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Peter S. Thorne [a], Jeffrey L. Lange [a], Peggy Bloebaum [a] & Gregory J. Kullman [a]
[a] Department of Preventive Medicine and Environmental Health, The University of Iowa, Iowa City, IA
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BIOAEROSOL SAMPLING IN FIELD STUDIES: CAN SAMPLES BE EXPRESS MAILED?

Peter S. Thorne
Jeffrey L. Lange
Peggy Bloebaum
Gregory J. Kullman

Department of Preventive Medicine and Environmental Health, The University of Iowa, Iowa City, IA

Bioaerosol sampling for viable microorganisms was conducted in 25 dairy barns in summer and in winter to examine the relationship of sample storage and shipping in determining bioaerosol concentrations separately for yeasts, molds, mesophilic bacteria, and thermophilic organisms. The study also compared the performance of three sampling methods—(1) all-glass impinger (AGI) used with peptone solution in both seasons and (2) betaine solution in winter; and (3) the nuclepore filtration and elution (NFE) method, using air filtration with subsequent elution and culturing—which were studied in a pairwise fashion with duplicate, simultaneous, side-by-side sampling. For each sample, one duplicate was analyzed within two hours in a laboratory less than 50 km from the sampling site, while the other was express-mailed to the authors' laboratory. Concentrations of all microorganisms measured by the AGI peptone method were unaffected by mailing in winter, but mesophilic bacteria increased in summer. AGI betaine samples were unchanged except for increased concentrations of molds after mailing in winter. Yeasts and mesophilic bacteria significantly decreased after mailing of NFE samples. Pairwise comparison of the sampling methods in winter yielded no significant differences in airborne concentrations for the yeasts, mesophilic bacteria, and thermophilic bacteria. Both AGI betaine and NFE methods had significantly greater concentrations of molds than AGI peptone. In summer, concentrations of yeasts and mesophilic bacteria were significantly greater with AGI peptone, as were molds with the NFE method. Overall, this study supports the idea that bioaerosol samples can be collected reliably in the field and shipped on ice overnight with certain restrictions. The NFE method is recommended for molds and thermophilic organisms; the AGI peptone method was also good. For yeasts and mesophilic bacteria, the AGI was better than the NFE. Differences of performance between collection media in AGI were minimal.

Dairy farmers are exposed to excessive concentrations of agricultural dust during feed handling, application of bedding materials, manure removal, and milking. The character of this dust is dependent on the vegetable materials being handled, crop growing conditions, geographic location, prevailing storage practices and moisture content, and the degree of fecal contamination.[1] The microbial constituents of agricultural dust include a complex mixture of viable and nonviable organisms[2–3] capable of yielding inflammatory toxins and serving as antigens to provoke immune responses in farmers. Some of these microorganisms have been implicated in the etiology of hypersensitivity pneumonitis and organic dust toxics syndrome.[4]

Accurate quantification of agricultural dust constituents is important for assessing whether a health hazard exists, determining dose-response relationships, and for evaluation of remedial actions. However, the quantification of airborne viable microorganisms in agricultural environments poses special challenges. Microbial sampling is complicated by the high concentrations frequently encountered, which limit the use of air to agar samplers, such as the Andersen Microbial Sampler, due to the potential for overloading. Thus, the all-glass impinger (AGI) and nuclepore filtration and elution methods (NFE) are the most commonly selected alternatives.[5] When sampling at distant field sites, interpretation is further complicated by having to mail samples overnight to a laboratory before they can be plated, giving organisms the opportunity to multiply or die in transit. Previous investigations have addressed this problem by plating shortly after collection in a mobile lab unit[6] or by refrigerating samples until they can be plated.[7–8]

This study was performed in conjunction with a large epidemiologic study of pulmonary risk factors among dairy farmers. The primary objective was to determine if overnight express mailing of samples back to the authors' home laboratory would produce valid results. The secondary objective was to systematically compare the field performance of the AGI with peptone or betaine collection media, and the NFE method for yeasts, molds, mesophilic bacteria, and thermophilic bacteria measured in 25 barns during summer and winter. Bacteria collected in the AGI may be subject to osmotic shock on rehydration in the liquid collection media. Betaine has been proposed as a useful osmoprotectant that may increase the recovery of viable airborne bacteria in environmental sampling.[9] Both the NFE method and the AGI with

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peptone collection media have been used widely to sample a variety of occupational environments.[5,7]

MATERIALS AND METHODS

Airborne viable microorganisms were sampled at 25 randomly selected barns located in the counties of Clark, Marathon, and Wood in central Wisconsin during the summer of 1992 (mean indoor conditions: 17.8°C, 75% RH; mean outdoor conditions: 13.5°C, 81% RH), and repeated the following winter (mean indoor conditions: 10.4°C, 76% RH; mean outdoor conditions: −11.6°C, 72% RH). Each stanchion barn housed an average of 52 milking cows (range 11 to 425, median 40) in two rows running the length of the barn. Feed was stored in one to four silos attached to the barns by a feed room. Hay, straw, or sawdust were used for bedding. During the summer months outside makeup air was introduced to the barns via open windows, doors, and axial fans. In sharp contrast, little makeup air was introduced in winter. The cows were in the barn from three to six hours a day in the summer and were inside nearly all winter.

All air sampling instruments ran simultaneously for the entire morning work shift (median 3 hours, range 2–6 hours). Work activities included milking, feeding, bedding, and manure removal. Air sampling instruments were hung in a metal basket approximately 1.5 m off the floor in the center aisle of each barn to represent worker breathing heights. The position of each basket in the barns was the same for both seasons. In the summer a single AGI using peptone collection media and two NFE samplers were used for bioaerosol sampling. In winter an AGI with betaine collection media was substituted for one of the NFE samplers.

NFE

NFE samples were collected in 25 mm open-face plastic cassettes loaded with polycarbonate membrane filters, 0.8 mm pore size (Poretics Corp. Livermore, Calif.), supported by polyvinyl chloride pads, all sterilized by UV light. Air was sampled at 2 L/min with constant flow air-sampling pumps (Gil-Air, Gilian Instrument Corp., Wayne, N.J.). After sampling, inlet and outlet connections to the cassettes were capped and cassettes were transported at ambient temperatures in an upright position. One NFE was processed within two hours of sampling at a remote laboratory located at the Marshfield Medical Research Foundation in Marshfield, Wis. (Field Lab). The second NFE was packed in "blue ice" freezer packs in a foam cooler and mailed by overnight express 400 km to the laboratory at the University of Iowa in Iowa City, Iowa (Home Lab). The temperature and condition of the samples on arrival were recorded, and the NFE filters were immediately processed as described previously.[5,10–11] Serial 10-fold dilutions of eluates in peptone water were plated in duplicate onto malt extract agar (MEA) incubated at 22 ± 2°C for yeasts and molds, trypticase soy agar (TSA) incubated at 22 ± 2°C for mesophilic bacteria, and TSA incubated at 53 ± 1°C for thermophilic bacteria (Difco Laboratories, Detroit, Mich.). TSA plates were counted daily for five days while MEA plates were counted for eight days.

AGI

AGI samples were collected with autoclaved all-glass impingers (AGI-30, ACE Glassworks, Vineland, N.J.) using 20 mL of sterile collection media at a flow rate of 12.5 L/min for 30 min at ambient temperature. To determine a time-weighted average of viable organisms for the entire work shift, at the end of each 30 min sampling period the lot of collection media was pooled into a sterilized collection bottle stored on ice and a fresh 20 mL lot of media was introduced to the AGI. Peptone collection media consisted of 1% peptone in distilled water, 0.01% Tween 80, and 0.005% antifoam A. Betaine collection media contained 10 mM phosphate buffer, 5 mM betaine, 0.01% Tween 80, and 0.005% antifoam A (Sigma Chemical Co., St. Louis, Mo.).

After sampling, pooled AGI samples were transported to the Field Lab on ice, and the curved inlet tubes of the AGI were washed with collection media and added to the pooled sample. Serial 10-fold dilutions of AGI peptone and betaine collection media aliquots (in peptone or betaine solutions) were plated in duplicate onto MEA and TSA culture media in the Field Lab (Day 0). The remaining undiluted AGI sample suspensions were then either refrigerated for 24 hours at 4°C in the field before plating again a day after sampling (Day 1, not mailed), or were express-mailed at 4°C to the Home Lab to be plated one, two, and three days after collection (Day 1, 2, or 3, mailed). For some of the summer samples the serially diluted media was plated in the Field Lab, and the plates were mailed to the Home Lab for incubation and enumeration.

Interinvestigator Validation

To validate the paired analysis of mailed versus not mailed bioaerosol samples, interinvestigator bias in the enumeration of colonies between the Field Lab and the Home Lab had to be assessed. Most other potential sources of systematic error were eliminated by having all laboratory materials prepared by one person, by using identical techniques in both laboratory settings, and by using the same lots of all laboratory supplies throughout the study. Interinvestigator validation was achieved by each investigator blindly counting 10 arbitrarily selected culture plates of each organism type in the same time frame. Results from nonparametric analysis showed that the counting of yeasts, molds, and mesophilic bacteria were all highly correlated ($r \geq 0.99$) and did not differ statistically between the two investigators ($p > 0.3$). Thus, comparison of sample concentrations between the two laboratories would not be confounded by differences in counting.

However, counting of the thermophilic bacteria was the most subjective, and Home Lab counts were significantly ($p = 0.02$) higher (4.2%) than Field Lab counts, while still highly correlated ($r = 0.99$). Since they were higher in every case, these data were divided by 1.042 for statistical tests that compared mailed versus not mailed thermophilic bacteria concentrations.

TABLE I. Comparison of Samples Processed in the Field Lab versus Those Shipped via Overnight Mail to the Home Lab

Organism	Season Sample Method	N pairs	Wilcoxon P Value	Difference Log_{10}[A]	Spearman r
Yeast	summer AGI peptone	16	0.07	0.18	0.55
	summer NFE	15	0.003	0.67	0.14
	winter AGI peptone	19	0.9	0.002	0.84
	winter AGI betaine	19	0.8	0.05	0.89
Mold	summer AGI peptone	16	0.7	0.06	0.75
	summer NFE	15	0.2	0.09	0.74
	winter AGI peptone	18	0.1	0.12	0.85
	winter AGI betaine	18	0.01	0.24	0.81
Mesophilic Bacteria	summer AGI peptone	16	0.008	0.33	0.41
	summer NFE	15	0.0002	0.73	0.79
	winter AGI peptone	19	0.7	0.07	0.62
	winter AGI betaine	19	0.9	0.009	0.64
Thermophilic Bacteria	summer AGI peptone	16	0.08	0.15	0.76
	summer NFE	14	0.9	0.07	0.75
	winter AGI peptone	15	0.4	0.13	0.83
	winter AGI betaine	14	0.08	0.15	0.91

[A] Difference in Log_{10} concentration of paired samples

Statistical Analysis

All statistical analyses were performed using SAS (version 6.04). Bioaerosol concentrations were quantified as colony forming units per m^3 of air (cfu/m^3) according to the following formula:

$$\text{Concentration} = \frac{\#\ \text{CFU} \times \text{Dilution Factor} \times \text{Final Solution Volume}}{\text{Volume of Aliquot Plated} \times \text{Sampled Air Volume}} [=]\ \text{CFU/m}^3$$

Colony concentrations from duplicate plates were not statistically different from one another ($p > 0.1$), and they all demonstrated high correlation ($r \geq 0.85$). Thus, for the paired data, concentrations were calculated by taking the means of duplicate plates. In a few cases where a duplicate plate was dropped, broken, or overgrown by a single spreading colony, the remaining plate value was used. The Shapiro and Wilk's W statistic was used to test normality of data after logarithmic transformation.[12] W statistic probability values less than 0.05 led to rejection of the hypothesis that the data were drawn from a normal distribution. In most cases the data were skewed such that the nonparametric Wilcoxon paired sample test was used to determine two-tailed p-values, which were considered significant at less than 0.05. Geometric mean differences in log_{10} were calculated between the concentrations to gauge practical significance for those samples that yielded statistically significant differences (e.g., a value of 1.0 indicated a 10-fold difference of cfu/m^3). Spearman rank correlation coefficients were used to measure the intensity of association between two variables. Values greater than 0.75 were considered to have good correlation, moderate correlation with values from 0.40 to 0.75, and poor correlation below 0.40.[13] Multiple sample testing used analysis of variance with repeated measures after applying Mauchly's criterion for test of sphericity.[14]

RESULTS

Viable bioaerosol concentrations were measured using AGI peptone, AGI betaine, and NFE methods in 25 dairy barns in two seasons. Paired data were examined to determine the effects of shipping and storage on microorganism concentrations and compare the performance of the three sampling methods.

Field Lab Use Versus Mailing To Home Lab

Bioaerosol sample concentrations can be confounded by a shipping variable when samples are mailed from the Field Lab to the Home Lab. In this study the shipping variable consisted of 20- to 28-hour storage on ice and agitation from physical handling during transit. The effect of shipping was tested for AGI peptone in the summer and winter, AGI betaine in the winter, and NFE in the summer. Identical procedures were followed in both laboratories and all reagents were drawn from the same lots.

For each sampling method in each season, the fourth column in Table I lists the p value for the comparison of the bioaerosol samples analyzed in the Field Lab versus those shipped overnight to the Home Lab. The fifth column lists log_{10} mean differences between the two concentrations to illustrate practical significance of the differences. The last column lists the coefficient of correlation (r) for mailed and not mailed concentrations.

The data summarized in Table I are plotted in Figures 1 and 2. Figures 1a and 1b present data for yeasts and 1c and 1d show data for molds. Figures 2a and 2b are for mesophilic bacteria and 2c and 2d present thermophilic bacteria data. For each pair, the summer data are shown on the left and winter data on the right. Diagonal lines in the plots are lines of identity, signifying equal concentration between mailed and not mailed samples. Hence, data points below and to the right of the line indicate an increase in concentration, while points

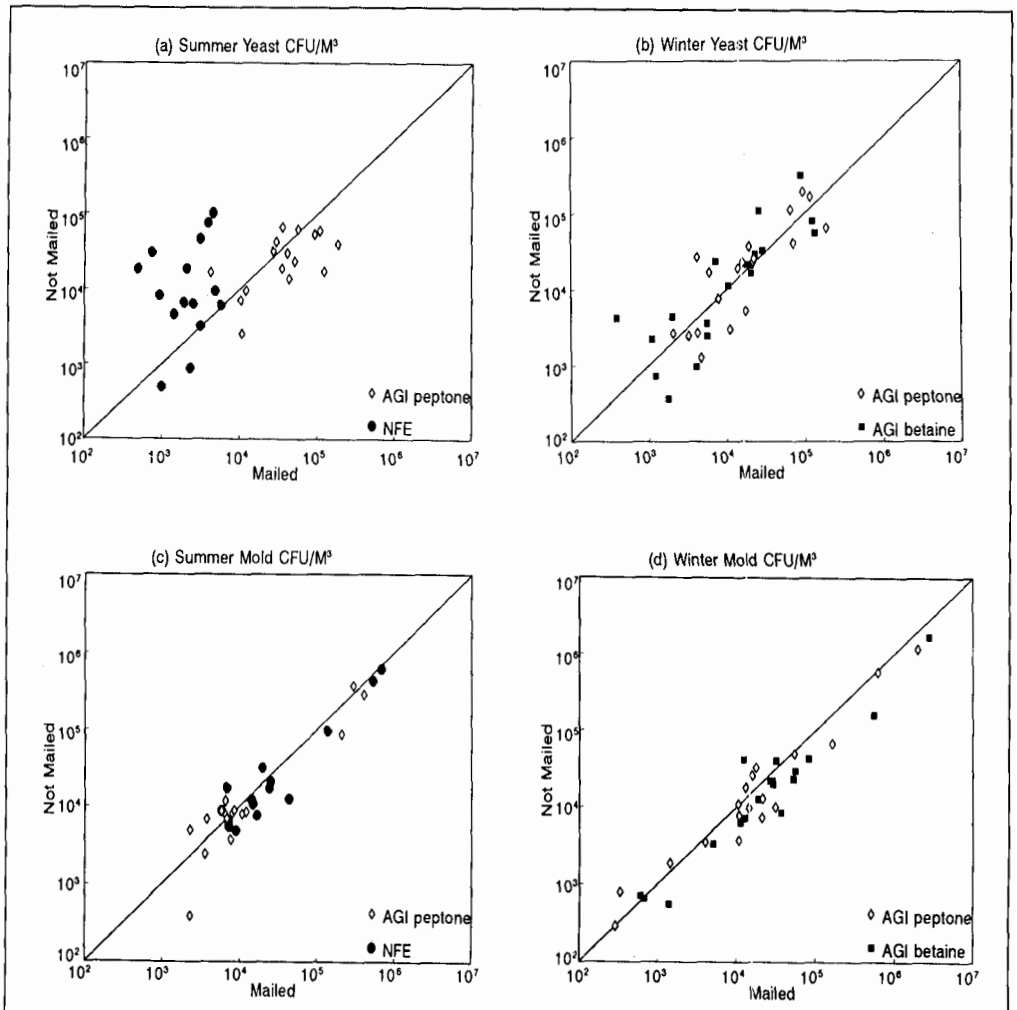

FIGURE 1. Evaluation effects for express mailing bioaerosol samples (yeasts and molds)

above and to the left represent a decrease in concentration on overnight mailing.

For yeasts, mailing of AGI peptone in the summer (Figure 1a) did not alter the concentration, but there was only moderate correlation ($r = 0.55$) between the mailed and not mailed samples. Mailing of the NFE during the summer led to a significant decrease ($p = 0.003$) in concentration with a mean difference of 0.67 log units and no correlation. The winter AGI peptone and betaine methods, which collect the organisms into a liquid medium (in contrast to the NFE), had high correlation (both $r \geq 0.84$), and their values were centered about the line of identity (Figure 1b).

Plots comparing mold concentrations are shown as Figures 1c and 1d. Summer AGI peptone, winter AGI peptone, and NFE sample methods did not differ as a result of shipping and were all highly correlated ($r \geq 0.74$). Mold dispersal units are hardy spores that tolerate desiccation better than yeasts. The winter AGI betaine, with 83% of the data points below the line of identity, had a significant increase ($p = 0.01$) on mailing (mean = 0.24 log units). The mailed and not mailed concentrations for this sample method were highly correlated ($r = 0.81$) for nearly four orders of magnitude.

For the mesophilic bacteria the summer AGI peptone had all but two data points below the line of identity (Figure 2a), signifying an increase ($p = 0.008$) in concentration of a mean 0.33 log units with overnight mailing. Correlation for this method was rather poor ($r = 0.41$). For the NFE data, 14 of 15 points were above the line of identity, demonstrating a highly significant decrease on mailing ($p = 0.0002$) with a mean 0.73 log units. Unlike the summer AGI peptone, the winter AGI peptone was centered about the line of identity with moderate correlation ($r = 0.62$). The winter AGI betaine data also showed no difference in concentration after mailing and demonstrated moderate correlation ($r = 0.64$).

Plots of thermophilic concentrations are shown in Figures 2c and 2d. For all sample methods in both summer and winter the thermophilic bacteria did not change in concentration between mailed and not mailed, plus, all yielded high correlation ($r \geq 0.75$).

Longer-term storage of samples was tested for AGI peptone and AGI betaine methods. Figure 3 illustrates geometric mean concentrations of these samples ($n = 9$) plated on three successive days after shipment to the Home Lab. Trends in the data shown in Figure 3 suggest growth for yeasts and mesophilic bacteria and loss for molds. However, for all organisms and both sample methods, only the yeasts for the AGI betaine increased significantly ($p = 0.01$) in concentration over the three days. For the AGI peptone yeast and mold samples, large variations in recovery were apparent (mean square error = 1.02–1.30) and differences did not achieve significance.

The effect of shipping samples from the Field Lab to the Home Lab after plating was examined in the summer for 10 sites. For each organism there was no difference ($p > 0.1$) in concentrations for plates processed and enumerated at the Field Lab or those mailed to the Home Lab and enumerated. Correlation was excellent for molds ($r = 0.85$) and mesophilic bacteria ($r = 0.92$), moderate for yeasts ($r = 0.42$) and thermophilic bacteria ($r = 0.54$).

Sample Storage and Shipping Effects Studied Independently

In the testing presented above it was not possible to separate the effects of a one-day delay in plating and the effects

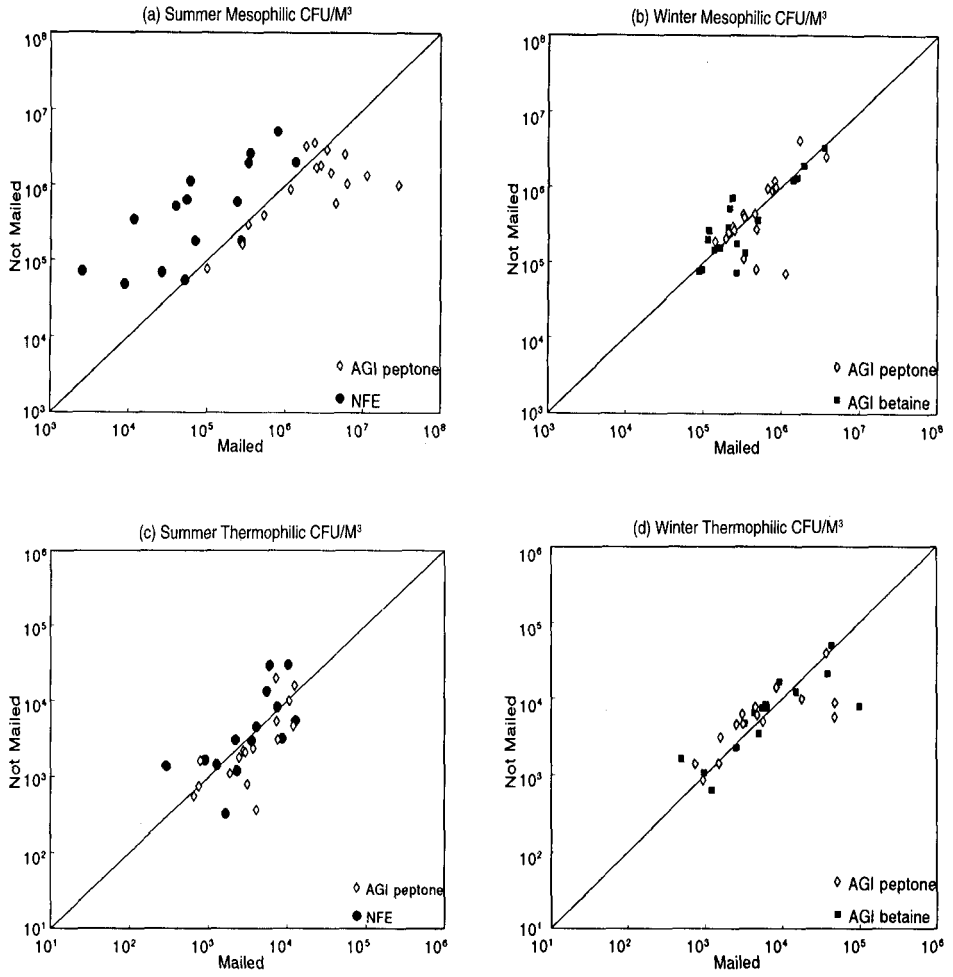

FIGURE 2. Evaluation effects for express mailing bioaerosol samples (mesophilic and thermophilic bacteria)

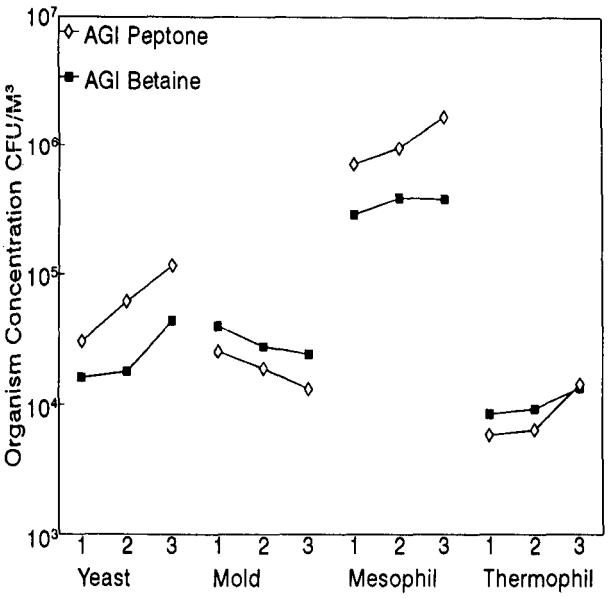

FIGURE 3. Evaluation of storage of bioaerosol samples

of mailing. The data presented in Table II separate these effects for AGI sampling methods, and show that no significant differences existed between any Day 0–Day 1 paired concentrations for the AGI peptone and AGI betaine. Spearman correlation coefficients were all very good ($r \geq 0.87$). Assessment of the effect of physical handling in shipping separate from storage time is shown in the bottom half of Table II. Day 1 Field Lab and Day 1 Home Lab samples were ostensibly held at 4°C for 24 hours before plating, although there may have been some fluctuation in temperature of mailed samples. AGI betaine mold concentrations were significantly higher ($p = 0.01$) for the mailed Home Lab samples. Thus, the increase in concentration for AGI betaine sampled mold shown in Table I can be attributed not to differences in storage time, but to changes induced by shipping (Table II).

Comparison of AGI versus NFE

Results across sampling method (Table III) were compared using data collected from the Field Lab (not mailed) for samples processed on the day of sampling (Day 0). In summer the AGI peptone method yielded significantly more yeasts ($p = 0.004$) than the NFE, with a mean difference of 0.30 log units. Significantly more mesophilic bacteria were isolated by the AGI peptone than by the NFE ($p = 0.009$), with a mean difference of 0.34 log units, perhaps because aggregates are more likely to be separated during liquid impingement or because of decreased viability of these fragile organisms due to desiccation on the surface of the nuclepore filters. The NFE yielded highly significantly greater mold concentrations ($p = 0.0001$) than the AGI peptone, with a mean difference of 0.26 log units. There was no difference in concentrations of thermophilic bacteria for the two sample methods. Between these methods, the sampling of yeasts, molds, and thermophilic bacteria had excellent correlation (all $r \geq 0.74$), while correlation for the mesophilic bacteria was moderate ($r = 0.56$).

Comparison of the winter AGI peptone sampling to the AGI betaine method for molds demonstrated marginally higher recovery by the AGI betaine method ($p = 0.05$). However, the mean difference was only 0.10 log units. Correlation of these sampling methods was excellent for the yeasts, molds,

TABLE II. Comparisons for Winter Samples Processed at the Field Lab on Separate Days and Between the Field Lab and Home Lab on the Same Day for the AGI Methods

Organism	Sample Method	N pairs	Wilcoxon P Value	Difference Log_{10}[A]	Spearman r
Day 0 Field Lab vs. Day 1 Field Lab					
Yeast	AGI peptone	19	0.2	0.12	0.87
	AGI betaine	19	0.3	0.0009	0.94
Mold	AGI peptone	19	0.6	0.06	0.95
	AGI betaine	19	0.9	0.005	0.89
Mesophilic	AGI peptone	18	0.4	0.01	0.96
Bacteria	AGI betaine	19	0.1	0.07	0.94
Thermophilic	AGI peptone	16	0.2	0.13	0.91
Bacteria	AGI betaine	15	0.9	0.0009	0.88
Day 1 Field Lab vs. Day 1 Home Lab					
Yeast	AGI peptone	18	0.07	0.44	0.88
	AGI betaine	17	0.2	0.26	0.93
Mold	AGI peptone	18	0.7	0.05	0.90
	AGI betaine	17	0.01	0.30	0.84
Mesophilic	AGI peptone	17	0.7	0.22	0.80
Bacteria	AGI betaine	18	0.6	0.11	0.75
Thermophilic	AGI peptone	16	0.4	0.27	0.85
Bacteria	AGI betaine	16	0.5	0.07	0.94

[A] Difference in Log_{10} concentration of paired samples

and thermophilic bacteria ($r \geq 0.82$), but only moderate for mesophilic bacteria ($r = 0.45$). For winter sampling, the AGI peptone mold concentrations were lower ($p = 0.05$) than the NFE with a mean difference of 0.27 log units. Correlation between the two sampling methods was poor for yeasts ($r = 0.30$) and mesophilic bacteria ($r = 0.13$), moderate for the molds ($r = 0.48$), and excellent for the thermophilic bacteria ($r = 0.79$). Comparison of winter sampling with the AGI betaine method and the NFE method showed no significant concentration differences. Spearman correlation coefficients were high for the yeasts ($r = 0.82$), moderate for the molds ($r = 0.67$) and thermophilic bacteria ($r = 0.64$), and low for mesophilic bacteria ($r = 0.37$).

DISCUSSION

Validity of Shipping Bioaerosol Samples

The validity of overnight-mailing viable bioaerosol samples has not previously been investigated. Therefore, the relationship of overnight shipping to microorganism concentrations and the performance of several bioaerosol sampling methods were evaluated under field sampling conditions using samples collected in 25 Wisconsin dairy barns. The AGI with peptone, AGI with betaine, and NFE methods were compared in summer and winter for yeasts, molds, mesophilic, and thermophilic bacteria covering two to five orders of magnitude concentration range. Mailing samples from the Field to the

TABLE III. Comparison of Bioaerosol Concentrations[A]

	Yeast			*Mold*			*Mesophilic Bacteria*			*Thermophilic Bacteria*		
	Wilcoxon P Value	*Difference Log_{10}[B]*	*Spearman r*	*Wilcoxon P Value*	*Difference Log_{10}*	*Spearman r*	*Wilcoxon P Value*	*Difference Log_{10}*	*Spearman r*	*Wilcoxon P Value*	*Difference Log_{10}*	*Spearman r*
Summer AGI Peptone vs. Summer NFE												
	0.004	0.30	0.74	0.0001	0.26	0.76	0.009	0.34	0.56	0.3	0.14	0.80
Winter AGI Peptone vs. Winter AGI Betaine												
	0.3	0.18	0.82	0.05	0.10	0.87	0.2	0.10	0.45	0.1	0.07	0.84
Winter AGI Peptone vs. Winter NFE												
	0.9	0.12	0.30	0.05	0.27	0.48	0.2	0.22	0.13	0.3	0.003	0.79
Winter AGI Betaine vs. Winter NFE												
	0.6	0.03	0.82	0.2	0.18	0.67	0.9	0.11	0.37	0.9	0.11	0.64

[A] N=20-24 for each organism class and each method in each season
[B] Difference in Log_{10} concentration of paired samples

Home Lab incurred a 24-hour delay in plating and contributed sample agitation during shipping. Only limited information on the effect of storage time on the NFE sample method has been presented to date. It was previously reported that bacterial concentrations from swine barns determined with NFE samples stored at room temperature were reasonably stable up to 38 hours after sampling.[5] Palmgren and colleagues concluded that storage time on dry filters was responsible for loss of viability using the NFE method, though decreases could not always be expected as some organisms had stable viability with time delay between sampling and plating.[10]

In this study a one-day delay in NFE plating due to mailing did not affect the viable concentration of the hardy molds and thermophilic bacteria, and both organisms showed good correlation ($r = 0.74$–0.75) between mailed and not mailed. The one-day delay in plating did cause a highly variable ($r = 0.14$) and significant ($p = 0.003$) decrease in the concentration of viable yeast that was most likely due to desiccation of these organisms on the dry surface of the NFE filters. Mesophilic bacteria in this study decreased very significantly ($p = 0.0002$) when shipped on ice but remained highly correlated ($r = 0.79$). These organisms may have also suffered viability loss from desiccation. Since correlation of these samples was high this suggested a concentration-independent loss of viability.

For all organism types and both AGI media there were only two conditions where significant differences arose that were attributable to shipping or a 24-hour delay in sample processing (Tables I and II). The comparison of Day 0 Field Lab versus Day 1 Home Lab data (Table I) for the mesophilic bacteria and AGI peptone method in the summer showed that the mailed samples exhibited higher concentrations compared to those processed immediately after sampling, even though these samples were shipped on ice and all arrived cold. Peptone is a nutritive collection medium with bovine protein and a nitrogen supply that could support bacterial growth. Therefore, the increase in concentration could be the result of actual growth supported by the nutritive media or a differential increase in viability of rested organisms stressed from sampling. No such differences were observed for AGI peptone winter sampling. Possible explanations for differential results between summer and winter AGI peptone methods include the change in seasons causing a corresponding change in species of airborne microorganisms with different growth characteristics, or alternatively, the difference in sampling temperature between seasons altering the stress of microorganisms or sampling efficiency of the AGI. Unfortunately, organisms were not speciated to determine if the bacterial flora differed between summer and winter. A significant difference was also detected for the AGI betaine method for molds in winter (Table I and Figure 1d) where the difference was 0.24 log units, and the Home Lab concentrations were highly correlated with the Field Lab determinations. Data in Table II suggest that this difference was attributable to effects of shipping ($p = 0.01$) and not the delay in processing ($p = 0.96$).

For both the yeasts and mesophilic bacteria in the NFE method there were reductions in bioaerosol concentrations for the samples mailed to the Home Lab versus those processed immediately. Mold and thermophilic bacteria samples were less susceptible to artifacts induced by shipping and a one-day delay in sample processing. Tables I and III illustrate that for the thermophilic organisms, no significant differences were observed for overnight mailing and delay, delay alone, or mailing alone. Further, all correlation coefficients exceeded 0.74. In a previous investigation of delayed plating for the AGI sampling of thermophilic organisms, Morey, with a limited sample size, noted that three days of successive plating on unspecified media with storage at 4°C did not significantly change the concentration.[15] In the present study, comparison of the mailed versus not-mailed samples for both the NFE and AGI methods agreed favorably for thermophilic bacteria. These samples were stable for two to three days after bioaerosol sampling (Figure 3).

Comparison of Sampling Methods

Matched-paired comparisons between the AGI peptone, AGI betaine, and the NFE sample methods were employed to assess differences and correlations in yeasts, molds, mesophilic bacteria, and thermophilic bacteria concentrations. It is intuitive that at least a good correlation should exist between sample methods for them to be considered acceptable.

Bioaerosol sampling with the AGI sampler and peptone, a commonly used nutritive collection medium, and with betaine, a reportedly osmoprotective medium, have not previously been reported in the reviewed literature. At the onset of this study, the authors hypothesized that betaine would yield higher concentrations of mesophilic bacteria than the peptone. However, results indicate there was no statistical difference between the two collection media. A key difference between this study and work done by Marthi and colleagues[9] is that they compared a phosphate buffer with or without betaine, while the present study compared betaine to a nutritive collection medium. Correlation of AGI peptone and AGI betaine sample methods plated on the same day of sampling was very good for yeasts, molds, and thermophilic bacteria (all $r \geq 0.82$). Concentrations of yeasts and thermophilic bacteria were not different between the two sample methods.

It was reported previously that AGI peptone and NFE sample methods were highly correlated for bacteria ($r = 0.86$) and fungi ($r = 0.78$) during fall and winter sampling in swine barns.[5] In addition, there were no statistical differences in concentrations between the two sample methods. In the present study, fungi were separated into yeasts and molds. A key distinction between yeasts and molds affecting sampling efficiency is that yeasts are prone to desiccation, while mold spores are more resilient. Consistent with previous results for fungi in swine houses,[5] moderate to good correlation existed between AGI peptone and NFE for molds (summer $r = 0.76$, winter $r = 0.48$) and somewhat lower values for yeasts (summer $r = 0.30$, winter $r = 0.74$) (Table III). The AGI peptone method yielded higher concentrations of yeasts in summer ($p = 0.004$), while the NFE method gave higher concentrations of molds in summer ($p = 0.0001$) and winter ($p = 0.05$). A disadvantage to the AGI is its inability to retain in suspension spores that are hydrophobic or made buoyant by trapped air. These mold spores can be re-entrained into the exiting air

stream. Since the AGI peptone and NFE sample methods yielded viable colonies of yeasts and molds with different recoveries, it proved important to count these organism types separately. Lumping them together as one group would have masked the true relationship of sampling efficiency.

CONCLUSION

Overnight mailing on ice of viable agricultural bioaerosol samples did not significantly alter the quantification of collected microorganisms for the AGI peptone during the winter. However, concentrations of mesophilic bacteria did increase with the AGI peptone in summer. All concentrations as determined with the AGI betaine did not change, except for a significant increase in molds. Yeast and mesophilic bacteria concentrations had highly significant decreases occur after mailing with the NFE, while mold and thermophilic bacteria concentrations remained steady. Thus, this study supports the notion that bioaerosol samples can, under certain circumstances, be reliably collected in the field and shipped on ice overnight to a distant laboratory.

Comparison of the three sample methods (AGI peptone, AGI betaine, and NFE) on the same day of sampling in the winter determined no significant differences in concentrations for the yeasts, mesophilic bacteria, and thermophilic bacteria. Both the AGI betaine and NFE produced significantly higher concentrations of molds than the AGI peptone.

In the summer only the AGI peptone and NFE were compared. Concentrations of yeasts and mesophilic bacteria were significantly higher with AGI peptone, while the NFE had higher concentrations of molds.

The NFE method is recommended for molds and thermophilic organisms although the AGI peptone method also was good. For yeasts and mesophilic bacteria concentration measurements, the AGI was the better method. No clear advantage was found of the betaine sampling media over the peptone solution.

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