic species. MPN spore data are usually reported as counts per ml instead of 100 ml, and sometimes recalculated for the basic unit, "per square inch."

MPN coliform procedures were limited to "wet out" effluents or rinse-extracted soiled towels. When employed, the methods were by standard procedures.[7]

Impression sampling utilized Rodac plates made of TSA and TSA-LN. Contact was at 5-8 psi for five seconds.

Assay Materials

Sources for the bacteria of concern to this study were either effluents from each step in a commercial washing cycle or suspensions obtained by "rinse extraction" of swatches from continuous white or blue toweling.

Hot effluents were collected as one-pint samples directly from the washer dump valve at a time judged to represent a midpoint sample or at the end of a defined step. The samples were split immediately. Portions were measured into sterile disposable plastic tubes containing chilled (4° C) diluent, and the balance was measured for "field" pH and dump valve temperatures. The portion intended for the viable count was chilled immediately to 4° C and such specimens were processed within four hours of collection.

In a laminar air flow clean room† swatches were cut aseptically from specimen lengths of continuous white or blue toweling—each of which contained only one woven hem or edge—using a sterile stainless steel six-inch square template. The swatches were folded and placed individually in sterile Petri plates, being handled by a gloved and gowned operator. After weighing, the swatches were minced with sterile shears into a one-quart, new, sterile paint can.

Five hundred ml of diluent were added to the minced cloth and processed as "rinse-extract" procedures for ten minutes at 26° C on a Red Devil paint shaker.‡ Fluid specimens were withdrawn by 10-ml wide-bore pipettes, delivered into two sterile 125x15 mm

* Baltimore Biological Laboratories, Division of BioQuest, Cockeysville, Md.

† Model 43 Laminar Flow Clean Work Station. Agnew-Higgins, Inc., Garden Grove, California.

‡ Red Devil Paint Conditioner. London P. Smith, Inc., Irvington, New Jersey.

screw-capped tubes; one was chilled immediately (10° C), and the other was heat-shocked for ten minutes at 80° C to destroy viable vegetative cells.

Decimal dilutions were prepared with suitable chilled diluent (10° C) and samples were either streaked in duplicate onto selective plates as 0.1 ml quantities with sterile angled glass rods, or processed as "pour plates" with 1.0 ml/plate, in duplicate. Incubation was at 35° C for 12 to 15 hours, then at 24° C for 24 hours.

MPN growth media received 1.0 ml inoculations; three tubes of TSB/YE for each decimal dilution, and three tubes of thioglycollate medium, similarly. After suitable incubation, growth was recorded as the number of positives per total number inoculated in that dilution. Subcultures were made, as streaked blood agar plates, to determine the types of bacteria which survived the heat-shocked treatment. Percentile population densities were derived from these observations. (No great confidence is placed in the MPN values obtained, but the sequence did permit scoring population densities of spores as a decimal fraction of the total viable count.)

Results and Discussion

Impression Sampling

Impression sampling, especially by Rodac plate, has become a routine bacteriological technique in modern American environmental assay. The limitations of Rodac impression sampling are usually considered to be unimportant, relative to the advantages to be gained. It is unlikely that 100 per cent of the recoverable bacterial population is attained from impervious surfaces by impression methods, even under optimal contrived conditions. It follows that quantitative microbial analyses require attention to be given the limitations or disadvantages of any method, and for impression sampling, some of the limitations include:

(a) uncertainty of percentile efficiency of viable particle recovery;

(b) uncertainty of amount of bacteriostasis generated by newly solubilized chemical residues from sampled surfaces;

(c) The lack of qualitative discrimination among various bacterial genera or species.

Although these issues have been recognized or studied by innumerable investigators, they must be reexamined for each new environmental application not yet thoroughly documented.

Qualitative bacterial recoveries from noncoated fabrics are monumentally difficult to achieve by impression sampling. Table 1 indicates that fabric-impinged bacteria do not imprint on Rodac plates with any degree of predictable regularity. Low-count washed cottons show no more relationship of Rodac data to other quantitative methods than those from the most extreme of random soiled towels, in the same context.

It is obvious from Table 1, when using Rodacs, that the best range of bacterial recovery from washed cotton toweling is 0.35 to 0.07 per cent of the total available bacteria; and from 0.05 to 0.00001 per cent for random soiled cotton towels. *Such data remove all hope for any bacteriologic value to be accrued by Rodac impressions from noncoated fabrics.*

The lack of "quantitation" is attributable to the strong adhesion and entrapment of microbial cells or spores by the amount and roughness of the surface areas of cotton fibers. Lubricants, in the final rinse, prepare the external, or exposed, two planar surfaces in such a manner that Rodac recoveries generally are limited to random particle "fallout" or bacteria deposited by human handling. The recovered populations have no necessary relevance or correlation to the entrapped biota within the fabric—by numbers or by types—

Table 1—Bacterial enumeration fallacies by impression sampling

Paired sets	Identity	% area	No. of plates*	No. (+) plates	Count range	Total count	Average/ sq. in.	Rinse extract avg./sq in.	
								Total	Spores
1	New, prewash	56.8	10	3	0–3	5	0.12	56	<2
	New, washed	56.8	10	7	0–3	10	0.25	351	194
2	Random soiled	56.8	10	10	10–28	196	4.79	9,236	1,417†
	Random washed	56.8	10	7	0–4	10	0.25	236	14
3	Exptl, pre-6th washed	56.8	10	10	18–48	348	8.51	16,146	7,986†
	Exptl, 6th washed	56.8	10	5	0–3	8	0.20	63	313†
4	Random 6th soiled	56.8	10	10	30–54	389	9.51	3,142	361†
	Random 6th washed	56.8	10	5	0–2	6	0.15	73	35
5	Random 7th soiled	56.8	10	10	>200	>2,000	>49.00	4.44×10^8	>69†
	Random 7th washed	56.8	10	7	0–2	9	0.22	253	188
6	New 2 washed	56.8	10	6	0–4	13	0.32	260	35
	Desized 2 washed	56.8	10	7	0–2	13	0.32	184	56
7	New 3 washed	56.8	10	5	0–2	7	0.17	21	49
	Desized 3 washed	56.8	10	6	0–1	6	0.15	108	<4

* Equal sets TSA and TSA-LN.
† Clostridium sp. present.

and it is the embedded populations which must be studied to evaluate the efficiency of any wash formula. In this regard our conclusions are at considerable variance to those of Robinton and Mood.[8]

It is known that very few of the available selective media have quantitative relevance when utilized in impression sampling. There is apparently some confusion in the minds of many who enumerate environmental bacteria, in their belief that Rodac results will correlate well with those derived from membrane technology. This belief is manifestly not factual. One tends to overlook the fact that membrane techniques permit unlimited dilution, washing, chemical neutralization, and preenrichment before the use of selective components. None of these valuable procedures can be applied readily or efficiently to Rodac populations. Further, even one of the original intents of impression sampling, i.e., replication, largely fails in applications—apart from pure culture studies, or for very few bacterial species, such as Bacillus spp, Staphylococcus aureus, Pseudomonas aeruginosa, and several others.

In the face of possible resolubilization of disinfectant or chemical bacteriostatic residues, usual good practice calls for incorporation of chemical neutralizers into the recovery medium. However, some chemical neutralizers cannot work efficiently in solidified substrates.

Likely materials for chemical neutralization include: thiosulfate, asolectin-polysorbate mixture, serum, and so on. Unless positive knowledge is at hand for (a) tetrathionate sensitivity, as per Mycobacterium tuberculosis, or (b) letheen sensitivity, as per some varieties of Streptococcus pyogenes, it is urged that convincing and suitable controls be structured carefully around the use of chemical neutralization.

In Table 1, half the Rodac plates were compounded of TSA-glucose, the other half of TSA-glucose-LN. Inspection of the results obtained from cotton swatches by Rodac plates reveals that little difference is obtained by either recovery medium when the final sour or rinse included one or more of the following: phenylmercuric acetate (PMA), phenylmercuric propionate (PMP), quaternary ammonium salts (QAC), tributyltin (TBT), and sodium phenylphenolate.

In the belief that, in freshly laundered cottons, there is an acceptable homogeneity in bacterial distribution by numbers and by types, and with the certain knowledge that bacteriostats, lubricants, and softeners are used, one may recalculate the table. This reveals that 432 colonies were obtained by TSA-LN and 426 colonies by TSA, all from 12 swatches of cotton toweling, using 60 plates of each medium. It appears that the LN neutralizer is unnecessary or nonfunctional under the conditions of use, an observation in agreement with the limitations of this material in solidified substrates.

To assume there was no resolubilization of fabric-adsorbed chemical residue is erroneous in the face of occasional moderate-to-heavy surfactant levels in the "rinse-extract" technique. Conclusions derived from the recorded data direct one to believe that either all of the released and enumerable bacteria were not sensitive to the resolubilized chemicals or else that those bacteria which were sensitive to the systems had already died or were irreversibly damaged, leaving only nonsensitive survivors. The latter judgment gains substantial support from the variable temperature systems employed by Quan, et al.[9]

From various hospitals and institutions, a number of representative cotton fabrics were evaluated by impression sampling and by "rinse-extract" methods. The cotton items include diapers,

Table 2—Description of hospital and institutional fabrics

Specimen No.	Specimen identity	Specimen size (sq in.)	Specimen weight (gm)	Impression sampling			
				Area sampled %	No. of plates	No. (+) plates	Count range
1*	Baby shirt	36	5.4186	56.8	10	0	0
2*	Diaper	36	3.9468	56.8	10	1	0-1
3	Diaper	36	3.7801	56.8	25	1	0-1
4	Absorption pad†	36	2.9311	16.0 (est.)	12	3	0-31
5	Washcloth	36	7.2357	56.8	20	7	0-3
6	Pillowcase	36	3.9104	56.8	20	10	0-6
7	Sheet	36	5.2952	56.8	10	10	11-26
8	Sheet	36	4.7679	56.8	10	3	0-1
9*	Sheet	36	3.8987 (no hem)	56.8	10	0	0

* From "sterile" packs.
† Absorption pad (disposable) sampled after stripping outer layers.

Table 3—Quantitative microflora of fabrics (counts per square inch)

Specimen No.	Total Rodac count	BAP	PEA	TSA	TSA-LN	(80 C/10 min)		
						TSA-LN	Thio	TSB
1*	0	<35†	ND	7	63	<4	<1.2	<1.2
2*	1	<35	ND	21	56	<4	<1.2	<1.2
3	1	70	ND	56	21	<4	<1.2	<1.2
4	35	<35	ND	52	142	7	9‡	9
5	12	347	ND	205	236	<4	5	<1.2
6	20	139	ND	600	254	49	93	28
7	183	1460	<35	368	438	7	5	9
8	3	<35	<35	76	63	<3.5	<2.3	<2.3
9*	0	<35	<35	56	77	<3.5	<2.3	<2.3

* From "sterile" packs.
† <n = limit of system; "n" may be zero.
‡ Cl. perfringens present.
ND = not done.

washcloths, Turkish towels, baby shirts, laboratory coats, pillowcases and sheets. Some were soiled, most were "central supply" items, and a few were from sterile packs. Tables 2 and 3 detail the important criteria or description of selected items and the quantitative microbial recoveries taken therefrom.

Table 4 shows the lack of fit of impression sampling data when contrasted to "rinse-extract" results. This table indicates in several instances—i.e., in numbers 3, 5, and 6—that multiple replication of the same site may or may not lead to increased bacterial recoveries. Consistent with data presented by the senior author earlier this year,[10,11] the issue of concern when sampling

fabric by Rodac pivots on the interpretation of negative plates. There will be an unmistakable presumption by nontechnical professionals to believe that a negative Rodac equates to a sterile surface. This is not the case at all. Even carefully controlled "rinse-extract" procedures will show a viable, albeit low, bacterial population to be derived from standard packs of "sterile" supplies (Table 4). It may be assumed that a closely controlled "macerate" system would recover even higher values than does the "rinse-extract" system (Wetzler,[12] Nicholes[13]).

The foregoing data clearly establish the incapacity of Rodac plates to accommodate recoveries of fabric-entrapped bacteria or spores. Similar conclusions have been reached by Nicholes[13] while comparing Rodac recoveries to "macerate" enumerations. Therefore, "impression" sampling cannot be relied upon as a device to establish whether or not there is a mathematical correlation of fabric-associated bacteria with those recoverable from wash effluents; this is because impression systems cannot provide an acceptable enumeration of fabric-associated bacteria, whether by bacterial types or by numbers (bacterial colony-forming units).

There may be intense criticism of our conclusions on the nonpertinence or fallacy of Rodac sampling of noncoated fabrics. We suggest to those concerned with environmental surveillance responsibilities that there are few, if any, inexpensive rapid field monitors for quantitatively assaying noncoated fabrics in a nondestructive manner. Perhaps the alternative is to direct emphasis to more critical areas amenable to Rodac sampling, when destructive sampling is ruled out.

As will be established later, one should be concerned more with the types of recoverable bacteria than with a gross nonspecific total count.

Experimental Wash Formula for Continuous Towels

It is the consensus of opinion in the rental laundering industry that the most

Table 4—Quantitative summation of hospital fabric bacteria

Specimen No.	Total available area (sq in.)	Area sampled by Rodac (sq in.)	"Rinse-extract" bacteria available (per sq in.)	"Rodac" bacteria available (per sq in.)
1*	72	40.9	63	0.000
2*	72	40.9	56	0.024
3	72	40.9	56	0.000 0.024 0.000
4	280	40.9	142	2.934
5	72	40.9	236	0.195 0.073
6	72	40.9	600	0.195 0.293
7	72	40.9	438	4.474
8	72	40.9	76	0.734
9*	72	40.9	77	0.000

* From "sterile" packs.

Table 5—Denver experimental "use-wash" series (counts per 0.1 gram)

No.	BAP (1)	PEA (2)	MYCO (3)	TSA (4)	LN (5)	Avg (4+5)	Δ (80°/10 min) LN	Thio	TSB
D–I (4.7093 gm) (new)	159	159	<27*	58	27	43	<3	———α ———β ———γ	———α ———β ———γ
D–I–C (5.7395 gm) (washed)	174	87	<22	222	218	220	122	+—— ——— ———	+++ ++— +——
DT–I–S (5.2154 gm) (soiled)	4,794	2,636	<24	6,711	5,369	6,040	978	+++ +++ ———	+++ +++ +——
DT–I–C (5.4283 gm) (washed)	230	230	<23	184	129	157	9	——— ——— ———	+++ +—— ———

* <n may be zero count.
$\alpha = 10^{-1}$
$\beta = 10^{-2}$
$\gamma = 10^{-3}$

difficult item to launder is a continuous towel. These items are usually 10 inches wide and 50 yards long when new. As received from the mills, such towels are relatively spore-free and possess a very low total bacterial count. In this initial condition, the cotton towels have excess sizing and finishing chemical residues which tend to "scavenger" fine soil particles and bacteria during the first several washes. A notable yellowing is grossly visible during this early time period. Much of this can be easily obviated by prewashing before initial use.

Of all the soiled continuous towels, experience clearly incriminates the "slaughterhouse" towel as the most challenging, due largely to blood, particles of tissue and hair, tanbark, and so on. All slaughterhouse towels in this study were from Denver, Colorado.

A "heavy" soil-washing formula must be applied to a towel in this state of soiling. Severe constraints are imposed due to high tensile strength losses by indiscriminate use of oxidizing wash supplies. In general, a typical wash formula includes caustic, high-titered soap, temperatures in the range 182° to 190° F, hypochlorite bleaching and fungicidal additives after souring. The values of hypochlorites both for bleaching and for microbial destruction are considerable[14,15]; but some bacterial spores possess extreme resistance to hypochlorite action.[16]

Continuous towels are washed as tied bundles and present technical difficulties involving wash chemicals, penetration of supplies into bundle interiors, deflocculation and multiple rinses to remove chemical residues. Once initially wetted, such continuous towels retain approximately 50 per cent to 55 per cent bound water by weight. This may complicate perfusion of soluble wash supplies into bundle interiors, especially with oxidatively reactive hypochlorite bleaches.

Table 5 presents bacterial recoveries as counts per 0.1 gram. Although it may be inferred correctly that a square inch of dry clean toweling will approximate

0.12 grams on the average, and the data will correlate extremely well with that reference unit, it is our belief that the baseline, 0.1 gram, will prove to be more functional in time. This is a proposed arbitrary convenience, but it does accommodate the future obvious differences which will be encountered with different fabrics, weave, thread weight, age of fabric, and so on. Table 5 is quite typical of the results which may be anticipated from any procedure that is programed for quantitative (and qualitative) data.

At one time or another, we have utilized selective and differential media not listed in Table 5, such as: Tergitol-7, TTC; MacConkey agar, cysteine tellurite trypticase agar, Sabouraud dextrose agar, Herman and Morelli egg yolk medium, and others. Generally these media have not been productive in our hands.

We believe it is essential to obtain several sets of values, by multiple systems, and that they should have sufficient overlap in order to serve as system controls. Therefore, Table 5 presents an adequate illustration of the planning which can be structured for microfloral recovery:

(1) The evaluative fabric specimen may be thoroughly sampled by an "impression" technique in order to elucidate environmental "fallout" and human handling of finished product.

(2) The "rinse-extract" or "macerate" methods of fabric disintegration and release of microflora lend themselves directly to "fluid" manipulations.

(3) Given a fluid sample, a variety of direct systems can be organized to provide total viable counts: "pour plate" embedment (at multiple temperatures); "spot plate," millipore membrane application; or "spread plate" devices. These are always qualitative as to type of colonial recovery, and may be reproducibly quantitative.

(4) Indirectly, fluid specimens lend themselves readily to "most probable number" (MPN) devices, and in the data presented here, we do utilize this system in order to establish that the population which exists as spores can be so quantified.

(5) Direct side-by-side evaluations of test systems are possible and economical. Table 5 presents data recoverable with and without the intervention of a common "neutralizer," i.e., letheen.

(6) The system is thoroughly controlled and easily indicts human errors in manipulations. Chance and erratic contamination errors are readily noted when they occur.

Table 6—Experimental wash series

Step	Procedure	Viable count*		Δ-Shocked spore count*		
		Streak	Embedded	Embedded	TSB†	Thio†
1	"Wet Out"	1.01×10^6	6.4×10^5	4.1×10^4	>11,000	>11,000
2	Flush	3.5×10^4	4.95×10^4	1.71×10^4	11,000	2,400
3A	Wash supplies	2.38×10^4	2.05×10^4	1.65×10^4	11,000	2,400
3B	Steam and wash	3.13×10^3	4.65×10^3	4.05×10^3	4,600	230
4	Flush	—	—	—	—	—
5	Flush	—	—	—	—	—
6	Flush	309	210	216	750	9
7	Bleach	13	39	45	240	4
8	Rinse	—	—	—	—	—
9	Rinse	—	—	—	—	—
10	Sour finish	42	18	14	43	<3

* = per ml effluent.
† = 3-tube MPN spores recorded/1.0 ml.

Table 7—Summary of quantitative bacteriology

Item and state	Units	Total count	Δ–Spore count
Slaughter-house	1 sq in.	1.75×10^8	1.39×10^4
pre-wash*	0.1 gm	1.17×10^9	4.30×10^4
Slaughter-house	1 sq in.	3.89×10^2	5.60×10^1
post-wash*	0.1 gm	3.00×10^2	4.10×10^1
"Wet out" effluent†	1 ml	1.00×10^6	4.10×10^4
"Souring" effluent†	1 ml	1.80×10^0	1.40×10^0

* = 36 sq in., rinse-extracted, 10 min, 25° C., PBS (pH 7.0).
† = Chemically neutralized, buffered (pH 7.0), chilled (10° C).

A number of our pilot studies, as well as some published in the literature, point to environmental factors that affect efficiency of wash supplies and wash supply sequences. Considerably more study is required before we can predict validly the soil removal of any supply formula under variables of fabric type, fabric weight or volume, soil type, and so on.

Suggestive evidence is found in Table 6 that the combination of "festoons" of continuous towels in nets, when laundered in "open pocket" as opposed to "partition" type washers, presents results quite analogous to those expected in loose or piece work in identical types of machines. The data in this table corroborate the Arnold summaries[1] in the sense of virtually "no count" final effluents. Normally, bundles of continuous towels laundered in partition washers, with adequate supplies, give counts of at least 1×10^3/ml under the same sampling conditions.

Monitoring effluents does achieve a value, in the sense that the data indicate the relative efficiency of wash supplies to penetrate bundled towels. There is a kind of correlation of effluent microbiologic data to the chemistry available from the refined field analyses of Luechauer.[17]

A negative, or "no count," effluent does not mean that the laundered fabric will be sterile. The relative rate of penetration, emulsification, and defloc-

Figure 1—Distribution of typical slaughterhouse towel bacteria prior to washing

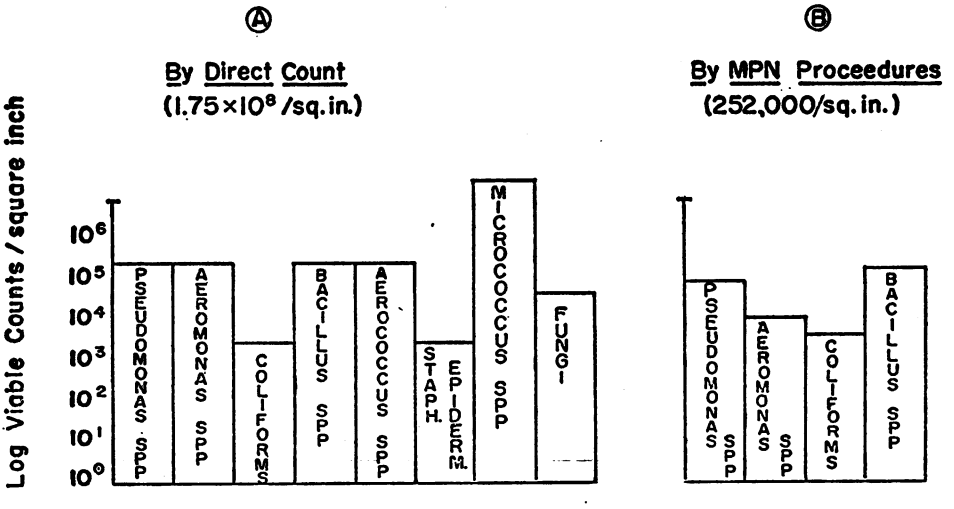


Table 8—Specific identification of bacteria from Figure 1

1. Pseudomonas aeruginosa: pyocin type 1, 3, 7 or nontypable
2. Coliforms: atypical E. coli, E. coli, C. freundii, Ent. liquefaciens, Ent. aerogenes, K. pneumoniae
3. Bacillus species: cereus, cereus-megatherium intermediates, megatherium, polymyxa, circulans, alvei, and five unidentified species
4. Fungi: Penicillium, Aspergillus, Mucor, and Rhizopus.

culation of wash supplies tends to lag in bundle interiors and does not reflect exact exterior criteria whether by chemistry or by bacteriology.

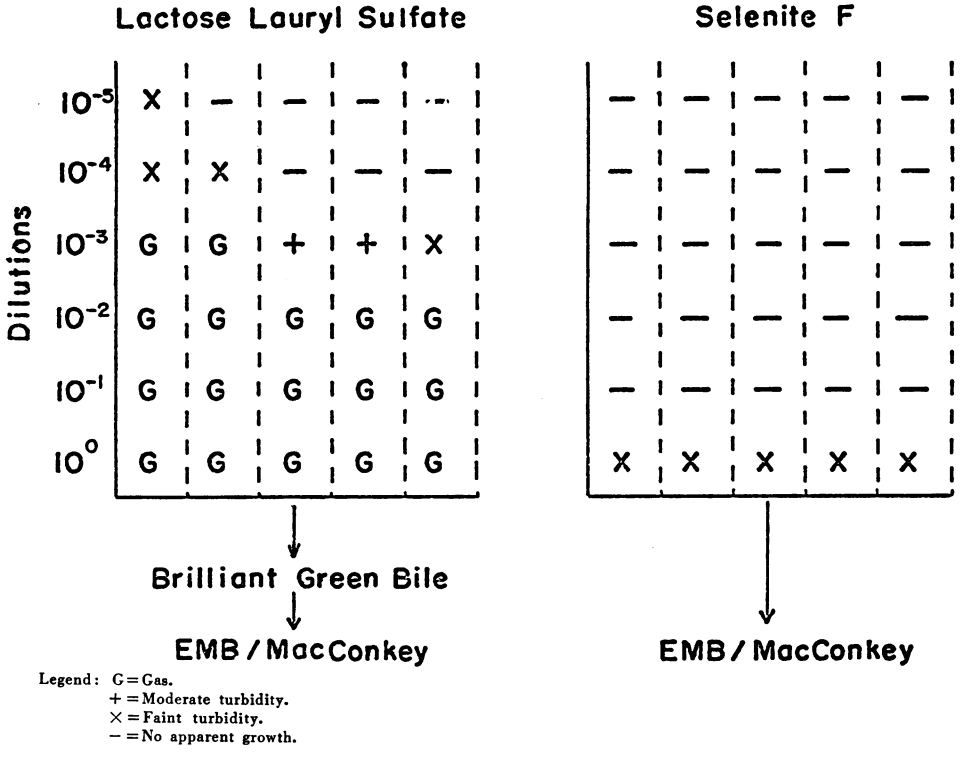
Qualitative Bacterial Composition of Soiled versus Washed Slaughterhouse Towels

It was deemed advisable to discover the microbial soil load (qualitatively and quantitatively) of a typical bloody slaughterhouse towel. Several 36-square-inch swatches were prepared for rinse-extraction elutions using LN diluent. After washing with an experimental Sanistat® washing program, the towels were extracted and ironed. Again, several 36-square-inch swatches were rinse-extracted in the described manner.

Table 7 presents average quantitative data relative to the soiled slaughterhouse towel, i.e., $1.17 \times 10^9/0.1$ gram of which $4.3 \times 10^4/0.1$ gram were spores.

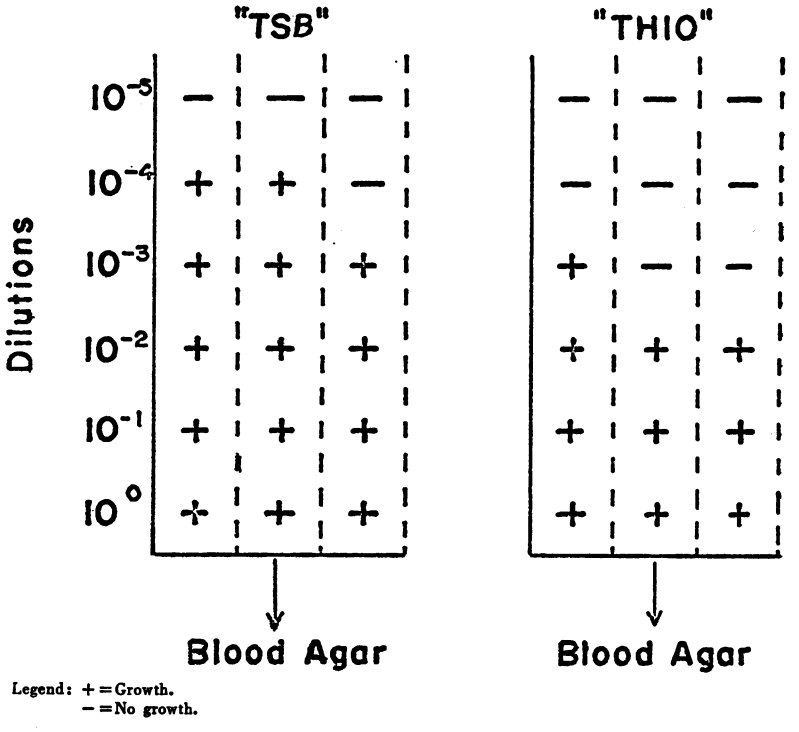
During the Sanistat® wash, the "wet-out" effluents revealed 1×10^6/ml of

Figure 2—MPN (coliform) slaughterhouse towel effluent



Legend: G = Gas.
+ = Moderate turbidity.
× = Faint turbidity.
– = No apparent growth.

Figure 3—MPN (spores) slaughterhouse towel effluent (Δ 80° C/10 min)



which $4.1x10^4$ were spores; whereas the final effluent rinses gave 2/ml which were spores. The finished towel, upon rinse-extraction, provided 300/0.1 gram of which 41/0.1 gram were spores.

Figure 1 presents the major types of bacteria which could be identified from the rinse-extracted, soiled, slaughterhouse towel. The gross counts of these genera are also estimated. It is noted that the predominant organisms were several Micrococcus spp., followed by Pseudomonas, Aeromonas, Bacillus, and Aerococcus genera, with the remainder comprising fungi, coliforms, and Staphylococcus epidermidis.

A conventional five-tube MPN procedure was provided using Lactose Lauryl Sulfate broth, and controlled with a five-tube selenite F selective broth series. Incubation for both sets was at 35° C; the former retained for 48 hours and the latter for 15 to 18 hours. Thereafter, all five tubes of all six dilutions were subcultured to Brilliant Green Bile broth for a presumptive and confirmed coliform MPN, and all these latter tubes were completed on EMB and MacConkey agars. All colonial types were identified and quantified.

By direct count procedures using surface-streaked enriched media we were able to establish an average of $1.75x10^8$/square inch; whereas by MPN procedures (taken from MPN coliform and MPN spore counts) we could establish only $2.52x10^5$/square inch.

Figure 1 shows that the numerical composition of several genera correlated rather closely for both procedures. Table 8 identifies the various isolates generically noted in Figure 1. Figure 2 outlines the laboratory manipulations which permitted our MPN values for coliforms (gas producers), for Aeromonas spp. (moderate turbidity, anaerogenic), and

for Pseudomonas spp. (faint turbidity, anaerogenic). From the selenite F series, we could only recover Pseudomonas spp. and then only from the nondiluted rinse-extraction fluids. This last system was highly inhibitory to the populations under study.

Figure 3 demonstrates typical data for MPN spores using TSB and Thioglycollate media. Many Bacillus megatherium spores will not germinate in thioglycollate medium, and we have found repeatedly that variations encountered, between TSB and thioglycollate recovery media, point conclusively to this fact.

Figure 4 is a graphic thermal-chemical bacterial destruction curve which we expect of a good washing formula that intends to provide a hygienically acceptable continuous towel. Naturally, a considerable dilution effect is ever present; between washing steps there should be a 44 per cent to 50 per cent dilution by effluent replacement. This is impossible for us to challenge mathematically because all viable particles in a bundled continuous towel are not available at the initial "wet-out" effluent stage.

Unless one has a carefully selected and controlled wash supply sequence, it is not unusual to wash only several

Figure 4—Temporal destruction of towel microflora in available effluents by Sanistat® process

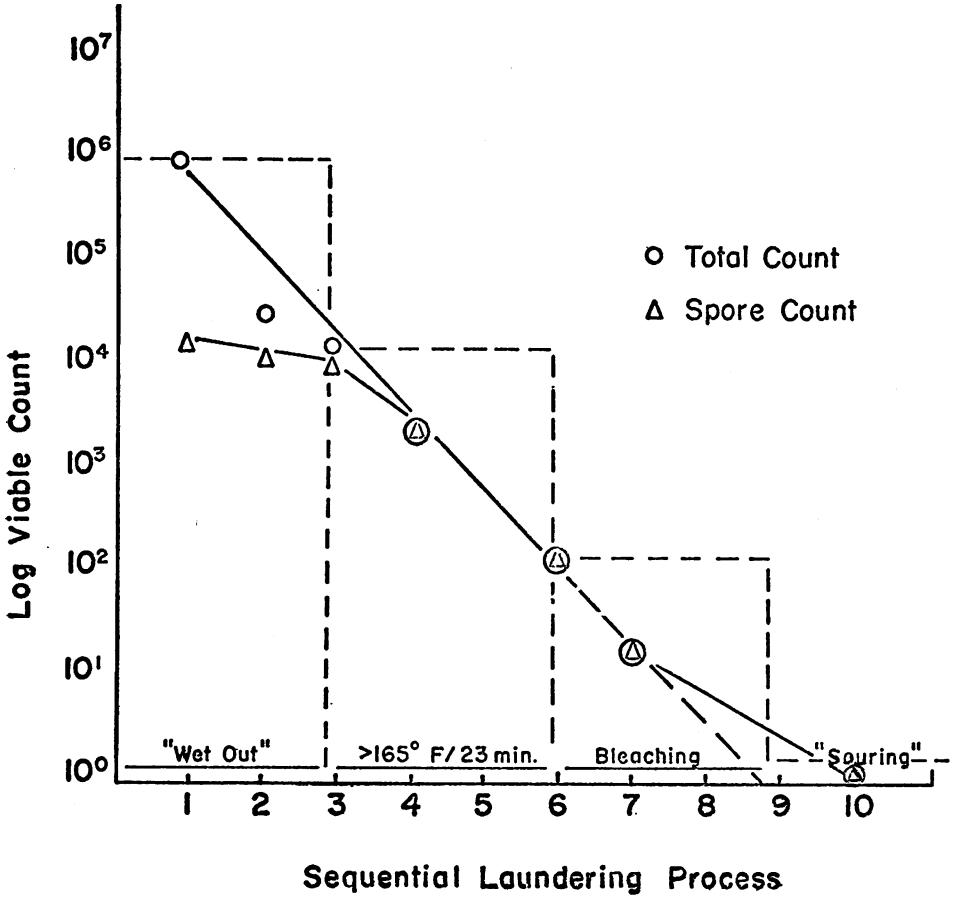
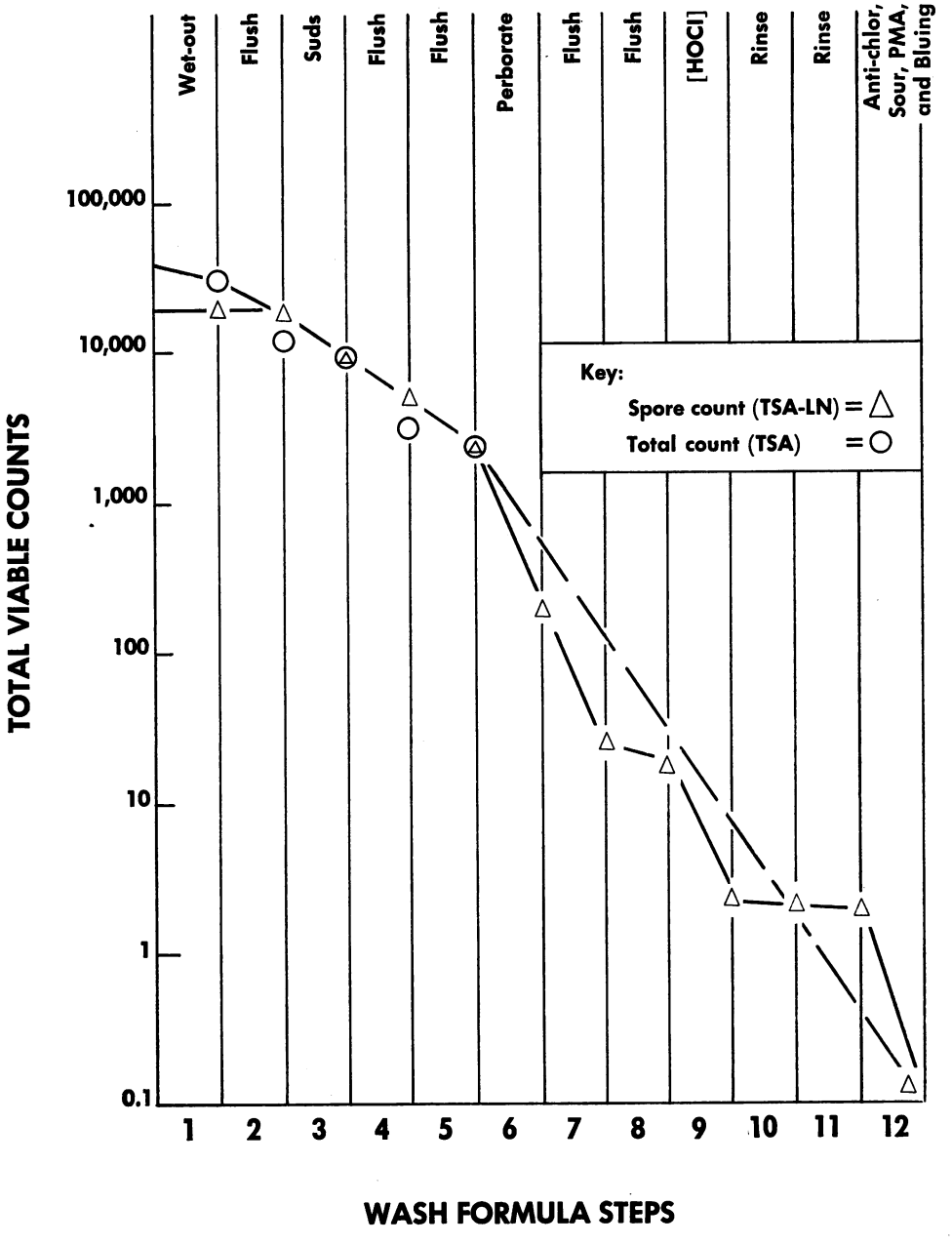


Figure 5—Microbial extinction by an experimental wash procedure for diapers



thicknesses of toweling, leaving bundle interiors virtually untouched.

An inordinantly heavy duty formula may lead directly to tensile strength losses of 10 per cent to 15 per cent.

Experimental Wash Formulae for Commercially Laundered Diapers

A typical Sanistat® formula was applied in a field trial in Chicago in June,

Table 9—Microbial reduction in "12-step" experimental wash applied to commercial diaper processing (counts per ml of effluents)

Sequence	No neutralizer		Buffered thiosulphate		Avg (1–4)	Per cent reduction by "step"	Δ TSA–LN (80°/10 min)
	TSA (1)	TSA–LN (2)	TSA (3)	TSA–LN (4)			
1	44,000	46,000	45,000	36,000	42,750	42,750 (initial)	41,000
2	49,000	42,000	27,000	27,000	36,250	12.9	39,000
3	25,000	25,000	13,500	14,450	19,500	54.4	18,000
4	7,900	9,000	6,900	7,900	7,925	81.5	7,100
5	3,300	4,200	4,300	4,400	4,050	90.5	4,400
6	—	150	—	100	125	99.7	135
7	—	60	—	50	55	99.87	46
8	—	50	—	45	48	99.888	23
9	—	2	—	3	2.5	99.99994	4
10	—	1	—	3	2	99.99995	8
11	—	3	—	4	3.5	99.99992	1
12	—	<0.25	—	<0.25	<0.25	>99.99999	<0.25

1969, at a commercial diaper service.

Figure 5 graphically illustrates the microbial destruction obtained by the supplies and the process. It should be noted that the vegetative populations never survived the flushes of the caustic-soap-heat combination, an observation consistently found with the Sanistat® process. This latter observation enables the incrimination of post-laundering contamination of a product by environmental "fallout" or by human handling.

Only spores survive the caustic-soap-heat process, and considerable evidence is now at hand that under field conditions *suitable oxygen bleaches are significantly more sporicidal than are the hypochloride bleaches* to selected Bacillus spp.

Table 9 reveals that the Sanistat® process is significant in gross reduction of microbes—more importantly, that no vegetative organism survives the process. It is obvious that the microbial population left for measurement after a "post-suds" flush consisted solely of Bacillus spp. spores. (The predominant populations were Bac. megatherium, Bac. circulans, and Bac. polymyxa. For the various geographic areas sampled to date, the predominant Bacillus spp. consistently found on slaughterhouse towels are Bac. cereus and, secondarily, Bac. cereus-megatherium intermediates.)

One might be tempted to argue that the collection of a pint of wash effluent is not an adequate sample and may have little relevancy to that contamination distributed randomly throughout 340 to 7,500 pounds of water, virtually half of which is considered "bound." The truth of the issue may not be acceptably proved here, but certainly the sample size, compared to the total available, is at least as representative as the typical water sample routinely collected for bacteriological analyses of raw or potable water supplies.

Further, there is no obvious reason why wash effluents prior to bleaching

methods should be treated differently in the laboratory than surface or raw water supplies; and why those collected after hypochlorite or oxygen bleaches should be treated differently than finished or potable water supplies. It can be said categorically that there is no evidence during our investigation of staphylococcal, coliform, enteric, or pseudomonal survival in either the post-bleached effluents or from the finished laundered fabrics.

Minimum Tolerance Standards

By way of a suitable summary, we should like to propose a type of Minimum Tolerance Standard which is achievable by commercial and institutional laundries and which does tend to define a "hygienically clean linen."

Qualitative Criterion

There shall remain on commercially laundered linens no vegetative bacterial or fungal population commonly held to be pathogenic. This implies that laundry washing, ironing, and packaging must guarantee a product with a zero tolerance for: all Enterobacteriaceae, Pseudomonas aeruginosa, Alcaligenes spp., and obligately pathogenic gram-negative bacteria; Staphylococcus aureus, Streptococcus pyogenes, and Corynebacteriaceae pathogenic for man; and dermatophytes of which Trichophyton spp. and Epidermophyton spp. are reasonable examples.

An acceptable, qualitative microbiologic assay will be required to assure this criterion.

Quantitative Criteria

An acceptable laundered linen will reflect both (1) a suitable "wash-extraction-ironing" process, and (2) protection of product from environmental bacterial deposition or human handling contamination by S. aureus, Strep. pyogenes, or Enterobacteriaceae.

Further, there shall be a limiting or maximum tolerance of $6x10^3$ Bacillus spp. spores/square inch, or per 0.1 gram, as obtainable from either "rinse-extraction" or "maceration" procedures, when these or comparable systems have established their capacity to yield quantitative data reproducibly.

Post-processing counts of ubiquitous Micrococcus spp. may occur in numbers not to exceed the tolerance for Bacillus spp. spores, it being understood: (1) that a clear-cut differentiation must be made between Micrococcus spp. and Staphylococcus spp., and (2) that plant managers recognize such contamination is unnecessary, since it is relatively simple and reasonably inexpensive to control.

Quantitative microbiologic procedures will employ those systems or devices known to give maximal results, e.g., trypticase soy-yeast extract (w/wo glucose) or TSA/YE blood agars. Suitable chemical neutralization devices will be used according to finishing additives. The predominant subpopulation of every bacteriologically "positive" specimen will be identified precisely, so that control measures for any excessive counts may be readily applied.

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