Journal of Food Protection, Vol. 56, No. 6, Pages 518-524 (June 1993)

Computer Simulation of Microbiological Sampling of Liquid Foods

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(Received for publication October 6, 1992)

ABSTRACT

A computer program that simulates the process of randomly selecting sample packages from a large lot and examining a portion of the package contents for microorganisms was developed. The program can be used to evaluate the theoretical effects on results (mean, standard deviation, and detection or nondetection) of various choices for the number of sample packages selected, the sample volume withdrawn for examination, the package size, and the behavior of the organisms at different microorganism concentrations. The results can be used to compare the theoretical effectiveness of and the risk inherent in various sampling schemes. The simulation program was used to systematically study the effects of varying the number of sample packages selected, the distribution of samples between packages, and the sample volume examined. The results indicate, for example, that finding no counts when 5-ml volumes are drawn from each of 16 sample packages is no assurance of sterility, but only indicates about a 50% chance that the average microbial concentration is less than 1 cell per 100 ml. Increasing the number of packages examined from 16 to 32 should decrease the likelihood of finding a nonzero result to approximately 25%. For 16 samples of 100 ml and the same organism concentration, however, a zero result at this organism loading is very unlikely. The total volume examined is the most important factor for most practical sampling situations. This finding implies that taking fewer samples of larger volume is the more cost effective strategy to reduce risk.

Different types of fluid foods impose different constraints on microbiological examination. Relatively large quantities (100 ml or more) of some liquids can be passed through a membrane filter, which is then placed on a nutrient medium and incubated before colony counting. Liquids that contain particles (such as pulp in some fruit juices) or emulsions (many dairy products), or are viscous (i.e., concentrates and syrups) readily block filters or filter very slowly. Direct plating of small volumes of such products is possible but is typically limited by the amount of liquid which agar can absorb; for a standard size agar plate the maximum liquid volume that can be used with direct plating is about 5 ml. Traditionally, many procedures for dairy samples use a 1-ml volume sample. Solid particles that may be mistaken for colonies, such as the fruit pulp particles in juice-based drinks, can make enumeration difficult, slow, or unreliable. Because of the differences between products, a number of different sampling methodologies are often used in practice even within a single company. Comparing results obtained with different methods is not usually a problem if the levels of microbial contamination are high or moderate. With zero or low count results, however, differences in methodology make it quite difficult to assess risk and compare the implications of results obtained with different sampling schemes (*11*).

It should be clear that it is not possible to have a fractional number of microorganism cells in a package. The distribution of integer numbers of cells over discrete unit volumes (such as packages) should follow the Poisson distribution (*1*). This corresponds to a random dispersion of cells within the total liquid volume. While this is not, strictly speaking, the case for organisms that form chains or clumps, each such group of cells would lead to formation of a single colony and thus a single count with the more commonly used evaluation techniques (either direct plating of liquid or membrane filtration followed by plating). Techniques such as ATP bioluminescence that respond to the total number of cells in a sample could give different results for such organisms. Some environmental samples have been shown to have microorganism distribution patterns that deviate from the Poisson distribution (*2,3*), but these are often subject to contamination from point sources and stratification. Fluid foods are typically well-mixed, and most studies have found that the distribution pattern of bacteria is either not significantly different from the Poisson distribution (*4,5,7,12*) or is quite close to it (*6*). It is, in fact, common practice to evaluate microbial enumeration methods by testing them for agreement with the Poisson distribution (*8-10*).

A computer program was developed to simulate the process of microbial sampling. It was designed to facilitate comparisons of various sampling strategies, to determine the risk associated with given results, and to explore the effects of various scenarios of organism growth, death, or stasis. The computer program was used to make theoretical comparisons of a number of sampling schemes (not all of which are technically feasible for all samples) and to evaluate the degree of safety inherent in zero and low count results obtained under various conditions.

DESCRIPTION OF PROGRAM

The program (see flow chart in Fig. 1) performs a simulation of the sampling and enumeration process. As such, it does not calculate an exact answer for a given set of conditions, but rather produces results with the same pattern of variation expected in practice. Many replications can be quickly simulated to determine the average result and typical variation. In each simulation the

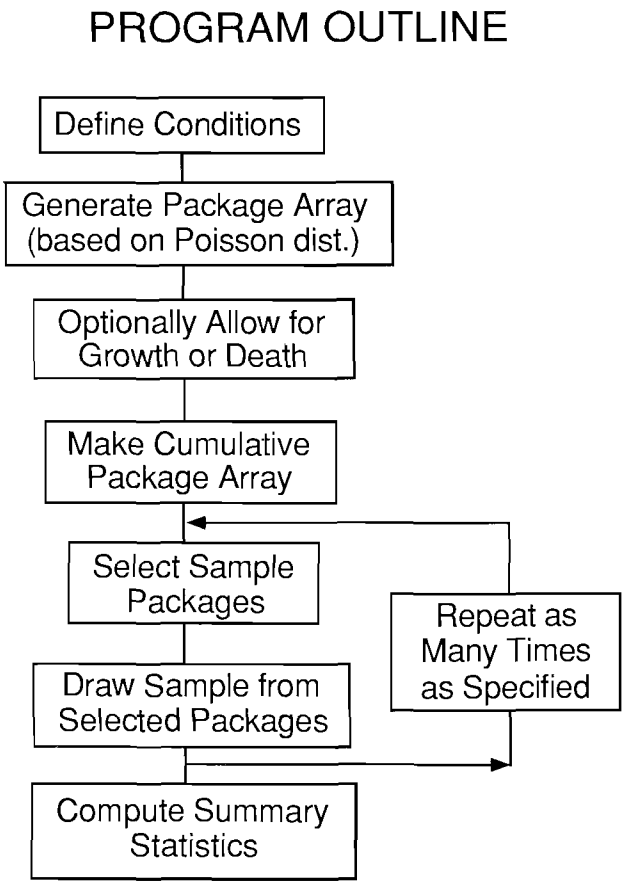

Figure 1. *Flow diagram of computer program which performs simulation of package sampling and microbiological examination.*

distribution pattern of organisms between packages is constant for a given set of conditions (number of packages, average microbial load, and growth assumptions). The particular packages selected as samples, however, are different in each repetition. Similarly, the simulation of sampling liquid from the selected sample packages is also different in each repetition. In order to accomplish this, statistical distribution functions and the random number generator of the computer are used.

The program was written in BASIC and compiled with Microsoft QuickBasic (Microsoft Corp., Bellevue, WA). Program output is displayed on the microcomputer screen and written to a file for subsequent examination or printing. A patent application describing the concept embodied in and use of the program to improve microbial sampling schemes has been filed.

The conditions that can be varied are the average viable microorganism level (expressed as the number of cells per 100 ml), the package volume, the number of packages in a lot (or production run), the number of packages to be sampled, the volume of liquid withdrawn for each examination, the number of subsamples to be taken from each package, and the number of repetitions to be performed under a set of conditions. The conditions for each execution can either be read from a file or entered directly and are listed in the printout.

The program can simulate organism growth or death between the time the microbial population is defined and sampled. It is known that either death or growth can occur with particular organisms and substrates, but intimate knowledge of product/contaminant situations would be needed to use this feature meaningfully other than to make worst-case estimates.

Generation of the package array

The program generates the distribution of organisms between packages using the Poisson distribution to calculate the probability of occurrence of packages with each integer number of organisms given a specified average organism concentration. The total number of packages is then multiplied by these probabilities, and the results are truncated to arrive at the number of packages with each number of organisms. For large numbers of organisms per package, the Poisson distribution is approximated by the normal distribution.

A summary of the distribution pattern that lists the numbers of packages with each number of organisms is displayed (see Fig. 2). The pattern is constant for a given organism concentration, package volume, and number of packages. The total number of organisms summed across packages is compared with the "theoretical" result calculated from the product of the average loading, the package volume, and number of packages. The two results typically differ slightly because one is a real number and the other the sum of a distribution of integer results.

```
count   packages
  0       27
  1       162       **
  2       477       *****
  3       938       *********
  4      1383       **************
  5      1632       ****************
  6      1605       ****************
  7      1353       **************
  8       998       **********
  9       654       *******
 10       386       ****
 11       207       **
 12       102       *
 13        46
 14        19
 15         8
 16         3
 17         1
 18         0

Total Count   59003                Theor. Count   59000
```

Figure 2. *A segment of the simulation computer program output which depicts the number of packages with each number of organisms per package (count).*

Growth, death, or stasis

Optionally, an allowance for growth or death of the organisms can be made by specifying a factor. A factor of 0.5, for example, indicates that on average, half of the organisms initially present die. A 1.0 value indicates that, on average, the population remains the same. A factor >1.0 indicates growth; for example, 2.0 is equivalent to an average doubling of the population. For the packages with each contamination level, the Poisson distribution is used to produce a new distribution with the correct average value. For example, if there are 825 bottles that contain two organisms each and the growth or death factor is 1.2, the new average for this group will be 2.4 organisms per bottle (1.2 x 2), and the number of bottles over which these organisms are distributed remains 825. This should be realistic in that even when the net effect is for organisms to grow, some individuals actually die, some remain static, and some grow more than the average amount. Packages that had no counts before this operation still have no counts afterwards. The new distribution can then be displayed. The new total number of organisms expected is calculated by:

(No. of packages) x loading x (package volume) x (growth/death factor)

Cumulative array

A cumulative distribution package array is produced beginning with the packages with the lowest count. This is used later to simulate selection of particular packages. For example, if there are 512 packages with 0 organisms, 431 with 1, and 213 with 2, the cumulative array would be as follows:

Array positions	Count
1-512	0
513-943	1
944-1156	2

Package selection function

The selection of sample packages is accomplished as follows. A random number between 0 and 1 is generated and multiplied by the total number of packages. This is truncated to arrive at an integer and added to 1. For an array of 10,000 bottles, this generates a number between 1 and 10,000. The program finds the count value corresponding to this randomly selected bottle number in the cumulative array. The corresponding count value determines the number of organisms in the selected package. A higher bottle number thus corresponds to a package with a higher organism loading while lower numbers correspond to lower loadings. The counts in all the selected packages are summed to arrive at the total number of organisms in all of the packages chosen.

Sampling from selected packages

For each selected package, the volume to be examined is withdrawn and the number of organisms in the sample is calculated. A cumulative array of the Poisson probabilities of occurrence of each discrete result is prepared using the number of organisms in the picked package and the package and sample volumes to define the expected result. A random number in the range of 0-1 is then used to index this array. This calculation gives the result expected from the ratio (sample volume)/(package volume) times selected-package-count most of the time, but it also gives both lower and higher results with increasingly smaller frequencies of occurrence the farther the number diverges from the ratio.

If two or more subsamples are to be taken from a package, the number of organisms and volume available are decremented and the process is repeated. For each of the other selected packages, the withdrawing of aliquot(s) is repeated. The results are summarized to indicate the total number of organisms in the sampled packages and in the volumes examined (see Fig. 3).

```
Total count in packages picked   67    In volumes examined    2

Total count in packages picked   76    In volumes examined    2

Total count in packages picked   87    In volumes examined    3

Total count in packages picked   66    In volumes examined    0

Sample total count      mean  65.25        std. dev.   11.96491
Colony total count      mean  1.04         std. dev.   .9631598
Percentage of  Zero counts   34            Non-zero   66
```

Figure 3. *A segment of the simulation computer program output where each line at the top indicates the results from one simulation, showing the total number of organisms in all selected packages and the total number of organisms found in all the volumes examined. The bottom shows a statistical summary for all the replications made for a set of conditions. The sample total count is for the total organisms in all packages selected. The colony total count results are for the total organisms found in the volumes examined.*

The simulation of package selection and liquid sampling can be repeated as many times as desired. Since the distribution of organisms over packages, and the effects of growth or death are the same for the same conditions (number of packages; organism loading; and, if used, the growth/death factor), the cumulative array is not recalculated for repetitions with the same conditions.

Statistical summary

The results for the total count in each replicate determination of all packages sampled and in all volumes examined are used to calculate summary statistics (see Fig. 3). The mean values and standard deviations for the total organisms in all the packages sampled and in all the volumes examined in each replication are calculated and displayed. The percentage of occurrence of zero counts is also reported.

VARIABLES TESTED

The microbiological count simulation computer program was used to perform a number of simulations. At least 100 replicate simulations were made for each set of conditions. The simulations were all for 10-oz (295-ml) packages and samples were drawn from 10,000 package lots in each case. The assumptions were that no growth or death occurred between the specification of organism concentration and sampling and that the organisms were randomly distributed among packages and within the volume of the packages.

The effects of the sample volume taken for examination, the distribution of the total sample volume between packages, and the numbers of packages sampled were examined at various microbial concentrations. For the sample volume study, simulations for volumes of 1, 2, 5, 10, 20, 50, 100, and 250 ml from 8- and 16-package sets were carried out for average microbial loadings of 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 organisms per 100 ml. In the package number study, simulations of sampling 5-ml volumes from 1, 2, 4, 8, 16, 32, and 64 selected packages and 100-ml volumes from 1, 2, 4, 8, and 16 selected packages over the same range of loadings were performed. The distribution study examined the effects of taking a total of 16 samples of 5 ml from different numbers of selected packages; this ranged from 16 aliquots all from the same package through one aliquot from each of 16 selected packages. Another distribution study compared the results of taking 100 ml of total sample volume from different numbers of packages (ranging from one sample of 100 ml through combinations of 2 x 50 ml, 5 x 20 ml, 10 x 10 ml, 20 x 5 ml, 50 x 2 ml to 100 packages with 1-ml samples). The combinations of parameters to carry out these studies were supplied to the computer program and simulations were carried out. Results for mean count found, standard deviation, and percent zero results (the incidence of nondetection of contamination) were noted in each case.

RESULTS AND DISCUSSION

The study design described above was intended to provide a systematic examination of what could be expected under a variety of conditions. It was also intended to evaluate the influence of each factor on the mean result, precision, and risk.

Sample volume plated

The first variable examined was sample volume. For each of a number of average microbial loadings in the range 0.1-4.0 microorganisms per 100-ml sample, simulations were performed for sets of 8 and 16 selected packages and a number of sample volumes in the range 1 ml through 250 ml. The data are shown in Tables 1 and 2 and can be graphed in a number of ways. Fig. 4 and 5 show the mean results and the variation depicted as the mean plus or minus 1 or 2 standard deviations for the 5- and 100-ml sample sizes. It is quite apparent that the variation is relatively large with the smaller sample volumes. For 1-ml samples, the standard deviation is as large as the mean result (see Table 1). The standard deviation becomes smaller relative to the mean as the sample size increases. For any single Poisson distribution the variance is approximately equal to the expected result (*1*). This would be consistent with a standard deviation that decreases as the mean increases. The results from the simulation study are in general agreement with a variance/mean ratio of 1.0.

Perhaps the simplest expression of the degree of risk implicit in these results is the frequency of occurrence of zero results in all the samples in a set (designated here the predicted percent zero). This was plotted for selections of 8 and of 16 samples (see Fig. 6 and 7). At the 0.1 cell per 100 ml level [roughly equivalent to an average of one cell in

TABLE 1. *Results for simulated examinations of 16 sample sets for indicated microbial loadings (cells/100 ml) and sample volumes.*

	Results for 100 simulations of indicated conditions							
cells/	Sample volume (ml) examined							
100 ml	1	2	5	10	20	50	100	250
0.10	0.01 ± 0.099[a]	0.03 ± 0.171	0.10 ± 0.333	0.17 ± 0.428	0.39 ± 0.618	0.87 ± 0.971	1.60 ± 1.393	3.67 ± 2.084
	99[b]	97	91	85	68	47	25	10
0.20	0.02 ± 0.141	0.08 ± 0.307	0.13 ± 0.367	0.41 ± 0.712	0.56 ± 0.729	1.43 ± 1.265	2.95 ± 1.904	7.99 ± 2.904
	98	93	88	70	57	26	6	0
0.50	0.10 ± 0.302	0.12 ± 0.356	0.37 ± 0.614	0.72 ± 0.805	1.44 ± 1.225	3.77 ± 2.242	8.29 ± 3.859	19.19 ± 4.287
	90	89	69	45	22	5	0	0
1.00	0.22 ± 0.524	0.35 ± 0.609	0.89 ± 0.973	1.86 ± 1.333	3.09 ± 1.870	7.98 ± 3.357	15.78 ± 4.113	39.98 ± 6.168
	82	72	43	14	4	0	0	0
1.50	0.32 ± 0.548	0.52 ± 0.717	1.18 ± 1.158	2.25 ± 1.666	4.49 ± 2.067	12.21 ± 3.777	24.34 ± 5.562	60.41 ± 8.034
	72	60	37	13	1	0	0	0
2.00	0.27 ± 0.548	0.60 ± 0.791	1.36 ± 1.330	3.17 ± 1.870	6.34 ± 2.724	16.17 ± 4.233	31.86 ± 6.883	80.09 ± 7.810
	77	54	33	6	0	0	0	0
2.50	0.46 ± 0.593	0.91 ± 0.965	1.98 ± 1.206	4.21 ± 2.240	7.94 ± 2.752	20.20 ± 5.538	39.82 ± 7.770	100.66 ± 10.268
	59	41	10	3	0	0	0	0
3.00	0.56 ± 0.756	0.91 ± 1.045	2.23 ± 1.496	4.89 ± 2.326	9.53 ± 3.401	24.29 ± 5.734	48.15 ± 8.539	119.99 ± 11.288
	56	44	12	2	0	0	0	0
3.50	0.45 ± 0.642	1.03 ± 1.039	2.83 ± 1.682	5.78 ± 2.549	11.23 ± 3.499	27.78 ± 6.475	53.38 ± 7.858	140.30 ± 11.400
	63	39	3	1	0	0	0	0
4.00	0.61 ± 0.764	1.23 ± 1.024	3.01 ± 1.667	6.60 ± 2.704	12.45 ± 3.735	32.96 ± 5.666	64.62 ± 9.153	159.38 ± 13.442
	53	26	2	0	0	0	0	0

[a] Means and standard deviations for 100 simulations.
[b] Percent of 100 simulations with zero results for all samples.

TABLE 2. *Results for simulated examinations of eight sample sets for indicated microbial loadings (cells/100 ml) and sample volumes.*

	Results for 100 simulations of indicated conditions							
Cells/	Sample volume (ml) examined							
100 ml	1	2	5	10	20	50	100	250
0.10	0.00 ± 0.000[a]	0.00 ± 0.000	0.05 ± 0.219	0.13 ± 0.367	0.16 ± 0.443	0.45 ± 0.730	0.75 ± 0.978	1.81 ± 1.398
	100[b]	100	95	88	86	67	51	16
0.20	0.02 ± 0.141	0.01 ± 0.099	0.08 ± 0.273	0.14 ± 0.349	0.20 ± 0.449	0.95 ± 1.019	1.54 ± 1.374	4.30 ± 2.130
	98	99	92	86	82	42	25	1
0.50	0.01 ± 0.099	0.13 ± 0.367	0.16 ± 0.368	0.36 ± 0.595	0.73 ± 0.897	1.94 ± 1.462	3.88 ± 2.375	9.77 ± 2.920
	99	88	84	69	50	14	5	0
1.00	0.05 ± 0.219	0.17 ± 0.428	0.37 ± 0.630	0.72 ± 0.866	1.67 ± 1.198	4.30 ± 2.272	7.81 ± 3.678	19.91 ± 4.551
	95	85	70	49	14	1	0	0
1.50	0.14 ± 0.377	0.25 ± 0.500	0.70 ± 0.810	1.17 ± 1.101	2.32 ± 1.663	6.13 ± 2.646	12.25 ± 4.098	29.86 ± 5.784
	87	77	49	35	9	0	0	0
2.00	0.19 ± 0.465	0.29 ± 0.556	0.75 ± 1.019	1.36 ± 1.219	3.09 ± 1.804	7.52 ± 3.380	15.61 ± 4.077	39.08 ± 6.462
	84	76	55	24	2	0	0	0
2.50	0.31 ± 0.526	0.47 ± 0.688	0.73 ± 0.886	2.11 ± 1.421	3.92 ± 2.097	10.14 ± 3.473	19.99 ± 5.697	50.78 ± 7.437
	72	63	46	9	2	0	0	0
3.00	0.32 ± 0.548	0.44 ± 0.671	1.41 ± 1.198	2.27 ± 1.413	4.96 ± 2.283	11.36 ± 3.656	23.51 ± 5.866	60.50 ± 7.324
	72	65	24	10	0	0	0	0
3.50	0.30 ± 0.522	0.59 ± 0.805	1.34 ± 1.320	2.57 ± 1.533	5.43 ± 2.836	14.76 ± 4.245	28.08 ± 5.937	69.89 ± 9.305
	73	55	27	7	1	0	0	0
4.00	0.31 ± 0.581	0.61 ± 0.852	1.68 ± 1.278	3.21 ± 1.966	6.35 ± 2.488	15.82 ± 4.115	31.38 ± 6.987	78.88 ± 8.834
	74	59	16	5	1	0	0	0

[a] Means and standard deviations for 100 simulations.
[b] Percent of 100 simulations with zero results for all samples.

every third 10-oz (295-ml) package], 5-ml platings of 16 packages would yield no counts approximately 90% of the time. The likelihood of finding no counts at this organism loading falls to about 25% with 100-ml samples. With average loadings of 0.2 cells per 100 ml, examination of 100-ml platings from 16 packages would nearly always yield some counts. With 5-ml samples, it can be seen that a zero result for all 16 plates could very easily occur even with rather high loadings. For reference, a loading of 0.33 cells per 100 ml is approximately one organism per 10 oz (295 ml) package,

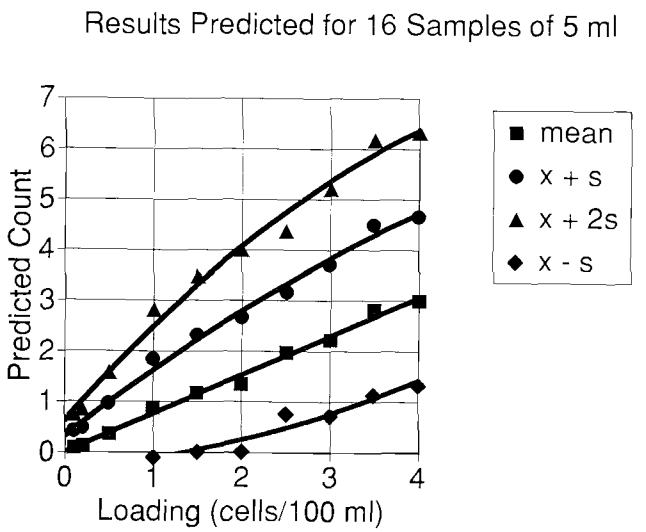

Figure 4. *Predicted total count results (the sum of all colonies seen on all plates) from 100 replicate simulations in which one 5-ml sample from each of 16 packages was examined for each indicated organism concentration are shown. The mean of the 100 replicates expresses the central tendency. The expected variation is expressed as the mean result plus and minus one or two standard deviations.*

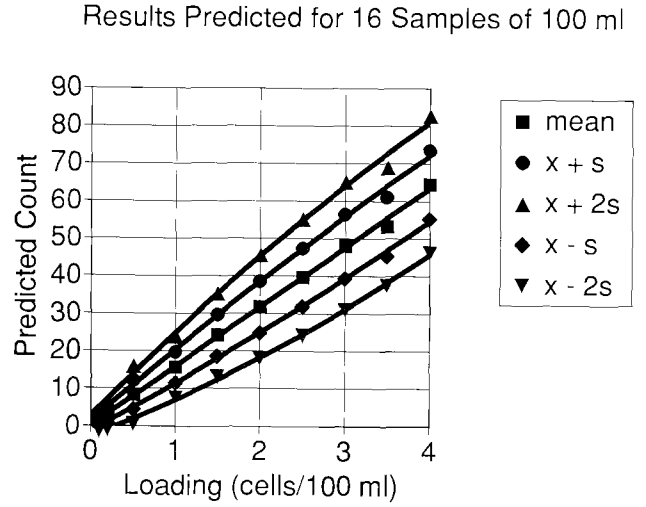

Figure 5. *Predicted total count results (the sum of all colonies seen on all plates) from 100 replicate simulations in which one 100-ml sample from each of 16 packages was examined for each indicated organism concentration are shown. The mean of the 100 replicates expresses the central tendency. The expected variation is expressed as the mean result plus and minus one or two standard deviations.*

while 1 cell per 100 ml is about three organisms per package. Clearly, the volume of sample examined has a very large effect on the likelihood of finding microbial contamination. Sample volumes of 1 ml or 2 ml, even when taken from 16 packages, can easily fail to detect quite high organism concentrations.

Number of packages examined

The effect of the number of packages examined with 5-ml and 100-ml platings was also investigated. The results are shown in Fig. 8 and 9. With 5-ml samples, examination of a single package offers very little likelihood of detecting even the highest levels of contamination; 16 packages are needed to have close to a 50% chance of detecting 1 cell per 100 ml

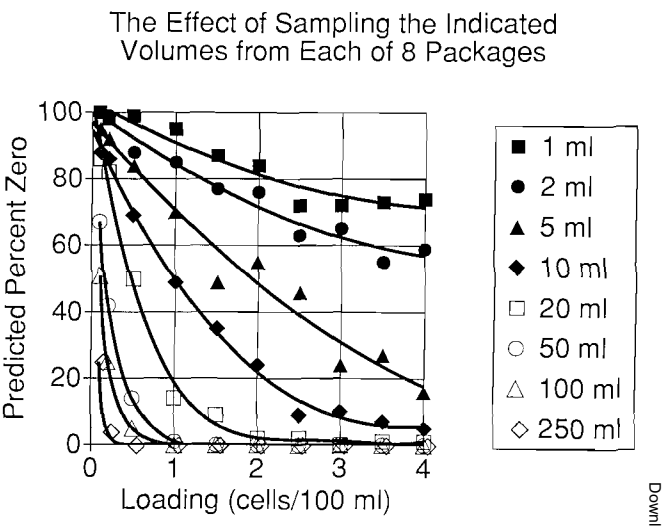

Figure 6. *Predicted frequency of finding no counts in any sample in 100 simulations where the volumes indicated in the legend were withdrawn from each of 8 packages at each indicated organism concentration.*

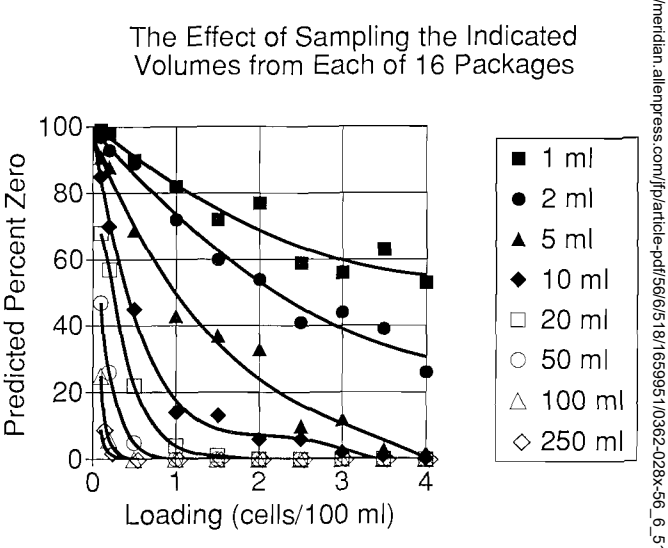

Figure 7. *Predicted frequency of finding no counts in any sample in 100 simulations where the volumes indicated in the legend were withdrawn from each of 16 packages at each indicated organism concentration.*

[about 3 cells per 10 oz package (295 ml)]. It is clear that a zero result is no assurance of sterility. With 64 samples, an average loading of 1 cell per 100 ml would be detected over 90% of the time. Fig. 9 indicates that with platings that correspond to 100 ml of sample, fewer samples are needed to achieve equal or better detection efficiency. It can be seen that a single 100-ml sample should be better than 16 samples of 5 ml each. This makes inherent sense, since 16 samples of 5 ml each contains a total of only 80 ml. This was confirmed by comparing results for a single 100-ml sample with 20 samples of 5 ml (data not shown); the results were very similar in mean and precision. In this sample volume range, the results appear to be mainly dependent on the total sample volume examined rather than the number of samples if the random distribution assumption holds. With larger volume samples, the benefit of increasing the number of samples appears to diminish at some point (compare results for 8 and 16 packages in Fig. 9).

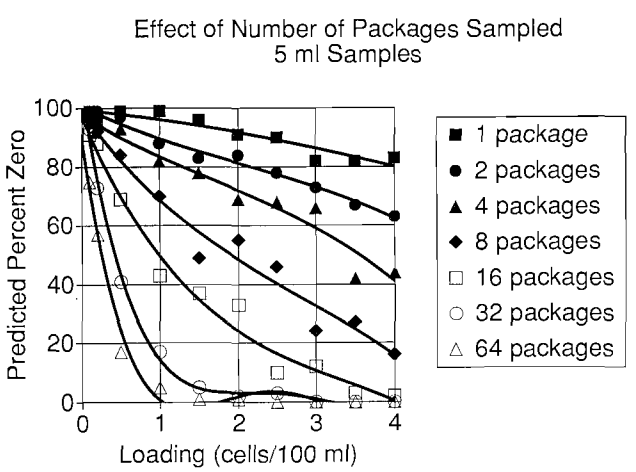

Figure 8. *Predicted frequency of finding no counts in any sample in 100 simulations in which one 5-ml sample was taken from each of the number of packages indicated in the legend for each indicated organism concentration.*

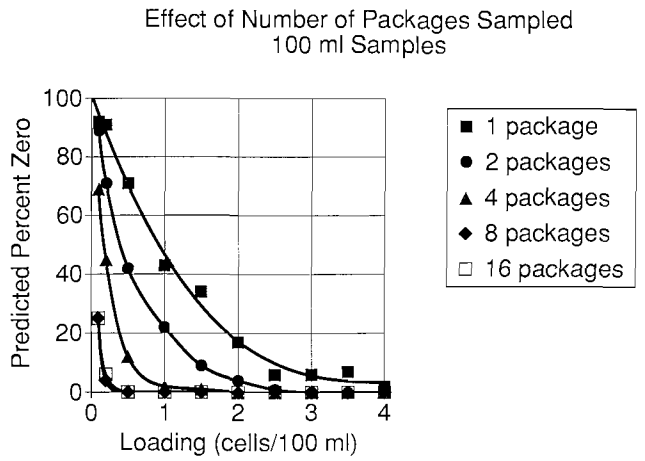

Figure 9. *Predicted frequency of finding no counts in any sample in 100 simulations in which one 100-ml sample was taken from each of the number of packages indicated in the legend for each indicated organism concentration.*

Rearranging the sampling pattern

Simulations in which the same total sample volume was distributed in several ways were carried out. In one case, 16 samples of 5 ml each were taken from 1, 2, 4, 8, or 16 packages. The results are shown in Fig. 10 and indicate virtually no effect of the sampling pattern on detection efficiency.

A comparison in which different numbers of samples of varying size, each resulting in a total examined volume of 100 ml, were simulated is shown in Fig. 11. Once again, the results were similar in mean and standard deviation (data not shown) as well as percentage of zero counts.

General

Clearly, the total volume examined is the factor with the strongest influence on results. Changing the distribution of the sampled volume between packages has little effect. For a given volume examined per package, the number of packages sampled appears mainly to exert its effect through its influence on the total volume sampled. However, with the largest examined volumes, the benefit of sampling larger numbers of

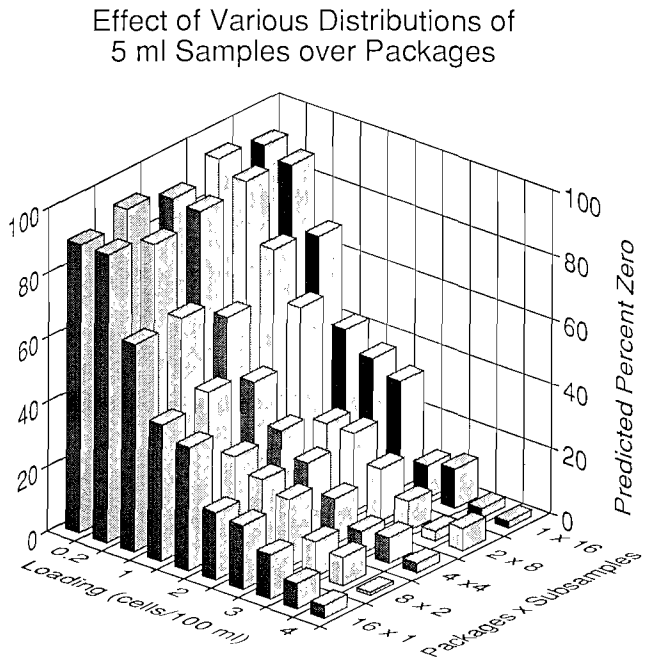

Figure 10. *Predicted frequency of finding no counts in any sample in 100 simulations in which 16 total samples of 5 ml were taken from 1, 2, 4, 8, or 16 packages for each indicated organism concentration.*

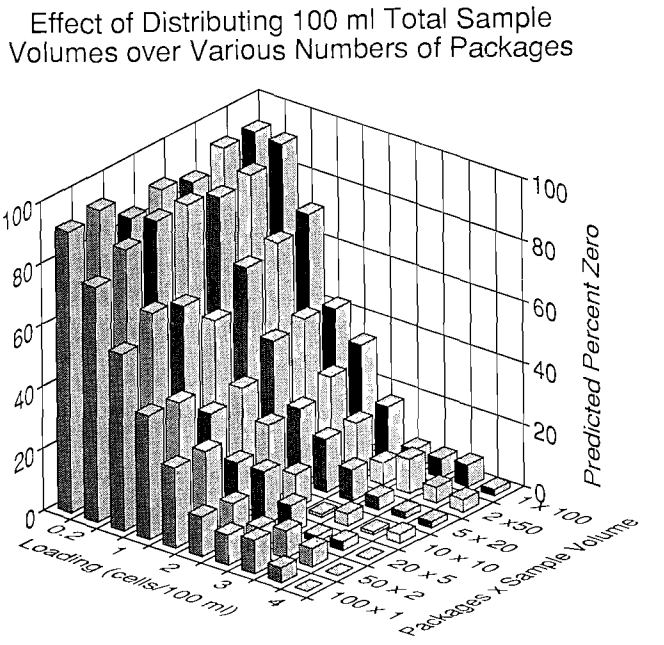

Figure 11. *Predicted frequency of finding no counts in any sample in 100 simulations in which a total of 100 ml was taken from 1, 2, 5, 10, 20, 50, or 100 packages.*

packages declines. For most practical samples, the total volume examined will be the important factor, provided that the counts are randomly distributed over the entire package population. This has important implications both for the choice of sampling pattern employed and for the cost of sampling.

The principle that the number of packages examined should be a function of the size of the lot makes sense in situations where the organism distribution pattern is expected to deviate from a random distribution. Otherwise, the total

volume examined should be the key factor. For processes involving aseptic filling, the assumption that organisms are randomly distributed across packages is more likely to be correct early in a filling run when the equipment should be cleaner. Sometime after cleaning, some spouts of a multispout filler may become infected, and this could lead to a less random distribution of organisms across packages. Such divergences from a random distribution pattern are thus likely to increase with the length of a filling operation or the interval between filler cleanings. Where tunnel pasteurizers are employed following filling, differences between packages caused by contamination of individual filler spouts are likely to be small, particularly for low or modest levels of contamination.

CONCLUSIONS

Increasing the volume basis of the sample applied to a plate has a large effect on the likelihood of detecting organisms and also improves precision. Sample permitting, the largest practical volume that can be examined should be used. It is of considerable interest to develop approaches to examine larger sample volumes; procedures that increase the volume basis for difficult samples should be particularly useful.

The number of packages examined influences results, but the effect is not as strong as that of the sample volume examined. The amount of additional work required to examine enough additional samples to improve the confidence of the results appears to be large compared to the improvement that could be gained through increasing the volume of each sample. In some cases, increasing the number of samples examined may be the only available option to reduce risk or improve the assessment.

If organisms are distributed randomly across all packages, varying the number of packages or subsamples from which the same total sample volume is drawn would not be expected to change the pattern of results.

It is clearly apparent from the above that if the random distribution assumption holds, the preferred sampling strategy, where the nature of the sample permits, is to take fewer samples of larger volumes. This would be cost effective in two ways; it should reduce product loss due to the destructive nature of microbial sampling, and it should reduce the labor cost that is usually a function of the number of samples. Substitution of various combinations of sampling approaches, such as taking fewer samples of larger volume, will have to be tested on a case-by-case basis. The count simulation program makes theoretical comparisons of this sort readily possible.

REFERENCES

1. Dowdy, S., and S. Wearden. 1991. Statistics for research, 2nd ed. Wiley-Interscience, New York. pp. 87-103.
2. Haas, C. N., and B. Heller. 1986. Statistics of enumerating total coliforms in water samples by membrane filter procedures. Water Res. 20(4):525-530.
3. Haas, C. N., and B. Heller. 1988. Test of the validity of the Poisson assumption for analysis of most-probable-number results. Appl. Environ. Microbiol. 54:2996-3002.
4. Hildebrandt, G., and H. Weiss. 1984. (Calculating mean value when determining bacterial counts in a sample homogenate.) Mittelwertbildung bei der Keimzahlbestimmung an einem Probenhomogenisat. Fleischwirtschaft. 64(1):83-86.
5. Hildebrandt, G., H. Weiss, and L. Hirst. 1986. A propos Farmiloe's formula. Calculating mean values when determining bacterial counts in a sample homogenate. Fleischwirtschaft 66(7):1128-1130.
6. Hurka, H., G. Arndt, and G. Hildebrandt. 1986. (Model experiments on distribution of bacterial counts in parallel random samples from one batch.) Modellversuche zur Verteilung von Keimzahlen in Parallelstichproben aus einer Charge. Fleischwirtschaft 66(12):1757-1762.
7. Mueller, A., and G. Hildebrandt. 1989. (The accuracy of culture methods for determination of microbial counts. II. Original investigations.) Die Genauigkeit der kulturellen Keimzahlbestimmung. II. Eigene Untersuchungen. Fleischwirtschaft 69(5):925-930.
8. Piton, C., and R. Grappin. 1991. A model for statistical evaluation of precision parameters of microbiological methods applications to dry rehydratable film methods and idf reference methods for enumeration of total aerobic mesophilic flora and coliforms in raw milk. J. Assoc. Off. Anal. Chem. 74:92-103.
9. Robaye, E., P. Pohl, and J. Thomas. 1969. (Simple method of calculating error limits in bacterial counts.) Revue des Fermentations et des Industries Alimentaires 24(5):177-81.
10. Sakazaki, R., H. Pivnick, G. Jarvis, M. Goddard, Y. Asakawa, G. Barrow, L. Beuchat, R. Colwell, T. Gleeson, et al. 1986. International commission on microbiological specifications for foods methods studies. XVI. Comparison of salt polymyxin broth with glucose salt teepol broth for enumerating vibrio-parahaemolyticus in naturally contaminated samples. J. Food Prot. 49:773-780.
11. Spicher, G., and J. Peters. 1973. (Can the existence of a sterile state be demonstrated by a sterility test?) Laesst sich Sterilitaet durch eine Sterilitaetspruefung beweisen? Branntweinwirtschaft 113(16):384-387.
12. Thomas, M. J. 1970. (Determination of the bacterial content of milk by the most probable number principle.) Zur Bestimmung des Milchkeimgehaltes nach dem MPN-Prinzip. Thesis. Tieraerztliche Hochschule, Hannover, Germany.