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Positive-Hole Correction of Multiple-Jet Impactors for Collecting Viable Microorganisms

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Multiple-jet impactors, typically with 200 or 400 holes, are used widely for collecting aerosols of living bacteria and fungi. In this type of impactor, the air jets impinge directly onto nutrient agar in a petri dish which is incubated after sampling until collected cells multiply into colonies. The observed number of colonies can be adjusted for the probability that more than one viable particle was collected through a sampling hole and merged with other microorganisms at an impaction site to produce a single colony. A "positive-hole" correction table has been published for a 400-hole impactor, but none has been produced previously for the 200-hole impactor. The expected number of sampled particles required to fill each of 1 through 200 and 1 through 400 impaction sites and the standard deviations of these values were calculated from probability theory. The results were compared with a Monte Carlo simulation. By using correction tables (which include the standard deviation of an expected value) an investigator can report the most probable viable particle count and a 95% confidence interval (mean ± 2 standard deviations). The range of collected particles that could have produced an observed number of colonies increases as the number of collected particles increases, and investigators should acknowledge the uncertainty associated with adjusted counts. It is advisable to use an impactor with the greatest practical number of sampling holes because this decreases the likelihood that multiple particles are deposited at the impaction sites. The assumption that all jets in a single-stage, multiple-jet sampler are equally likely to collect particles was tested experimentally with an aerosol of bacterial spores and found to be reasonable.

Introduction

A six-stage, 400-hole impactor[1] was recommended in 1964 as a standard or a reference sampler for collecting airborne microorganisms[2] and has been considered the most practical and aerodynamically sound sampler for this type of work.[3] A modified version of the 400-hole impactor is recommended for measuring human exposure to airborne microorganisms in indoor environments.[4,5] The present discussion deals specifically with the impactors of one manufacturer (Andersen Samplers, Inc., Atlanta, Ga.), although the information also applies to similar multiple-jet impactors.

During sampler operation, a vacuum pump is used to draw air at a rate of 28 L/min through the 400 holes of the impactor. The 400 air jets impinge on nutrient agar in 10-cm diameter petri dishes, or plates, which are positioned beneath the six stages. Particles that escape impaction on the first plate proceed with the airstream to the next stage. The diameter of the exhaust section of each stage is slightly larger than a plate, allowing the airstream to flow around the plate to the next stage. On each stage, the 400 holes are arranged in 11 concentric rings. The number of holes per ring, moving from the center to the outer edge, is 10, 15, 20, 26, 31, 37, 42, 48, 53, 58, and 60. All holes on a stage are the same diameter, but the size of the holes decreases progressively from Stage 1 to Stage 6, resulting in the separation of particles into six aerodynamic size fractions[6] (Table I).

To obtain a size distribution of viable particles, a sampler is loaded with six glass petri dishes, each containing 27 mL of sterile nutrient agar suitable for the organism(s) to be collected. In some cases it is more convenient to use disposable plastic petri dishes; in which case, they are filled with 45 mL of medium in order to maintain the correct distance between the impactor stage and the collecting surface. See Andersen,[1] Cipriano,[7] and Furuhashi and Miyamae[6] for further discussions of the effects of changing the jet-to-plate distance and Andersen[1] and Cipriano[7] on the use of plastic dishes. After sampling, the plates are removed, covered immediately, and incubated for a time and at a temperature suitable for the organisms collected. A bacterial or fungal colony forms at each impaction site where one or more particles were deposited. Only particles containing at least one viable microorganism can be detected.

The manufacturer also produces one-, two-, and six-stage samplers specifically designed for use with commercially-prepared plastic petri plates. Any number and combination of stages can be used (see below).

Positive-Hole Correction

Andersen[1] provided a positive-hole correction or conversion table for use with the 400-hole impactor. The table was based on the principle that as the number of viable particles impinging on a plate increased, the probability of particles entering unoccupied holes decreased. The values in the table were calculated from the following formula:[8]

$$P_r = N\left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \ldots + \frac{1}{N-r+1}\right] \quad (1)$$

where P_r is the expected number of viable particles required to produce r positive holes, and N is the total number of holes per stage.

Andersen[1] stated that the positive-hole correction is not needed for Plates 1 and 2 because the deposition of particles

TABLE I
Theoretical Particle Collection Efficiency for a Six-Stage, 400-Hole Impactor[6]

Stage No.	Jet Diameter (cm)	Jet Velocity (cm/sec)	d_{50}[A] (μm)
1	0.118	108	7.2
2	0.091	180	4.8
3	0.071	297	3.2
4	0.053	528	2.1
5	0.034	1279	1.0
6	0.026	2331	0.6

[A] d_{50} = effective 50% cutoff diameter, *i.e.*, diameter for which collection efficiency is 50%.

does not follow the jet patterns. When the positive-hole correction is used for the lower four plates, colonies outside the hole pattern are not counted. Andersen claimed that observed counts up to 390 colony-forming units (cfu) per stage are reliable, *i.e.*, a corrected count of approximately 1500 total viable particles. If higher counts are encountered, Andersen advised that colonies be counted by microscope.

For counting by microscope, plates are incubated for a short time, after which the microcolonies at a number of impaction sites are counted and the total for a plate is calculated. This method is time consuming, however, and it is more difficult to discern microscopic colonies than it is to count macroscopic cfu. Also, only the central deposition sites can be examined easily.

Uniformity of Particle Deposition with a Six-Stage, 400-Hole Impactor

May[9] collected dye aerosols on glass discs in petri dishes and noticed variations in the density of the stains at the impaction sites on Plates 1 and 2. The variation on Plate 3 was less noticeable, and Plates 4, 5, and 6 appeared to be uniform. May reported that particle collection at the upper stages was affected by differences in airflow rates between the inner and the outer holes, by nonuniform interferences between adjacent holes, and by particle size.

May[9] suggested reducing the number of holes to 200 to allow more space between holes, aligning the holes to avoid interference between impaction jets, and tapering the holes to assure the smooth entry of air. He also recommended using the positive-hole correction table for the 400-hole sampler to correct colony counts from the 200-hole sampler by doubling the observed count, reading the appropriate corrected number from the table, and reporting half this value. May's method does not appear to have been adopted by many investigators.

Two-Stage, 200-Hole Impactors for Collecting Airborne Microorganisms

For many industrial hygiene applications, a simple separation of particles into nonrespirable and respirable fractions is sufficient.[10] Gillespie et al.[10] noted that this separation could be obtained using a six-stage sampler by summing the cfu on Plates 1 and 2 as nonrespirable and those on Plates 3 through 6 as respirable. Andersen Samplers, Inc., developed a disposable polystyrene, and later an aluminum, two-stage cascade impactor with 200 tapered holes in a radial pattern per stage, following May's[9] recommended design (discussed above). The hole diameter on the first stage is 0.15 cm and on the second, is 0.04 cm (compare with Table I). The two-stage sampler is operated in the same manner as the six-stage impactor.

Curtis et al.[11] measured lower concentrations of airborne bacteria with a disposable, two-stage, 200-hole sampler than with an eight-stage, 400-hole impactor. The investigators,[11] however, used the positive-hole correction on counts for the lower five stages of the eight-stage sampler "when indicated" but did not use any correction for the two-stage sampler. Because the two-stage sampler gave lower results in all cases, the investigators concluded that the difference between the two samplers was not an artifact of correcting only the counts from the eight-stage sampler. The authors suggested that a correction table similar to that for the 400-hole sampler was needed for the 200-hole sampler when used in environments in which the air concentration was relatively high.

Gillespie et al.[10] compared a two-stage and a six-stage impactor using naturally occurring aerosols at waste water and waste water sludge treatment facilities. The authors explained that the positive-hole correction was not performed for the six-stage, 400-hole sampler because such a procedure had not been developed for the two-stage, 200-hole sampler and because rapid fungal growth prevented a determination of the hole pattern. They observed that the two-stage sampler collected fewer cfu than did the six-stage sampler and was less efficient at collecting particles < 1 μm, partly because the two-stage impactor has larger sampling holes.

Single-Stage, Multiple-Jet Impactors

There are two sampling options when particle-size information is not needed: (1) collect air samples using all stages of a multiple-stage impactor and pool the counts from the plates as mentioned above or (2) use only one collection plate under the last stage of the sampler with or without the other stages in place. Using only a final collection plate with all six stages in place is known as the S-6 (or S_6) method[3] and collecting through only the sixth stage is referred to as the N-6 (or N_6) single-stage method.[12]

Two advantages of the S-6 and the N-6 methods are that shorter sampling times and less collection media are needed. There is a tendency for the very small holes of the lower stages to become occluded, however, and for the single collection plate to be overloaded. With the S-6 method, there also is more potential for wall losses on the upper stages.

Jones et al.[12] sampled fungi from naturally occurring aerosols with a standard six-stage, an S-6, and an N-6 impactor. In this case counting was done by microscope so that multiple colonies at impaction sites could be resolved without applying the positive-hole correction. The authors[12] found that the N-6 method was identical to the standard 6-stage

sampler, but the S-6 method recovered, on average, approximately half as many viable particles as the standard six-stage impactor.

Comparisons of Multiple-Jet Impactors and Other Air Samplers

Furuhashi and Miyamae[6] compared a six-stage impactor and a slit-to-agar sampler, using a laboratory-generated aerosol of *Serratia marcescens* (a rod-shaped bacterium, approximately 0.5 μm × 0.5 to 1 μm). The authors[6] used the positive-hole correction for the 400-hole impactor when determining the number of collected bacteria. They observed that when the air concentration was above 1400 cfu/m^3, the six-stage sampler collected significantly fewer cfu than did the slit sampler. The authors considered several possible explanations for the higher slit sampler counts, for example, the shattering of particles in the slit sampler and losses on the walls of the six-stage sampler. They concluded that the number of multiple viable particles forming single colonies in the impactor sampler was greater than the number obtained from the positive-hole correction table.

Zimmerman et al.[13] compared a two-stage, 200-hole impactor with a three-stage impinger using a simulated waste water irrigation spray of *Escherichia coli* (a rod-shaped bacterium, approximately 1 × 3 μm), aerosolized as predominantly single-cell droplets. The authors[13] noted that the disagreement between the impinger and the impactor measurements was greater when they observed more than 120 cfu on the impactor samples. Although they did not apply a positive-hole correction, Zimmerman et al.[13] discussed such adjustment, stating that it is based on probability statistics with little or no experimental evidence to support it.

The above discussions demonstrate that researchers have not applied positive-hole corrections uniformly to their data. Although May[9] suggested a reasonable method to adapt the 400-hole correction table for a 200-hole impactor, other investigators do not appear to know of this procedure. Even when investigators use the original correction table, they report corrected counts without any consideration of the range of numbers of collected particles that could have produced the observed number of cfu. The use of the most probable value alone confers a false precision to an adjusted measurement.

These deficiencies were addressed in the study reported here by calculating from probability theory: (1) the expected number of collected viable particles that would produce 1 through 200 and 1 through 400 observed cfu and (2) the standard deviations of these values. The results were compared with a Monte Carlo simulation in which repeated estimations of the values were made, from which averages and standard deviations were determined.

The assumption that all jets in a multiple-jet sampler are equally likely to collect particles was evaluated by collecting bacterial spores and recording where colonies developed on sampled plates. The 400-hole, N-6 impactor was chosen for evaluation because this sampler is one of the most widely recommended and used instruments for studying exposure to airborne microorganisms[4,5] and because May[9] already had demonstrated the uniformity of particle deposition for the 200-hole sampler.

Experimental Materials and Methods

Calculation of Correction Tables

Correction tables for 200- and 400-hole impactors were calculated from the following formulas:[14]

$$E(n_\kappa) = N\nu_\kappa^{(1)} \qquad \kappa = 1, \ldots, N \qquad (2)$$

and

$$\mathrm{var}(n_\kappa) = N^2\nu_\kappa^{(2)} - N\nu_\kappa^{(1)} \qquad (3)$$

where

$$\nu_\kappa^{(\alpha)} = \sum_{\ell=1}^{\kappa} \frac{1}{(N - \ell + 1)^\alpha} \qquad \alpha = 1 \text{ or } 2 \qquad (4)$$

N is the total number of holes, n_κ is the average number of particles that must be collected to fill κ holes, $E(n_\kappa)$ is the expected value of n_κ, and $\mathrm{var}(n_\kappa)$ is the variance of n_κ. $N\nu_\kappa$ in Equation 2 is equivalent to P_r in Equation 1.

For the hypothetical sampler described above, it was assumed that the collection of particles stopped at the instant a particle entered the κth hole. In reality, collection stops at random, and the expected number of particles collected if κ holes were filled would be equal to or greater than n_κ but less than $n_{\kappa+1}$. Therefore, the average, $(n_\kappa + n_{\kappa+1} - 1)/2$, is reported here.

The calculation was compared with a Monte Carlo simulation of the collection of airborne particles in a multiple-hole sampler. Particles were assumed dropped at random into N holes until κ were occupied. A hole was considered occupied after one or more particles had entered that cell. The desired number of holes to be filled was designated X. The program took N as a parameter and performed 1000 iterations, indexed by i. On each iteration, the program simulated dropping balls at random into the holes until κ were occupied for $\kappa = 1, \ldots, N$. The empirical mean of X over $i = 1, \ldots, 1000$ was used to estimate the expected value, and the standard deviation of these 1000 counts was used to estimate the standard error. If particle collection among the sampling holes was unequal (see below), the Monte Carlo simulation could have been weighted to account for these differences, and a correction table would have been produced that reflected the nonuniform particle collection.

Uniformity of Particle Collection

Although there did not appear to be any reason to doubt that particle collection was equal for all holes within each concentric ring of holes on an impactor stage, the possibility of differences between the rings needed to be checked for the single-stage, 400-hole sampler.

The assumption of uniform particle collection was tested by collecting aerosols of *Bacillus subtilis* spores (approximately 0.5 × 1.0 μm) and comparing the average number of colonies collected in each of the 11 rings of impaction sites. The concentration of the test suspension was adjusted to produce predominantly single-spore droplets from a collison

TABLE II
Positive-Hole Correction Table to Adjust Colony Counts from a 200-Hole Impactor for the Possibility of Collecting Multiple Particles through a Hole

i[A]	ii[B]	iii[C]	i	ii	iii	i	ii	iii	i	ii	iii
1	1.0	0.0	51	58.8	3.1	101	140.6	7.9	151	281.3	18.1
2	2.0	0.1	52	60.2	3.1	102	142.7	8.0	152	285.4	18.5
3	3.0	0.1	53	61.6	3.2	103	144.8	8.2	153	289.6	18.8
4	4.0	0.2	54	63.0	3.3	104	146.8	8.3	154	294.0	19.2
5	5.1	0.2	55	64.3	3.4	105	148.8	8.4	155	298.4	19.6
6	6.1	0.3	56	65.7	3.4	106	151.0	8.6	156	302.8	20.0
7	7.1	0.3	57	67.1	3.5	107	153.2	8.7	157	307.4	20.4
8	8.2	0.4	58	68.5	3.6	108	155.3	8.8	158	312.2	20.8
9	9.2	0.4	59	69.9	3.7	109	157.5	9.0	159	317.0	21.2
10	10.2	0.5	60	71.3	3.8	110	159.7	9.1	160	321.9	21.6
11	11.3	0.5	61	72.8	3.8	111	162.0	9.3	161	327.0	22.1
12	12.4	0.6	62	74.2	3.9	112	164.2	9.4	162	332.2	22.6
13	13.4	0.7	63	75.6	4.0	113	166.4	9.6	163	337.5	23.1
14	14.6	0.7	64	77.2	4.1	114	168.8	9.7	164	343.0	23.6
15	15.6	0.8	65	78.6	4.2	115	171.2	9.9	165	348.6	24.1
16	16.6	0.8	66	80.0	4.2	116	173.5	10.1	166	354.4	24.7
17	17.8	0.9	67	81.6	4.3	117	175.9	10.2	167	360.4	25.2
18	18.8	0.9	68	83.1	4.4	118	178.3	10.4	168	366.6	25.8
19	20.0	1.0	69	84.6	4.5	119	180.8	10.5	169	372.9	26.5
20	21.0	1.0	70	86.2	4.6	120	183.2	10.7	170	379.4	27.1
21	22.2	1.1	71	87.7	4.7	121	185.8	10.9	171	386.2	27.8
22	23.3	1.2	72	89.2	4.8	122	188.3	11.1	172	393.2	28.5
23	24.4	1.2	73	90.8	4.9	123	190.9	11.2	173	400.5	29.3
24	25.6	1.3	74	92.4	5.0	124	193.5	11.4	174	408.0	30.1
25	26.7	1.3	75	94.0	5.1	125	196.2	11.6	175	415.9	30.9
26	27.8	1.4	76	95.6	5.1	126	198.8	11.8	176	424.0	31.8
27	29.0	1.5	77	97.2	5.2	127	201.6	12.0	177	432.6	32.8
28	30.2	1.5	78	98.8	5.3	128	204.3	12.2	178	441.4	33.8
29	31.3	1.6	79	100.5	5.4	129	207.1	12.4	179	450.8	34.8
30	32.5	1.6	80	102.2	5.5	130	210.0	12.6	180	460.6	36.0
31	33.7	1.7	81	103.8	5.6	131	212.8	12.8	181	470.8	37.2
32	34.9	1.8	82	105.6	5.7	132	215.8	13.0	182	481.6	38.5
33	36.1	1.8	83	107.2	5.8	133	218.7	13.2	183	493.1	40.0
34	37.3	1.9	84	109.0	5.9	134	221.8	13.5	184	505.2	41.5
35	38.5	2.0	85	110.6	6.0	135	224.8	13.7	185	518.2	43.2
36	39.7	2.0	86	112.4	6.1	136	227.9	13.9	186	532.0	45.1
37	40.9	2.1	87	114.2	6.3	137	231.0	14.2	187	546.8	47.1
38	42.2	2.2	88	116.0	6.4	138	234.2	14.4	188	562.8	49.4
39	43.4	2.2	89	117.8	6.5	139	237.5	14.6	189	580.2	52.0
40	44.6	2.3	90	119.6	6.6	140	240.8	14.9	190	599.3	54.9
41	45.9	2.4	91	121.4	6.7	141	244.2	15.2	191	620.4	58.3
42	47.2	2.4	92	123.2	6.8	142	247.6	15.4	192	644.0	62.2
43	48.4	2.5	93	125.1	6.9	143	251.0	15.7	193	670.8	66.9
44	49.7	2.6	94	127.0	7.0	144	254.6	16.0	194	701.8	72.5
45	51.0	2.6	95	128.8	7.2	145	258.2	16.3	195	738.4	79.6
46	52.2	2.7	96	130.8	7.3	146	261.8	16.6	196	783.4	88.9
47	53.6	2.8	97	132.7	7.4	147	265.6	16.8	197	841.8	101.7
48	54.8	2.8	98	134.7	7.5	148	269.4	17.2	198	925.1	121.3
49	56.2	2.9	99	136.6	7.6	149	273.3	17.5	199	1075.1	156.9
50	57.6	3.0	100	138.6	7.8	150	277.3	17.8	200	1175.6	253.8

[A]i = the observed number of colony-forming units (cfu).

[B]ii = the expected number of cfu corrected for coincidence.

[C]iii = the standard deviation of ii (see text for a further explanation).

nebulizer (BGI, Inc., Waltham, Mass.).[15] The averages and the variances of the number of cfu in each ring for 13 samples were compared using one-way analysis of variance.

Results

The results of the exact calculations of particle coincidence (using Equation 2) are displayed in Table II for the case of a 200-hole sampler and in Table III for the case of a 400-hole sampler. The values in Table II agree with May's[9] method of using half the corrected value from the 400-hole table for twice the observed 200-hole sampler colony count. For example, the expected value for an observed colony count of 130 cfu collected with a 200-hole impactor is 210 cfu (Table II). This value could be derived from Table III by looking up the corrected value for 260 cfu (*i.e.*, 2 × 130) and dividing the number in column ii by two (*i.e.*, 420/2 = 210). The Monte Carlo simulation agreed very well with the exact calculation and is not presented here.

In the test of equal particle collection among the 11 rings of holes in the 400-hole impactor, the average numbers of cfu for rings 2, 3, 7, 8, and 10 were slightly higher than the overall average, while for rings 4, 5, 6, 9, and 11, the counts were slightly lower than the overall average (Table IV). A test of equal variance, however, found no significant difference (p value = 0.9766), and a test of equal means also showed no difference (p value = 0.9998).

Discussion

By using a correction table that includes an expected value and a standard deviation, an investigator can report the average corrected value along with a 95% confidence interval (mean ± 2 standard deviations). For example, when bacterial or fungal colonies are observed at 50 of 200 impaction sites, an average of 58 viable particles would have been collected. The 95% confidence interval, however, would be 52 to 64. If colonies were present at all 200 impaction sites, an average of 1176 viable particles would have been collected, and the interval from 668 to 1683 viable particles would have a 95% probability of containing the true value. Of course in actual use, a sample would be discarded as overloaded if colonies grew at every impaction site.

Tables II and III demonstrate that as the number of filled holes increases, the standard deviation also increases. For the case of the 200-hole sampler, the standard deviation is only 5.2% of the mean when 50 holes are occupied, but the coefficient of variation (CV) increases to 21.6% when all 200 holes are occupied. For the case of the 400-hole sampler, when 50 holes are occupied, the CV is 3.5%; when 200 holes are occupied, the CV is 4.0%; and when all 400 holes are occupied, the CV is 19.4%. This suggests that there is an advantage to using an impactor with 400 holes rather than one with 200 holes because the more sampling holes that are available to particles, the less likely it is that multiple particles will be collected at the same impaction site. For example, 400 viable particles collected with a 200-hole impactor would result in, on average, 173 cfu, *i.e.*, an average of 2.3 particles per impaction site. This same number of viable particles collected with a 400-hole sampler would result in, on average, 253 cfu, *i.e.*, an average of 1.6 particles per impaction site. Collecting viable particles singly increases the likelihood that all of the bacteria or fungi in a colony are the same, which decreases the chance that one species of microorganism overgrows others in a colony and masks their presence.

The test of the uniformity of colony distribution on a single-stage, 400-hole impactor agreed with what May[9] found, *i.e.*, that there is little variation in particle deposition across a plate when the jet velocity is above 500 cm/sec. Therefore, interference between holes in the single-stage, 400-hole impactor does not appear to be a problem. Colony counting, however, would be facilitated greatly if the 400 holes were arranged in a rectangular grid rather than in concentric rings, as May[9] also suggested.

When comparing concentrations of airborne bacteria or fungi, it is best to collect more than one sample at each location or under each set of environmental conditions to be compared. The corrected counts are read from the appropriate table and converted to concentration measurements by dividing by the sampled air volume. The standard deviations likewise are converted to concentration measurements. The means of two sets of measurements can be compared using a *t*-test:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_p[(1/n_1) + (1/n_2)]^{1/2}} \quad (5)$$

and a pooled standard deviation (s_p):

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \quad (6)$$

where

$$s_i^2 = \sum_{j=1}^{n_i} s_{ij}^2 \qquad i = 1, 2 \text{ and } j = 1, \ldots, n \quad (7)$$

Furuhashi and Miyamae[6] noted that the positive-hole correction appeared to underestimate the actual number of collected particles at the upper limit of the sampler's capacity. The accuracy of Tables II and III needs to be verified, perhaps by counting the number of microcolonies collected at impaction sites and comparing the totals with the theoretical corrected counts.

The difficulty that colony formation presents in quantifying the number of particles collected with an impactor is unique to the sampling of viable microorganisms. A multiple-jet impactor could be used to collect viable bacteria and fungi without correcting the observed colony counts for coincidence if investigators used sufficiently short sampling times so that the colony counts always were below the number at which correction for multiple hits became necessary. Using more than one stage also would reduce the number of cfu on each plate if the particle-size distribution of the sampled aerosol was sufficiently broad. A third alternative would be to count microscopic colonies before they merged to form single macroscopic cfu at the impaction sites. These practices, however, would limit severely the usefulness of multiple-jet impactors. Therefore, it is more

TABLE III
Positive-Hole Correction Table to Adjust Colony Counts from a 400-Hole Impactor for the Possibility of Collecting Multiple Particles through a Hole

i[A]	ii[B]	iii[C]	i	ii	iii	i	ii	iii	i	ii	iii
1	1.0	0.0	51	54.6	2.0	101	116.4	4.3	151	189.6	7.2
2	2.0	0.1	52	55.7	2.0	102	117.8	4.4	152	191.2	7.3
3	3.0	0.1	53	56.8	2.0	103	119.1	4.4	153	192.8	7.4
4	4.0	0.1	54	58.0	2.1	104	120.4	4.5	154	194.4	7.4
5	5.0	0.2	55	59.2	2.1	105	121.8	4.5	155	196.1	7.5
6	6.0	0.2	56	60.3	2.2	106	123.2	4.6	156	197.7	7.6
7	7.1	0.2	57	61.5	2.2	107	124.5	4.6	157	199.4	7.7
8	8.1	0.3	58	62.6	2.3	108	125.9	4.7	158	201.0	7.7
9	9.1	0.3	59	63.8	2.3	109	127.2	4.7	159	202.6	7.8
10	10.1	0.3	60	65.0	2.3	110	128.6	4.8	160	204.4	7.9
11	11.1	0.4	61	66.2	2.4	111	130.0	4.8	161	206.0	7.9
12	12.2	0.4	62	67.4	2.4	112	131.4	4.9	162	207.6	8.0
13	13.2	0.5	63	68.6	2.5	113	132.8	4.9	163	209.4	8.1
14	14.2	0.5	64	69.7	2.5	114	134.2	5.0	164	211.0	8.1
15	15.3	0.5	65	70.9	2.6	115	135.6	5.1	165	212.8	8.2
16	16.3	0.6	66	72.1	2.6	116	137.0	5.1	166	214.4	8.3
17	17.4	0.6	67	73.3	2.7	117	138.4	5.2	167	216.2	8.4
18	18.4	0.6	68	74.5	2.7	118	139.8	5.2	168	217.9	8.4
19	19.4	0.7	69	75.7	2.7	119	141.2	5.3	169	219.6	8.5
20	20.5	0.7	70	77.0	2.8	120	142.7	5.3	170	221.4	8.6
21	21.6	0.8	71	78.2	2.8	121	144.1	5.4	171	223.1	8.7
22	22.6	0.8	72	79.4	2.9	122	145.6	5.4	172	224.8	8.7
23	23.7	0.8	73	80.6	2.9	123	147.0	5.5	173	226.6	8.8
24	24.8	0.9	74	81.8	3.0	124	148.4	5.6	174	228.4	8.9
25	25.8	0.9	75	83.0	3.0	125	149.9	5.6	175	230.2	9.0
26	26.8	0.9	76	84.3	3.1	126	151.4	5.7	176	231.9	9.0
27	28.0	1.0	77	85.5	3.1	127	152.8	5.7	177	233.7	9.1
28	29.0	1.0	78	86.8	3.2	128	154.2	5.8	178	235.5	9.2
29	30.1	1.1	79	88.0	3.2	129	155.8	5.9	179	237.3	9.3
30	31.2	1.1	80	89.2	3.3	130	157.2	5.9	180	239.1	9.3
31	32.2	1.1	81	90.5	3.3	131	158.7	6.0	181	241.0	9.4
32	33.4	1.2	82	91.8	3.3	132	160.2	6.0	182	242.8	9.5
33	34.4	1.2	83	93.0	3.4	133	161.6	6.1	183	244.6	9.6
34	35.6	1.3	84	94.3	3.4	134	163.2	6.2	184	246.4	9.7
35	36.6	1.3	85	95.6	3.5	135	164.6	6.2	185	248.4	9.7
36	37.8	1.3	86	96.8	3.5	136	166.2	6.3	186	250.2	9.8
37	38.8	1.4	87	98.1	3.6	137	167.8	6.3	187	252.0	9.9
38	40.0	1.4	88	99.4	3.6	138	169.2	6.4	188	254.0	10.0
39	41.0	1.5	89	100.6	3.7	139	170.8	6.5	189	255.8	10.1
40	42.2	1.5	90	102.0	3.7	140	172.3	6.5	190	257.8	10.2
41	43.2	1.5	91	103.2	3.8	141	173.8	6.6	191	259.6	10.2
42	44.4	1.6	92	104.6	3.8	142	175.4	6.7	192	261.6	10.3
43	45.5	1.6	93	105.8	3.9	143	177.0	6.7	193	263.5	10.4
44	46.6	1.7	94	107.2	3.9	144	178.5	6.8	194	265.4	10.5
45	47.8	1.7	95	108.4	4.0	145	180.1	6.9	195	267.4	10.6
46	48.8	1.7	96	109.8	4.0	146	181.6	6.9	196	269.4	10.7
47	50.0	1.8	97	111.1	4.1	147	183.2	7.0	197	271.3	10.8
48	51.2	1.8	98	112.4	4.1	148	184.8	7.0	198	273.3	10.9
49	52.2	1.9	99	113.8	4.2	149	186.4	7.1	199	275.3	10.9
50	53.4	1.9	100	115.0	4.2	150	188.0	7.2	200	277.3	11.0

TABLE III—cont.

i[A]	ii[B]	iii[C]	i	ii	iii	i	ii	iii	i	ii	iii
201	279.3	11.1	251	395.0	16.6	301	558.6	25.5	351	839.9	44.7
202	281.3	11.2	252	397.8	16.8	302	562.6	25.8	352	848.2	45.3
203	283.3	11.3	253	400.4	16.9	303	566.7	26.0	353	856.6	46.0
204	285.4	11.4	254	403.2	17.0	304	570.8	26.3	354	865.2	46.7
205	287.4	11.5	255	405.9	17.2	305	575.0	26.5	355	874.0	47.4
206	289.4	11.6	256	408.7	17.3	306	579.2	26.8	356	883.0	48.1
207	291.5	11.7	257	411.4	17.5	307	583.6	27.0	357	892.2	48.9
208	293.6	11.8	258	414.2	17.6	308	587.8	27.3	358	901.6	49.7
209	295.6	11.9	259	417.1	17.8	309	592.2	27.5	359	911.2	50.5
210	297.8	12.0	260	420.0	17.9	310	596.6	27.8	360	921.1	51.3
211	299.8	12.1	261	422.8	18.0	311	601.2	28.1	361	931.2	52.2
212	302.0	12.2	262	425.6	18.2	312	605.6	28.4	362	941.6	53.1
213	304.2	12.3	263	428.6	18.3	313	610.2	28.6	363	952.2	54.0
214	306.3	12.4	264	431.6	18.5	314	614.8	28.9	364	963.2	55.0
215	308.4	12.5	265	434.5	18.6	315	619.6	29.2	365	974.5	56.0
216	310.6	12.6	266	437.4	18.8	316	624.2	29.5	366	986.1	57.1
217	312.8	12.7	267	440.4	19.0	317	629.0	29.8	367	998.0	58.2
218	315.0	12.8	268	443.5	19.1	318	633.9	30.1	368	1010.4	59.3
219	317.2	12.9	269	446.5	19.3	319	638.8	30.4	369	1023.0	60.5
220	319.4	13.0	270	449.6	19.4	320	643.8	30.8	370	1036.2	61.8
221	321.6	13.1	271	452.6	19.6	321	648.8	31.1	371	1049.7	63.1
222	323.8	13.2	272	455.8	19.8	322	653.9	31.4	372	1063.8	64.5
223	326.2	13.3	273	458.9	19.9	323	659.1	31.7	373	1078.3	65.9
224	328.4	13.4	274	462.1	20.1	324	664.3	32.1	374	1093.4	67.5
225	330.6	13.5	275	465.3	20.3	325	669.6	32.4	375	1109.1	69.1
226	333.0	13.6	276	468.5	20.5	326	675.0	32.8	376	1125.4	70.8
227	335.2	13.7	277	471.7	20.6	327	680.4	33.2	377	1142.4	72.6
228	337.6	13.8	278	475.0	20.8	328	685.9	33.5	378	1160.2	74.6
229	340.0	13.9	279	478.2	21.0	329	691.5	33.9	379	1178.8	76.6
230	342.2	14.0	280	481.6	21.2	330	697.2	34.3	380	1198.4	78.8
231	344.6	14.2	281	485.0	21.3	331	703.0	34.7	381	1218.9	81.2
232	347.0	14.3	282	488.3	21.5	332	708.8	35.1	382	1240.6	83.8
233	349.4	14.4	283	491.7	21.7	333	714.7	35.5	383	1263.4	86.5
234	351.8	14.5	284	495.2	21.9	334	720.8	35.9	384	1287.7	89.5
235	354.2	14.6	285	498.6	22.1	335	726.8	36.3	385	1313.5	92.8
236	356.6	14.7	286	502.2	22.3	336	733.0	36.8	386	1341.1	96.5
237	359.1	14.9	287	505.6	22.5	337	739.4	37.2	387	1370.8	100.5
238	361.6	15.0	288	509.2	22.7	338	745.8	37.6	388	1402.8	104.9
239	364.0	15.1	289	512.8	22.9	339	752.2	38.1	389	1437.7	109.9
240	366.6	15.2	290	516.4	23.1	340	758.8	38.6	390	1475.9	115.6
241	369.0	15.3	291	520.0	23.3	341	765.6	39.1	391	1518.1	122.2
242	371.6	15.5	292	523.8	23.5	342	772.4	39.6	392	1565.3	129.9
243	374.1	15.6	293	527.4	23.7	343	779.4	40.1	393	1618.9	139.0
244	376.6	15.7	294	531.2	23.9	344	786.4	40.6	394	1680.8	150.1
245	379.2	15.8	295	535.0	24.2	345	793.6	41.1	395	1754.1	164.0
246	381.8	16.0	296	538.8	24.4	346	801.0	41.7	396	1844.1	182.3
247	384.4	16.1	297	542.7	24.6	347	808.5	42.3	397	1960.8	207.6
248	387.0	16.2	298	546.6	24.8	348	816.1	42.8	398	2127.5	246.5
249	389.7	16.4	299	550.6	25.1	349	823.8	43.4	399	2427.5	317.1
250	392.4	16.5	300	554.5	25.3	350	831.8	44.1	400	2628.0	510.1

[A]i = the observed number of colony-forming units (cfu).

[B]ii = the expected number of cfu corrected for coincidence.

[C]iii = the standard deviation of ii (see text for further explanation).

TABLE IV
Demonstration of Uniform Particle Collection for a Single-Stage, 400-Hole Impactor

Impactor Ring Number[A]	Number of Holes/Ring	Mean Number of cfu/Ring	Standard Deviation
1	10	0.346	0.2537
2	15	0.354	0.2040
3	20	0.365	0.2004
4	26	0.310	0.2473
5	31	0.341	0.2108
6	37	0.337	0.2422
7	42	0.366	0.2317
8	48	0.364	0.2206
9	53	0.331	0.2072
10	58	0.368	0.2419
11	60	0.320	0.1949
Overall	400	0.346	0.2168

[A]Ring 1 is at the center of the impactor stage, and Ring 11 is at the outer edge.

convenient to adjust sampling data using a positive-hole correction table, and it is preferable to use a table that includes an estimate of the standard deviations associated with the expected values.

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