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**AIRBORNE VIABLE MICROORGANISMS IN OFFICE ENVIRONMENTS:
SAMPLING PROTOCOL AND ANALYTICAL PROCEDURES**

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16. Abstract (Limit: 200 words) The sampling protocol and analytical procedures for airborne viable microorganisms in office environments are presented. Sampling strategy is outlined. The primary objective of sampling is to identify the sources of microorganisms so that corrective action may be taken. Sampling times, culture media, sample selection, calibration, and disinfection are discussed. Steps in the handling and processing of collected samples are described. These should have the input of a microbiologist or consultant experienced in microbiological techniques. Data interpretation is considered. Recommended remedial actions in cases where the total microorganism count exceeds 10,000 per cubic meter are given. These include removing stagnant water and slime from mechanical systems in the building, eliminating water spray systems in air handling units, substituting steam for cold water in humidifiers, eliminating the use of cold mist evaporators, maintaining the relative humidity below 70 percent, replacing heating, ventilation, and air conditioning system filters at scheduled intervals, providing adequate amounts of suitable outdoor air to reduce concentrations of microorganisms that may be shed by occupants, and reassigning affected or sensitized personnel to other work environments if necessary.		13. Type of Report & Period Covered		
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I. Rationale for Monitoring Airborne Viable Microorganisms in an Office

Environment: It is recommended that aerobiological sampling be initiated only after medical or clinical reports indicate the possibility of workplace-related illness that may be due to bioaerosols. It should be recognized that these types of illnesses may be caused by a diverse array of agents including bacteria, actinomycetes, and fungi [15,19]. Dose response data between exposure to those agents and human illness are unavailable. Nevertheless, the quantitative measurement of viable microorganisms, as described herein, is recommended as an initial step in assessing the hygienic status of an office environment.

II. Sampling Strategy

- A. Objective: The primary objective of sampling is to identify the source of airborne microorganisms so that corrective action may be undertaken.
- B. Presumptive Sources: Outdoor air; components of the heating, ventilation and air-conditioning (HVAC) system; office furnishings and equipment; portable appliances; occupants.
- C. Sampling Duration: For most situations 3 to 6 minutes sampling duration is adequate or at least 0.1 m^3 of air should be sampled. As a minimum, duplicate samples should be obtained for each culture medium at each location for each variable that may affect bioaerosol levels (e.g., humidifier on or off; fan coil unit operating with dirty or clean filter).
- D. Where to Sample:
1. Near potential sources such as diffusers, fan coil units, office furnishings, and portable appliances; within return chase and air supply plenum of HVAC system; near occupants.

2. For comparison, in the outdoor air away from obvious sources of bioaerosols and in offices where occupants do not have building-related illness.

E. When to Sample: Sampling should be carried out:

1. Before the HVAC system is turned on and before occupants arrive for work.
2. Before occupants arrive for work at a time when the HVAC system is operating.
3. At the time of maximal occupancy (HVAC system on).
4. After occupants leave (HVAC system off).
5. In relation to changes in HVAC system operational parameters or other building and office variables.

F. Quality Assurance: Culture media must be checked for sterility; culture plates must be inserted into sampler but not otherwise exposed to air flow through instrument; temperature of incubators must be checked; some culture plates should be inoculated with test organisms to assure laboratory quality control.

III. Culture Media

- A. Culture Plates: Use sterile 100 x 15 mm plastic Petri dishes and add 45 ml of media [4] or use sterile glass Petri dishes and add 27 ml of media [3].

- B. Media for detection and enumeration of fungi: malt extract agar - ingredients per liter.

Malt extract	20 g
Peptone	1 g
Dextrose	20 g
Agar	20 g

- C. Media for detection and enumeration of bacteria and actinomycetes.
BBL tripticase soy agar or equivalent - 40 g per liter.

- D. Additional Media: Other media for the culture of fungi, bacteria, and actinomycetes may be used. However, equivalency to the media listed in Section III 2-3 in terms of supporting growth of microorganisms collected in an office environment should be known (See Reference 6).

IV. Sampler Selection, Calibration, and Disinfection

A. Sampler Selection

1. N6-Andersen: The Andersen viable impactor is considered to be a standard instrument for collection of airborne viable particulate in environments (like offices) where the concentration of bioaerosols is normally low [5]. A modified version of the Andersen sampler [referred to as NIOSH-6 (N6)] procedure [13] is used in place of the original multistage impactor [3]. A Petri dish containing culture media is placed on top of the instrument base plate. Stage 6 (only) is then

placed on top of the base plate (spring clamps previously removed from base plate). Stage 6 is then sealed to the base plate (electrical tape does well). The standard Andersen inlet is not used. For further details see reference 13.

2. Use of Other Impactors: The utility of other viable impactors such as the Spiral Air System, Biotest, and Microban instruments is recognized especially where portability of the sampler is of importance with regard to the sampling strategy. These as well as other impactors may be used in place of the N6 sampler. However, their collection efficiency relative to the N6 instrument should be known. This may be accomplished by reference to manufacturer's information or published data [12]. If the efficiency of other instruments is significantly different than the N6 sampler, data should be adjusted to reflect airborne concentrations that would have been obtained if the N6 sampler had been utilized. In cases where medical reports indicate illness due to a lower or distal airways exposure, size classification of collectate into respirable and nonrespirable particulate may also be desirable [3,11].

3. Other Sampling Methods: Aerosol sampling devices which operate on the principles of filtration and impingement have many successful applications in aerobiology [7], but they are not recommended for this application because: very low

counts are expected in most office environments; results obtained with impingers are not directly comparable with impactor data; and there is loss of viability of some organisms collected on filters.

B. Sampler Collection: Samplers must be calibrated to recommended air flow rates. Air flow rate through samplers should be checked on a daily basis during use in the field.

C. Sampler Disinfection: Ideally the sampler sieve plate should be sterilized before use at each sampling location. For use in the field decontamination by immersion in 70% ethanol for one minute will suffice. Excess ethanol should be drained away and the sieve plate should be allowed to dry thoroughly uncovered before use. Other methods of disinfection as recommended by manufacturers are acceptable. It is advisable to clean the sampler and inspect sieve plate jets for blockage at the end of each day.

V. Handling and Processing of Collectate

A. Obtain input of microbiologist or consultant experienced in microbiological techniques. Make arrangements for sample analysis with a microbiological laboratory that is experienced in the handling and processing of airborne microorganisms prior to the initiation of sampling.

B. Obtain information on the procedures to be used for shipping and transport of collectate to the microbiological laboratory.

C. For enumeration of counts leading to the determination of total colony-forming units (CFUs) per m^3 , inform laboratory that there are 400 possible impaction sites (N6 sampler) for colony growth on culture plates utilized. Avoid counting duplicate colonies at single impaction sites. Avoid counting colonies that grow at sites other than the 400 impaction locations. For example, do not count colonies found along the margin of the culture plate or between obvious impaction sites. Adjust raw colony counts by using the positive hole table (table 1).

D. Total CFU per m^3 is determined as follows:

$$\text{Total CFU/m}^3 = \frac{\text{Adjusted* number of colonies on plate}}{\text{Total volume of air sampled in cubic meters}}$$

*Positive hole adjustment [3]

E. Specify that total count measurements for 25°C fungi, 35°C bacteria, and 55°C actinomycetes be made by the laboratory. The industrial hygienist should provide data to the laboratory on the air volume sampled in obtaining collectate. The laboratory is to provide a report on the total CFU per m^3 of viable particulate (average CFU per m^3 of 25°C fungi plus average CFU per m^3 of 35°C bacteria plus average CFU per m^3 of 55°C actinomycetes) at each sample site before proceeding with more analysis. Alternatively, the laboratory can provide the industrial hygienist with average plate counts for each sampling site and the industrial hygienist can calculate the total viable count.

TABLE 1

Positive hole conversion table: Positive hole counts (r) and corresponding corrected particle counts (P)

r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
1	1	41	43	81	91	121	144	181	208	201	279	241	308	281	485	321	648	381	931
2	2	42	44	82	92	122	146	182	208	202	281	242	372	282	488	322	654	382	942
3	3	43	45	83	93	123	147	183	209	203	283	243	374	283	492	323	658	383	942
4	4	44	47	84	94	124	148	184	211	204	285	244	377	284	498	324	664	384	953
5	5	45	48	85	95	125	150	185	213	205	287	245	378	285	498	325	670	385	974
6	6	46	49	86	97	126	151	186	214	206	288	246	382	286	502	326	675	386	986
7	7	47	50	87	98	127	153	187	216	207	292	247	384	287	508	327	682	387	988
8	8	48	51	88	99	128	154	188	218	208	294	248	387	288	508	328	688	388	1010
9	9	49	52	89	101	129	156	189	220	209	296	249	390	289	513	329	692	389	1023
10	10	50	53	90	102	130	157	170	221	210	288	250	392	290	518	330	697	370	1086
11	11	51	55	91	108	131	159	171	223	211	300	251	395	291	520	331	708	371	1080
12	12	52	56	92	108	132	160	172	225	212	302	252	398	292	524	332	708	372	1084
13	13	53	57	93	108	133	162	173	227	213	304	253	400	293	527	333	715	373	1078
14	14	54	58	94	107	134	163	174	228	214	306	254	403	294	531	334	721	374	1083
15	15	55	59	95	108	135	165	175	230	215	308	255	406	295	535	335	727	375	1109
16	16	56	60	96	110	136	166	176	232	216	311	256	408	296	538	336	733	376	1125
17	17	57	61	97	111	137	168	177	234	217	313	257	411	297	543	337	738	377	1142
18	18	58	63	98	112	138	169	178	236	218	315	258	414	298	547	338	746	378	1160
19	19	59	64	99	114	139	171	179	237	219	317	259	417	299	551	339	752	379	1179
20	20	60	65	100	115	140	172	180	239	220	319	260	420	300	555	340	758	380	1198
21	21	61	66	101	116	141	174	181	241	221	322	261	423	301	559	341	766	381	1219
22	22	62	67	102	118	142	175	182	243	222	324	262	426	302	563	342	772	382	1241
23	23	63	69	103	119	143	177	183	245	223	326	263	429	303	567	343	779	383	1263
24	24	64	70	104	120	144	179	184	246	224	328	264	432	304	571	344	786	384	1286
25	25	65	71	105	122	145	180	185	248	225	331	265	434	305	575	345	793	385	1314
26	26	66	72	106	123	146	182	186	250	226	333	266	437	306	579	346	801	386	1341
27	27	67	73	107	125	147	183	187	252	227	335	267	440	307	584	347	808	387	1371
28	28	68	75	108	126	148	185	188	254	228	338	268	443	308	588	348	816	388	1403
29	29	69	76	109	127	149	186	189	256	229	340	269	447	309	592	349	824	389	1438
30	30	70	77	110	129	150	188	190	258	230	342	270	450	310	597	350	832	390	1476
31	31	71	78	111	130	151	190	191	260	231	345	271	453	311	601	351	840	391	1518
32	32	72	79	112	131	152	191	192	262	232	347	272	456	312	606	352	848	392	1566
33	33	73	81	113	133	153	193	193	263	233	349	273	459	313	610	353	857	393	1619
34	34	74	82	114	134	154	194	194	265	234	352	274	462	314	615	354	865	394	1661
35	35	75	83	115	136	155	196	196	267	235	354	275	465	315	620	355	874	395	1734
36	36	76	84	116	137	156	198	198	269	236	357	276	468	316	624	356	883	396	1844
37	37	77	86	117	138	157	199	199	271	237	359	277	471	317	629	357	892	397	1961
38	38	78	87	118	140	158	201	198	273	238	362	278	475	318	634	358	902	398	2127
39	39	79	88	119	141	159	203	199	275	239	364	279	478	319	639	359	911	399	2427
40	40	80	89	120	143	160	204	200	277	240	367	280	482	320	644	360	921	400	*

All holes must be clean and open.

* Indicates quantitative limit of state (approx 2628 particles) is exceeded

VI. Interpretation Data

A. If total count at complainant's location equals or exceeds 10^4 per m^3 proceed to remedial actions in part VII. Proceed to part VI.B if total count is less than 10^4 per m^3 or if total count is equal to or greater than 10^4 per m^3 and clinical or research aspects of study would benefit by identification of etiologic agent.

B. Identification of microorganisms in collectate

1. Fungi: Specify that the laboratory identify and enumerate (CFU per m^3) the following fungi that may be present in the collectate:

Cladosporium spp.

Alternaria spp.

Aureobasidium spp.

Penicillium spp.

Mucor spp.

Stachybotris spp.

Aspergillus spp.

Other fungi

The presence of high levels of fungi or one or two types of fungi at complainant's but not at noncomplainant's or outdoor locations may indicate a building-related source. Proceed to remedial actions in Section VII.

2. Bacteria: As an initial screen specify that the laboratory identify and enumerate (CFU per m³) the following:

Gram positive cocci

Bacillus spp.

Gram negative rods

Other predominant bacteria

High levels of Bacillus spp. or gram negative rods at the complainant's as opposed to other indoor sites may indicate a building-related source. Proceed to remedial actions in Section VII or continue analysis of bacteria (below). As a second screen specify that the laboratory identify and enumerate (CFU per m³) the following:

Staphylococcus aureus

Staphylococcus epidermidis

Other Staphylococcus spp.

Streptococcus salivarius

Other Streptococcus spp.

Corynebacterium spp.

Acinetobacter spp.

Pseudomonas spp.

Micrococcus spp.

Other bacteria

High levels of one or more of these bacteria at complainant's but not at noncomplainant's locations may be indicative of a problem. For example, airborne Streptococcus salivarius has been suggested as an indicator of respiratory pollution [23].

Obtain from the laboratory a report listing the types of potentially pathogenic bacteria found at each sampling site. Proceed to remedial actions in Section VII.

3. Actinomycetes: Specify that the laboratory identify and enumerate (CFU per m³) the following actinomycetes that may be present in the collectate:

Thermoactinomyces vulgaris

Micropolyspora faeni

Other actinomycetes

The presence of the two aforementioned actinomycete species in indoor environments has been associated with outbreaks of hypersensitivity lung illness [10]. Proceed to remedial actions in Section VII.

VII. Recommendations for Remedial Actions [1,16-18] and types of Microorganisms (Fungi = F, Bacteria = B, Actinomycetes = A) Affected

A. Source Control: Sources of microorganisms should be removed. The following actions are recommended.

1. Remove stagnant water and slimes from building mechanical systems (F,B,A).
2. Eliminate water spray systems in air handling units (B,A).
3. Substitute steam for cold water in humidifiers (F,B,A).
4. Eliminate the use of cold mist vaporizers (F,B).
5. Prevent water leaks or floods (F,B,A).
6. Keep indoor relative humidity below 70% (F).

B. Dilution Control

1. Replace HVAC system filters at scheduled intervals (F,B,A).
2. Upgrade the collection efficiency [2] of air handling unit filter banks.
3. Provide adequate amounts of suitable outdoor air to reduce levels of microorganisms that may be shed by occupants (B).

C. Administrative Control: Affected or sensitized personnel may have to be reassigned to another work environment.

VIII. Additional Tests

If the aforementioned sampling protocol and analytical procedures for airborne viable microorganisms fails to identify the source of bioaerosol contamination then consider sampling for each of the following: nonviable spores [14,22], protozoans [9], mycotoxins [8], endotoxins [21], and submicron particulate antigens [20].

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