Series 10-800

Instruction Manual
Single Stage Viable Sampler
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I. INTRODUCTION

The assay of the microbial content of the air has become increasingly more significant in the past decade as the need for "contamination-free" environments has become more apparent. The treatment of hospital patients, medical as well as surgical, who are high risk candidates for infection; the manufacture and processing of sterile materials and pharmaceuticals. The concern for indoor air quality; the massive productions and wide distribution of convenience foods; and the growing emphasis on consumer protection have all contributed to the need for controlled environments. Biological aerosols have been defined as viable biological contaminants occurring as solid or liquid particles in the air. These particles can very in size from viruses less than 0.1-micron in diameter to fungal spores 100 or more microns in diameter. They may occur as single, unattached organisms or as aggregates.

Viable particle samplers have been generally used to collect and assay aerobic species of bacteria and fungi. Even though many viable samplers, including the Thermo Scientific Samplers, will collect some virus particles, there is no convenient, practical method for the cultivation and enumeration of these particles.

Note there are two constraints on all viable particle samplers for which there is no analog in the assay of non-biological aerosol. First, the particle must be separated from the air for any viability assay. Second, the ability to reproduce (viability) must be demonstrated.

The purpose of this manual is to outline proper methods for the assay of biological aerosols using the Thermo Scientific Viable Particle Sampler.

II. ONE-STAGE VIABLE PARTICLE SAMPLER

A. Collection plates are prepared by aseptically pipetting 41ml of sterile culture media (45-50 degrees) into a 15mm plastic, disposable petri dish. We recommend either Fisher 8-757-13 or VWR 25384-070 petri dishes although any dish over 90mm in diameter is acceptable.

B. General detection and enumeration media are normally used in the collection of fungi, bacteria and the thermophilic Actinomycetes. Selective media is not recommended for initial assay collection since it inhibits the repair and growth of injured or stressed cells. Plates can be replicated on differential or selective media for identification after the organisms have been collected.

NOTE: General guidelines for media

Fungi: Traditionally, malt extract agar (MEA) has been recommended as a broad-spectrum media for the collection and enumeration of fungi. MEA is a generic term and formulation will vary from suppliers. Dichloran Gglycerol 18 agar (DG-18) is also newly recommended for most fungi including xerophilic fungi. DG-18 does not have the disadvantage of Rose Bengal Agar (RBA). Antibiotics such as streptomycin may be added to the medium to inhibit the growth of bacteria.

Bacteria: Tryptic Soy Agar (TSA) Casein Soy Petone agar (CSPA) and Nutrient Agar (NA) are broad-spectrum media for bacteria.

As with fungi, growth-restricting chemicals may be added.

Thermophilic Actinomycetes: CPSA and Tryptone Glucose Yeast Agar are standard methods agar (SMA). Standard plate count agars (SPC) are broad-spectrum media.

C. One collection plate, with the cover removed, is placed into the base of the sampling instrument.

D. The air to be sampled enters the sampler and accelerates through the jet orifices of the classification stage. Smaller particles are inertial impacted on the agar plate.

E. Viable particles are retained on the agar plate, and the exhaust air is carried through the outlet in the instrument base and the vacuum hose to the vacuum source (regulated pump or in-house vacuum system).

F. For maximum collection efficiency, a constant air sample flow of one (1) actual cubic foot per minute (ACFM) must be provided. Any vacuum source capable of an equivalent 10 inches of mercury or more will maintain a flow rate or 1 ACFM or 28.3 liters per minute (LPM).

G. After sampling is completed, the sampling time is recorded, the agar collection plate is removed from the sampling instrument and the cover is replaced on the Petri dish. Invert each covered plate to prevent condensation drip into the media.

H. Inoculated agar plates are incubated at the appropriate temperature for times ranging form hours for a fast-growing bacterium to develop a micro colony, to days for a fungus to develop into a visible colony and perhaps sporulate, to weeks for an organism such as a multi-drug resistant M. Tuberculosis to produce visible colonies. As a general rule, plates are incubated at:

Fungi
25°C or room temperature with natural light
Bacteria, environmental
25 to 30°C
Bacteria, human-source
35 to 37°C
Bacteria, themophilic Actinomycetes
50 to 56°C

I. Following incubation, the total concentration of culturable microorganisms is calculated by dividing the volume of air sampled into the total number of colonies observed on the plate. It is often necessary to use a dissecting-type microscope (10X-100X) to observe more than one colony at the same impaction point. Concentrations of culturable bioaerosol are normally reported as colony forming units (CFU) per unit volume of air. CFU is the number of colonies that replicate from individual or groups of bacterial cells, bacteria endospores or fungal spores.

J. Knowing the air sampler flow rate and the sampling time, the mean number viable particles (aerobic bacteria or fungi) per unit of air can be calculated.

III. AERODYNAMIC PARTICLE SIZING

The design concept of the Thermo Scientific Viable Samplers evolved from the following information:

The human respiratory tract is an aerodynamic classifying system for airborne particles. A sampling device can be used as a substitute of the

respiratory tract as a collector of viable airborne particles and as such, it should reproduce to a reasonable degree the lung penetration by these particles. The fraction of inhaled particles retained in the respiratory system and the site of deposition vary with all the physical properties (size, shape, density) of the particles, which make up the aerodynamic dimensions (Figure 1). Because the lung penetrability of unit density particles is known and since the particles sizes that are collected on each stage of the Thermo Scientific Viable Samplers have been determined, if used according to standard operating procedures, the stage distribution of the collected material will indicate the extent to which the sample would have penetrated the respiratory system.

Figure 1

Numerous small rounds jets improve collection (impaction) efficiency and provide a sharper cutoff of particle sizes on each stage of inertial impactors. Thus, the Six-Stage and Single Stage (N6) Samplers with 400 small round jets per stage and the Two-Stage Sampler with 200 tapered round jets per stage meet all the criteria for the efficient collection of airborne viable particles. Reports have discussed a reduced efficiency in cascade impactors when particles bounce off the impaction surface are, rein trained and lost in the exhaust air. This effect is minimized when a sticky agar surface is used as the collection medium.

Ranz and Wong conducted the earliest and most fundamental work in inertial impaction theory in the early 1950's. In this work, Ranz and Wong showed that the collection of a particle by an obstacle is a function of what is defined as the inertial impaction parameter:

$$K = \frac{C\,U D_p^2}{18 D_c}$$

Where U is the relative velocity, P is the particle density, Dp is the particle diameter, u is the gas viscosity, Dc is the diameter of the round jet, and C is the Cunningham slip correction factor.

Data from inertial impactors are normally presented as 50% effective cutoff diameters. For the Thermo Scientific impactors, containing round jets and flat collection surfaces, the 50% effective cutoff diameter would yield a value of 0.14 for the inertial impaction parameter K.

The Cumming ham slip correction factor is equal to:

$$C = 1 + 0.16 \times 10^{2} / Dp$$

for normal temperatures and pressures.

This factor corrects for the fact that as particle diameters approach the mean free path length of the gas molecules, they tend to "slip" between gas molecules more easily and are therefore more easily able to cross the bulk flow stream lines. The collection efficiency is therefore slightly greater than would be predicted by inertial impaction theory for particle diameters on the of 1 0r 2 microns. The overlapping of particle size between stages, which is naturally inherent in all cascade impaction devices, is minimized in Thermo Scientific's sampler by design. Ranz and Wong (1952) stated that as a particle passes through a jet, its nearness to the axis of the jet is one of the factors that determine whether or not the particle will reach the impaction surface. In contrast to competitive samplers that have larger rectangular jets in each stage, round jets. Travel of the particle is thus confined near the axis of the jets. The average distance of the particles from the axis of the jets is less than in other impactors. Ranz and Wong (1952) also

stated that round jets have sharper cutoffs than rectangular jets. The Thermo Scientific sampler, therefore, on a theoretical basis, should have a sharper cutoff.

Another inherent advantage of the Thermo Scientific Air Sampler over its competitors is that single circular orifice impactors by design must operate with higher orifice velocities. This results in more turbulent flow, greater re-entrainment, and a skewing of the size distribution toward the lower end (i.e., the indicated size distribution being smaller than it really is).

IV. IMPACTORS

1. Description

The Thermo Scientific N6 Viable Particle Sampler is constructed with aluminum Components that are held together by three spring clamps and sealed with O-ring gaskets. The impactor stage contains multiple precision-drilled orifices. When air is drawn through the sampler, multiple jets of air in the stage direct any airborne particles toward the surface of the agar collection surface beneath the stage. The range of particle size collected depends on the jet velocity of the stage.

The stage contains 400 orifices with diameters. The stage has a removable plastic Petri dish with cover. The exhaust section of the stage is approximately 19mm larger in diameter than the Petri dish, which allows unimpacted particles to go around the dish and be exhausted.

There is an optional carrying case, which will accommodate both the Thermo Scientific One Stage Viable Particle Sampler and vacuum pump for ease of portability. Case dimensions are 9 3/8” wide x 8 ¾” high x 5” deep. Complete sampler and vacuum pump weights including carrying case are 6 ¼ pounds and ½ pounds respectively.

A constant air sample flow of 1 ACFM is provided by a continuous duty vacuum pump. An adjustable valve on the pump controls flow rate and periodic calibration is recommended. Requirements for flow rate adjustments can be found in Section VI.

2. Assembly

The orifice stage should be cleaned and disinfected each time the instrument is used. A mild detergent and warm water are sufficient for cleaning. The soap can be removed by holding the stage under hot running water or immersing them in clean water or immersing them in clear water in an ultrasonic cleaner. The stage should be examined for any material in the jet holes. If holes are plugged, or partially plugged, a jet blast of dry air or a portable Freon gun is effective in cleaning them. Just before use, wipe all surfaces with 70% isopropyl alcohol using a gauze pad.

The complete impactor assembly consists of an inlet cone, one jet classification stage, and a base plate. The stage is inscribed with a serial number. Each stage contains an O0ring (neoprene standard, Teflon optional) for sealing. These O-rings should be checked regularly and replaced when they no longer provide an airtight seal.

The assembly of the impactor begins by placing an agar collection plate, uncovered; on the base plate so that the Petri dish rests on the three raised metal pins. Insert the jet classification stage. All the agar plates should be at room temperature before they are inserted into the sampling instrument.

3. Sampling

When ready to sample, the vacuum pump is turned on and a sample stream of 1 ACFM will flow through the sampler. Figure 5 shows how impaction occurs at the orifice-collector interfaces.

Normal sampling periods for viable aerosols will vary from a few minutes up to 30 minutes depending on the purpose for which the sample is collected and the type of air environment being sampled. It is important to collect sufficient viable particles in each sample to be statistically significant and representative, however, difficulty is encountered in counting agar plates, which contain more than 250-300 colonies.

After the sampling has been completed, the sampler is disassembled and the covers are replaced on each of the Petri dishes.

4. Calibration

*S*ince the orifices velocities determine the size fraction for a stage, it is important that the sampler be operated at 1 ACFM (28.3 liters per minute). For this reason, the unit should be periodically recalibrated and whenever non-standard temperatures and pressures are encountered, calibration should be performed at the sampling conditions. Do not use rubber tubing of smaller diameter or length different than that supplied with the impactor unless the flow rate is readjusted.

Each Thermo Scientific pump is equipped with an adjustable valve after the flow rate has been set. To adjust the flow, turn the screw in to increase flow and out to decrease flow.

Each Thermo Scientific pump-impactor assembly is calibrated before shipment to deliver 1 ACFM at ambient temperature and pressure levels in Franklin, Massachusetts. In order to recalibrate at your sampling environment, the following procedure is recommended:

Place a calibrated dry gas meter upstream from the sampler. Attach a short 1" I.D. hose with approximately ¼" wall to the inlet cone of the impactor and the other end to the outlet of the dry gas meter. Adjust the pump valve until you are pulling 1ACFM over a three minutes test period as determined with an accurate stopwatch. After maintaining 1 ACFM for three minutes, tighten the lock nut on the adjustment valve. Because of the 1.4 ACFM free floating rating of the motor and pump, up to 50 feet of tubing can be used between the sampler and pump while still maintaining 1 ACFM through the sampler.

The pump rate of the 12-volt DC pump will vary with voltage. One ACFM can be drawn through the impactor if the voltage is maintained near 12 volts.

V. ANALYSIS & INTERPRETATION OF DATA FROM VIABLE PARTICLE SAMPLERS

The number of viable particles per unit volume of air sampled is easily computed. After incubation, count the number of bacterial colonies (accepted microbiological theory assumes that each colony represents a single particle) on each sample plate. Sum the number of colonies on each plate to give a grand total for that particle sample. Divided this total by the total volume of air sampled in cubic feet (if a constant flow rate of 1 ACFM is maintained, the volume of air sampled is equal to the number of minutes sampled) to give the mean number of viable particles per cubic foot of air in the sample.

Note the number of viable particles in the air sample is not equal to the number of bacterial cells in the sample since a single viable particle may contain more than one cell. If the sample plates have been incubated aerobically, all the colonies must be considered as aerobic or facultative anaerobic bacteria.

It is not possible to determine the exact density or shape of viable particles, which are collected with any cascade impactor including the Thermo Scientific Viable Particle Samplers.

Positive Hole Correction Method

Agar plates containing more than 300 colonies may be counted by a "positive Hole" method, which is less accurate than optically counting each colony, and is rarely used today. However, since some people still use this technique, the following discussion is included:

The positive hole method is essentially a count of the jets, which delivered viable particles to the Petri dish, and the conversion of this count to a particle count by using the "positive hole" conversion table (Table I). This table is based upon the principle that as the number of viable particles being impinged on a give plate increases, the probability of the next going into an "empty hole" decreases. For example, when 9/10 of the holes have each received one or more particles, the next particle has but one chance in ten of going into an empty hole. Thus, at this point, on the average, ten additional particles would be required to increase the number of positive holes by one, and before all holes becomes positives, some holes will receive a number of particles. The value in the table were calculated form the basic formula (Feller, 1950):

$$Pr = N[1/N + 1/N-1 + 1/N-2 + \ldots 1/N-r+1]$$

Where Pr is the expected number of viable particles to produce 'r' positive holes and 'N' is the total number of holes per stage (400). The above formula assumes that the flow of particles stops at the instant a particle enters the *n*th hole. Since, in the actual case of sampling, the flow of particles stops at random, the expected number of particles present if '' positive holes are observed, would be equal to or grater than Pr but less than Pr +1 and the average would be (Pr+Pr+1-1)/2. This correction has been applied in the construction of the table. In using the positive hole conversion table the number of positive holes must be precisely determined. A colony out of the hole pattern is not counted as a positive hole. By this method, counts up to 1200 or 1500 particles per stage are quite reliable. If higher counts are to be encountered the microscope method is employed.

(Table Follows)

VI. INSTRUCTIONS FOR THE VACUUM PUMP

Pump and motor require no lubrication

Do not use rubber tubing of smaller diameter or length than that supplied with the unit unless the flow rate is checked and readjusted.

The pump is equipped with an adjustable valve. Always tighten the lock nut on the adjustment valve after the flow rate has been set.

To adjust flow- turns screw in to increase the flow and out to decrease the flow. It is important the unit always operates at 1 ACFM. The unit should be periodically recalibrated. A dry gas

meter is recommended for this purpose. To calibrate – attach a 1' (I.D.) hose with approximately a ¼" wall to the inlet nozzle of the sampler and the other end to the outlet of a dry gas meter. Continue to adjust the valve until you are pulling 1 ACFM over a three minutes test period (determine by an accurate stopwatch). After this has been achieved, tighten the lock nut on the adjustment valve.

The pump and motor are guaranteed by the original manufacturer and should not be disassembled for any reason.

Due to the 1.4 ACFM rating of the motor and pump, up to 50 feet of hose can be used between the sampler and the motor and still pull 1 ACFM.

12-VOLT PUMP OPERATION

Battery required: 12-volt automotive type, minimum 69 amp. Hour capacity.

1. Connect clip of red-shielded pump wire to positive (+ or Red) battery terminal.

2. Connect clip of black-shielded wire negative (-) terminal, pump should start immediately.

3. If pump does not start, check battery voltage, should be not less than 12-volts under light load, 13 volts no load.

4. If pump does not operate with fully charged battery, check battery clip connections and wires for poor connections.

5. Should pump fail to operate after steps 1-4 are completed, refer to manufacturers instructions.

6. Pumping rate if the 12v DC unit will vary with voltage. Normal pump operation requires a current drew of approximately 11 amps. Continuous running in excess of 3 hour may result in reduced battery voltage and lower CFM through the Thermo Scientific Sampler.

7. Fully recharge battery between uses.

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Table 1

Positive hole conversion table: Positive hole counts (r) and corresponding correct particle counts (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	161	206	201	279	241	369	281	485	321	649	361	931
2	2	42	44	82	92	122	146	162	208	202	281	242	372	282	488	322	654	362	942
3	3	43	45	83	93	123	147	163	208	203	283	243	374	283	492	323	659	363	952
4	4	44	47	84	94	124	148	164	211	204	285	244	377	284	495	324	664	364	963
5	5	45	48	85	96	125	150	165	213	205	287	245	379	285	499	325	670	365	974
6	6	46	49	86	97	126	151	166	214	206	289	246	382	286	502	326	675	366	986
7	7	47	50	87	98	127	153	167	216	207	292	247	384	287	506	327	680	367	998
8	8	48	51	88	99	128	154	168	218	208	294	248	387	288	508	328	686	368	1010
9	9	49	52	89	101	129	156	169	220	209	296	249	390	289	513	329	692	369	1023
10	10	50	53	90	102	130	157	170	221	210	298	250	392	290	516	330	697	370	1036
11	11	51	55	91	103	131	159	171	223	211	300	251	395	291	520	331	703	371	1050
12	12	52	56	92	105	132	160	172	225	212	302	252	398	292	524	332	709	372	1064
13	13	53	57	93	106	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	1093
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	409	296	539	336	733	376	1125
17	17	57	61	97	111	137	168	177	234	217	313	257	411	297	543	337	739	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	21	60	65	100	115	140	172	180	239	220	319	260	420	300	555	340	759	380	1198
21	22	61	66	101	116	141	174	181	241	221	322	261	423	301	559	341	766	381	1219
22	23	62	67	102	118	142	175	182	243	222	324	262	426	302	563	342	772	382	1241
23	24	63	69	103	119	143	177	183	245	223	326	263	429	303	567	343	779	383	1263
24	25	64	70	104	120	144	179	184	246	224	328	264	432	304	571	344	786	384	1288
25	26	65	71	105	122	145	180	185	248	225	231	265	434	305	575	345	793	385	1314
26	27	66	72	106	123	146	182	186	250	226	333	266	437	306	579	346	801	386	1341
27	28	67	73	107	125	147	183	187	252	227	335	267	440	307	584	347	808	387	1371
28	29	68	75	108	126	148	185	188	254	228	338	268	443	308	588	348	816	388	1403
29	30	69	76	109	127	149	186	189	256	229	340	269	447	309	592	349	824	389	1438
30	31	70	77	110	129	150	188	190	258	230	342	270	450	310	597	350	832	390	1476
31	32	71	78	111	130	151	190	191	260	231	345	271	453	311	601	351	840	391	1518
32	33	72	79	112	131	152	191	192	262	232	347	272	456	312	606	352	848	392	1565
33	34	73	81	113	133	153	193	193	263	233	349	273	459	313	610	353	857	393	1619
34	36	74	82	114	134	154	194	194	265	234	352	274	462	314	615	354	865	394	1681
35	37	75	83	115	136	155	196	195	267	235	354	275	465	315	620	355	874	395	1754
36	38	76	84	116	137	156	198	196	269	236	357	276	468	316	624	356	883	396	1844
37	39	77	86	117	138	157	199	197	271	237	359	277	472	317	629	357	892	397	1961
38	40	78	87	118	140	158	201	198	273	238	362	278	475	318	634	358	902	398	2127
39	41	79	88	119	141	159	203	199	275	239	364	279	478	319	639	359	911	399	2427
40	42	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 (approximately 2628 particles) is exceeded*

SERVICE LOCATIONS

For additional assistance, Thermo Fisher Scientific has service available from exclusive distributors worldwide. Contact one of the phone numbers below for product support and technical information or visit us on the web at www.thermo.com/aqi.

1-866-282-0430 Toll Free
1-508-520-0430 International