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OPERATING MANUAL FOR ANDERSEN SAMPLER, INC.

VIABLE (MICROBIAL) PARTICLE SIZING SAMPLER

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DIRECTOR OF OPERATIONS

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NOVEMBER, 1984

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OPERATING MANUAL FOR ANDERSEN SAMPLERS, INC.
VIABLE (MICROBIAL) PARTICLE SIZING SAMPLERS

I. PREFACE

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, and the increased use of these products; the massive production 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 vary 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 Andersen Samplers, will collect some virus particles, there is no convenient, practical method for the cultivation and enumeration of these particles. There are two constraints on all viable particle samplers for which there is no analog in the assay of nonbiological aerosols. First, the particle must be separated from the air for any viability assay, and 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 Andersen 2000 Inc. Viable Particle Samplers.

II. INTRODUCTION

SIX-STAGE VIABLE PARTICLE SAMPLER

The Andersen 1 ACFM six-stage Viable Particle Sampler is a multi-orifice, cascade impactor which is normally used to measure the concentration and particle size distribution of aerobic bacteria and fungi in the intra-mural or ambient air. This instrument has been widely used as a standard for enumerating the viable particles in a microbial aerosol. Viable particles can be collected on a variety of bacteriological agar and incubated in situ for counting and identification.

This sampler was calibrated so that all particles collected, regardless of physical size, shape, or density are sized aerodynamically and can be directly related to human lung deposition.

TWO-STAGE ALUMINUM VIABLE PARTICLE SAMPLER

The Andersen 1 ACFM two-stage Viable Particle Sampler is also a multi-orifice cascade impactor. This unit is used whenever a size distribution is not required and only respirable-nonrespirable segregation or total counts are needed. Ninety-five to one hundred percent of the viable particles above 0.8 microns in an aerosol can be collected on a variety of bacteriological agar.

This sampler separates viable particles into two size ranges with the 50% cut-off diameter of Stage I at 8.0 microns for spherical particles of unit density.¹ The sampler operates like the two-stage disposable sampler. However, it is fabricated of aluminum and is reusable.

A brief description of the operation of the viable particle samplers follows:

1. Six-Stage Viable Particle Sampler

- a. Collection plates are prepared by aseptically pipetting 27ml of sterile bacteriological agar (45-50°C) into each of six glass Petri dishes supplied with the instrument. The Petri dishes must be sterilized prior to filling. Petri dishes, other than those supplied, cannot be used since this would alter the distance between the jet orifice and the collection surface of each stage.² Plastic Petri dishes should not be used because the static charge generated reduces the collection efficiency.³
- b. Any general purpose, solid bacteriological medium, such as trypticase soy agar or blood agar, can be used for the collection plates. Selective media are not recommended since they inhibit the repair and growth of injured or stressed cells.⁴
- c. One collection plate, with the cover removed, is inserted on each stage of the sampling instrument.
- d. The air to be sampled enters the inlet cone and cascades through the succeeding orifice stages with successively higher orifice velocities from Stage 1 to Stage 6. Successively smaller particles are inertially impacted onto the agar collection surfaces.

le particles are retained on the agar plates, and the
ust air is carried through the vacuum hose to the
um source (pump or in-house vacuum system).
maximum collection efficiency, a constant air sampler
of 1 ACFM must be provided. This constant flow is
ided with a continuous-duty vacuum pump, and is controlled
i adjustable valve on the pump. Periodic calibration is
mended (See Section VI). Another method of assuring a
ant flow would be to insert an airflow meter (not provided),
a minimum capacity of 1 ACFM (28.3 liters/min.) in the
um hose between the Sampler and the vacuum source. This
eter should be calibrated, using a Wet-Test or Dry-Test
eter, by the user.

sampling is completed, the sampling time is recorded, the
collection plates are removed from the sampling instrument,
he cover is replaced on each Petri dish. Identify each
as to sample and stage number (i.e., 1-1, 1-2, 1-3, etc.).
all agar plates, inverted to prevent condensation drip,
incubator at 35°C for 18 to 24 hours. Plates can be
ited at room temperature if the user is most interested in
nmental bacteria whose optimum growth temperature is lower
ody temperature or at 20° to 25°C for maximum recovery of

incubation, the number of colonies on each plate are
d, using a standard bacterial colony counter.

g the air sample flow rate and the sampling time, the
umber of viable particles (aerobic bacteria and/or fungi)
it volume of air can be calculated, and the percent of
les in each size range can be estimated.

2. Two-Stage Viable Particle Samplers

- a. Collection plates are prepared by aseptically pipetting 20ml of sterile bacteriological agar (45-50°C) into each of two sterile 100x15mm plastic, disposable Petri dishes. An anti-static chemical has been incorporated into the plastic used to fabricate the disposable sampler. Commercially available agar plates (20 ml agar) can be substituted for user-prepared collection plates.
- b. Any general purpose, solid bacteriological medium, such as trypticase soy agar, or blood agar, can be used for the collection plates. Selective media are not recommended since they inhibit the repair and growth of injured or stressed cells.⁴
- c. One collection plate, with the cover removed, is inserted on each stage of the sampling instrument.
- d. The air to be sampled enters the jet orifices of Stage I and cascades through the jet orifices of Stage II with a higher orifice velocity through Stage II than Stage I. Smaller particles are inertially impacted on the agar plate in Stage II than in Stage I.
- e. Viable particles are retained on the agar plates, and the exhaust air is carried through the outlet in the base of the instrument, and the vacuum hose to the vacuum source (pump or in-house vacuum system).
- f. For maximum collection efficiency, a constant air sample flow of 1 ACFM must be provided. To assure this constant flow, a critical orifice has been incorporated into the outlet in the base of the instrument. Any vacuum source which is the equivalent of 10 inches of mercury or more

will maintain a flow rate of 1 ACFM (28.3 liters/min).

An airflow meter is not required unless it is desired to sample at a flowrate less than 1 ACFM.

- g. After sampling is completed, the sampling time is recorded, the agar collection plates are removed from the sampling instrument and the cover is replaced on each Petri dish. Identify each plate as to sample and stage number (i.e., 1-1, 1-2).
- h. Place both agar plates, inverted to prevent condensation drip, in an incubator at 35°C for 18 to 24 hours. Plates can be incubated at room temperature if the user is most interested in environmental bacteria whose optimum growth temperature is lower than body temperature or at 20-25°C for maximum recovery of fungi.
- i. After incubation, the number of colonies on each plate are counted using a standard bacterial colony counter.
- j. Knowing the air sample flow rate and the sampling time, the mean number of viable particles (aerobic bacteria and/or fungi) per unit volume of air can be calculated and the percent of particles in the respirable (Stage II) and nonrespirable (Stage I) size ranges can be estimated.

III. AERODYNAMIC PARTICLE SIZING

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

The human respiratory tract is an aerodynamic classifying system for airborne particles⁵. A sampling device can be used as a substitute for 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 dimensions⁵ (Figure 1). Because the lung penetrability of unit density particles is known⁶ and since the particle sizes that are collected on each stage of the Andersen Viable Samplers have been determined, if a standard model of these samplers is used according to standard operating procedure, the stage distribution of the collected material will indicate the extent to which the sample would have penetrated the respiratory system.

Numerous small round jets improve collection (impaction) efficiency and provide a sharper cutoff of particle sizes on each stage of inertial impactors⁷. Thus, the Six-Stage Sampler 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. Recent reports have discussed a reduced efficiency in cascade impactors when particles bounce off the impaction surface, are reentrained and lost in the

exhaust air⁸. This effect is minimized when a sticky agar surface is used as the collection medium.

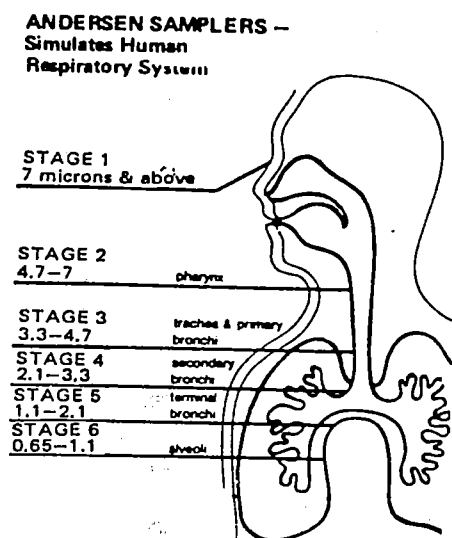


Figure 1. Andersen Sampler Simulates Human Respiratory System

The earliest and most fundamental work in inertial impaction theory was conducted in the early 1950's by Ranz and Wong.⁹ 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:

$$K = \frac{C_p U D_p^2}{18 \mu D_c}$$

Where U is the relative velocity, ρ is the particle density, D_p is the particle diameter, μ is the gas viscosity, D_c 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 Andersen 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 Cunningham slip correction factor is equal to:

$$C = 1 + 0.16 \times 10^{-4}/D_p \text{ for normal temperatures and pressures.}$$

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 order of 1 or 2 microns. The overlapping of particle size between stages, which is naturally inherent in all cascade impaction devices, is minimized in Andersen's samplers 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 determines whether or not the particle will reach the impaction surface. In contrast to competitive samplers which have larger rectangular jets in each stage, the Andersen sampler has 400 small, 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 Andersen sampler, therefore, on a theoretical basis, should have a sharper cutoff.⁷

Another inherent advantage of the Andersen Air Samplers over its competitors is that single circular orifice and multiple rectangular 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

A. SIX-STAGE VIABLE PARTICLE SAMPLER

1. Description

The Andersen 1 ACFM Viable Particle Sampler is constructed with six aluminum stages that are held together by three spring clamps and gasketed with O-ring seals (Figure 2). Each impactor stage contains multiple precision drilled orifices. When air is drawn through the sampler, multiple jets of air in each stage direct any airborne particles toward the surface of the agar collection surface for that stage. The size of the jet orifices is constant for each stage, but is smaller in each succeeding stage. The range of particle sizes collected on each stage depends on the jet velocity of the stage and the cutoff of the previous stage. Any particle not collected on the first stage follows the air stream around the edge of the Petri dish to the next stage.

Each stage contains 400 orifices with diameters ranging from 1.81 mm on the first stage to 0.25 mm on the sixth stage. Each stage has a removable glass petri dish with metal cover. The exhaust section of each stage is approximately 19 mm larger in diameter than the Petri dish which allows unimpacted particles to go around the dish and into the next stage (Figure 3).

The Andersen Six-Stage Viable Particle Sampler and Vacuum pump include their own carrying cases for ease of portability (Figure 4). Case dimensions are 9 3/8" wide x 8 3/4" high x 5" deep. Complete sampler and vacuum pump weights including carrying cases are 6 1/4 pounds and 12 pounds respectively.

A constant air sample flow of 1 ACFM is provided by a continuous duty vacuum pump. Flow rate is controlled by an adjustable valve on

the pump and periodic calibration is recommended. Requirements for flow rate adjustments can be found in Section VI.

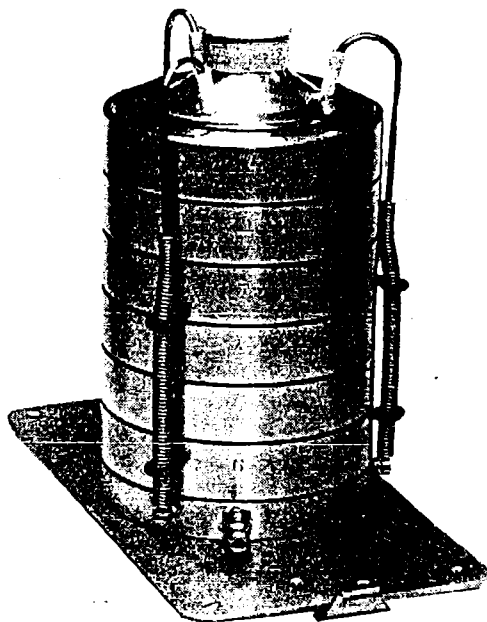


Figure 2. Andersen Six-Stage Viable Sampler

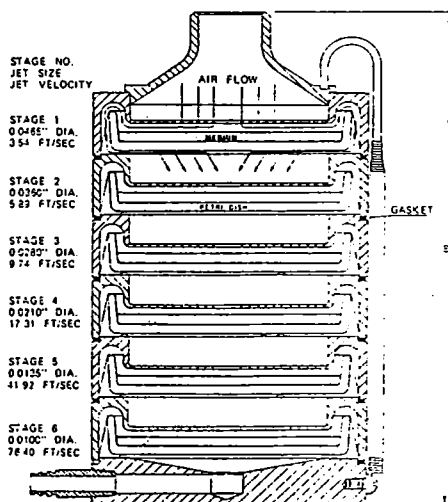


Figure 3. Schematic Diagram of the Andersen Six-Stage Viable Sampler

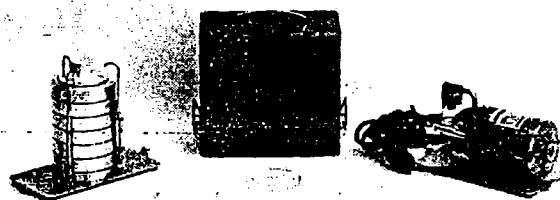


Figure 4. Andersen Viable Sampler with Vacuum Pump and Carrying Case (only one shown)

The jet orifice dimensions and particle size ranges for each stage are:

<u>STAGE</u>	<u>ORIFICE DIAMETER (mm)</u>	<u>RANGE OF PARTICLE SIZES (MICRONS)</u>
1	1.81	7.0 and above
2	0.91	4.7 - 7.0
3	0.71	3.3 - 4.7
4	0.53	2.1 - 3.3
5	0.34	1.1 - 2.1
6	0.25	0.65 - 1.1

2. Assembly

The orifice stages 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 stages under hot running water or immersing them in clean water in an ultrasonic cleaner. Each 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, six stages, and 12 glass Petri dishes (includes 6 spare dishes). The stages are inscribed 1, 2, 3, 4, 5, and 6. Each stage contains an O-ring (neoprene standard, Teflon optional) for sealing. These O-rings should be checked regularly and replaced when they no longer provide an air-tight seal.

The assembly of the six-stage 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 stage 6 over the petri dish. Place a second Petri dish on the top of stage 6 and continue in this manner until all six agar collection plates have been positioned in the sampler. The inlet cone is placed on top of stage 1. All the agar plates should be at room temperature before they are inserted into the Sampling Instrument.

When the Petri dishes supplied with the sampler are used and 27ml of agar medium is placed in each Petri dish, the three metal pins on each stage position the collection surface for the correct distance between the jet orifices and the agar surface.

After the Sampler has been assembled, connect the outlet nipple on the base plate to the vacuum pump or other vacuum source.

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.

The flow of high velocity sample air across the agar plates also tends to dehydrate and perhaps damage the viable particles which have already been collected¹⁰. Thus, extended sampling periods (over 30 minutes) are not recommended. If a larger sample volume is required, it is better to use two Sampling Instruments in parallel or to remove the agar plates representing one sample and insert fresh plates for a second sample.

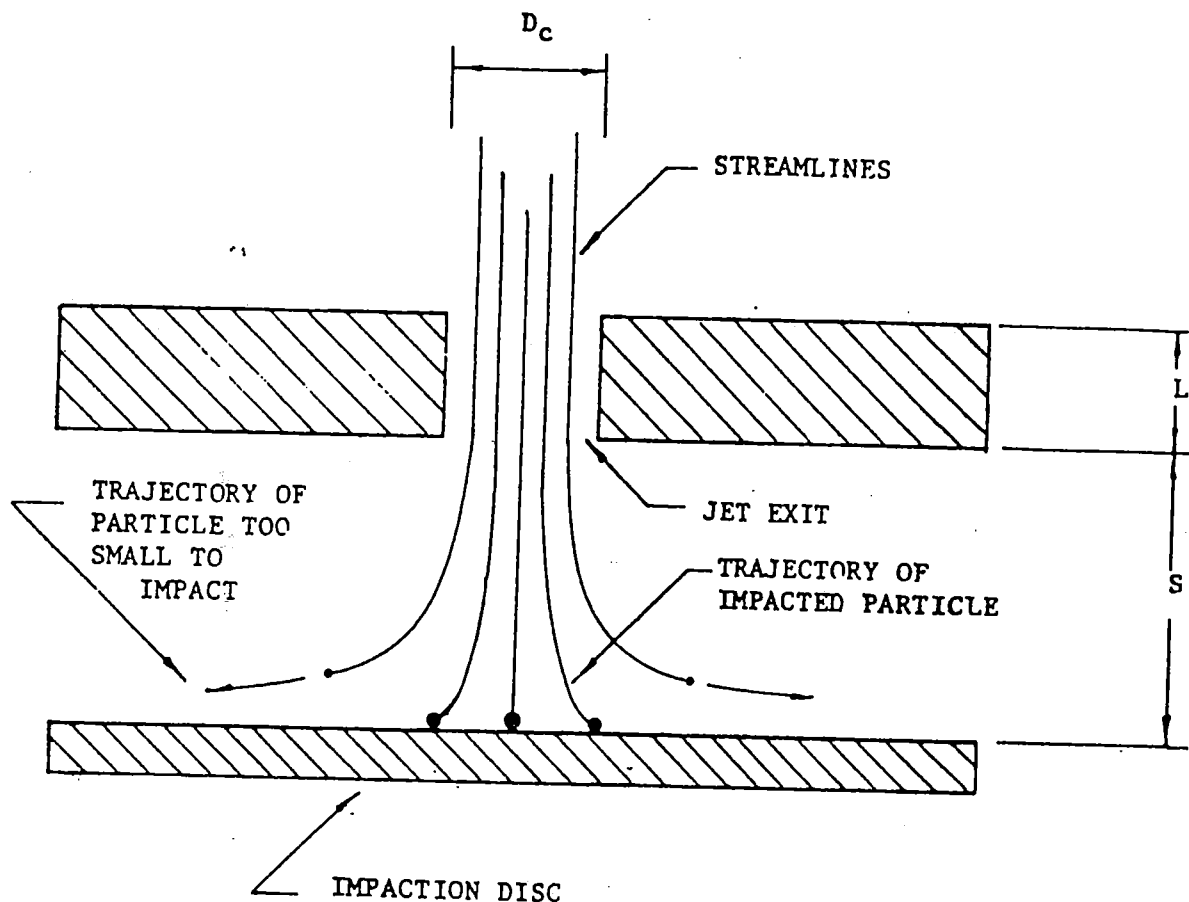


Figure 5. Schematic of Impactor Stage

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

4. Calibration

Since the size fraction for each stage is determined by the orifice velocities, it is important that the sampler be operated at 1 ACFM. 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 Andersen 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 the flow, turn the screw in to increase flow and out to decrease flow.

Each Andersen pump-impactor assembly is calibrated before shipment to deliver 1 ACFM at ambient temperature and pressure levels in Atlanta, Georgia. 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 1/4" 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 1 ACFM over a three minute test period as determined with an accurate stop watch. After maintaining 1 ACFM for three minutes, tighten the lock nut on the adjustment valve.

Because of the 1.4 ACFM free flow 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 and motor are guaranteed by the original manufacturer and should not be disassembled for any reason. The pump and motor require no lubrication.

The pump rate of the 12 volt D. C. pump will vary with voltage.

One ACFM can be drawn through the impactor if the voltage is maintained near 12 volts. Refer to the attached supplementary titled "Instructions For The New Andersen Pump" for detailed 12 volt D.C. pump operation.

B. TWO-STAGE ALUMINUM VIABLE PARTICLE SAMPLER

1. Description

The Andersen 1 ACFM Viable Particle Sampler is constructed of aluminum with two stages which are held together with three dowel pins and three teflon caps. Each impactor stage contains multiple precision drilled orifices. When air is drawn through the Sampler, multiple jets of air in each stage direct any airborne particles toward the surface of the agar collection surface for that stage. The size of the jet orifices is the same on each of the two stages but are smaller on the second stage. The range of particle sizes collected on each stage depends on the jet velocity of the stage and the cutoff of the previous stage. Any particle not collected on the first stage follows the air stream around the edge of the Petri dish to the second stage.

Each stage contains 200 tapered orifices. The diameter of the orifices on the first stage are 1.5 mm and 0.4 mm on the second stage. Standard 100 x 15 mm petri dishes are used for collecting surfaces on each stage. The exhaust section of each stage is approximately 19 mm larger in diameter than the Petri dish which allows unimpacted particles to go around the dish and into the next stage (Figure 3).

A continuous duty vacuum pump is available which will provide a constant sample flow of 1 ACFM. A 1 ACFM critical orifice is built into the base plate of this instrument. Any vacuum source, including equivalent of 14 inches of mercury pressure or greater. A flowmeter is not required with this instrument.

The 50% effective cutoff diameter of Stage I of this Sampler is 8.0 microns for spherical particles of unit density (or their aerodynamic equivalent).¹ A reasonable working interpretation would

conclude that non-respirable particles (do not penetrate the lower human respiratory tract) are collected on Stage I and respirable size particles (will penetrate the lower human respiratory tract) are collected on Stage II.

2. Assembly

The complete impactor assembly consists of a base, two stages, and three threaded teflon caps (Figure 7).

The assembly of the two-stage impactor begins by placing an agar collection plate, uncovered, on the base so that the Petri dish rests on the three post supports. Place Stage II carefully over the three dowel pins and slide into position over the Petri dish. Place the second Petri dish on the top of Stage II and carefully cover with Stage I. Screw the three caps onto the dowel pins and tighten by hand. Connect a vacuum hose (not supplied) to the brass nipple on the base of the instrument. Connect the vacuum hose to a vacuum source.

When a 100 x 15 mm Petri dish containing 20 ml of agar medium is correctly placed on the support posts of each stage, the correct distance between the jet orifices and the agar surface is maintained.

3. Sampling

When ready to sample, the vacuum source is turned on. A constant sample flow rate of 1 ACFM (28.3 liters/min.) will be maintained if the vacuum source is the equivalent of 14 inches of mercury pressure or greater. Accurately time the length of the sampling period.

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.

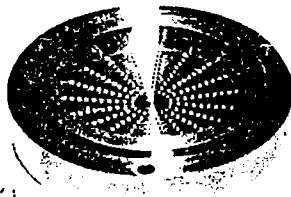
The flow of high velocity sample air across the agar plates also tends to dehydrate and perhaps damage the viable particles which have already been collected.¹⁰ If a larger sample volume or a longer sampling time (over 30 minutes) is required, it is better to use two or more Sampling Instruments in parallel or to sample sequentially.

After the sampling has been completed, the Sampler is disassembled. This is accomplished by unscrewing the teflon caps. Remove Stage I and the Petri dish on Stage I and replace its cover. Remove Stage II and the Petri dish on Stage II and replace its cover.

4. Calibration

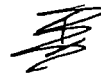
No calibration is required because of the built-in critical orifice. Fourteen inches of mercury pressure or greater must be maintained to insure critical flow. (It is impossible to obtain flows in excess of 1 ACFM when drawing greater than 14 inches mercury pressure because critical velocity is attained at 14 inches. Therefore, it is permissible to sample at any vacuum $\geq$ 14 inches mercury and be assured that 1 ACFM is flowing through the sampler).

50%
Cut-off
 $\geq 8\mu m$



Stage
 ϕ

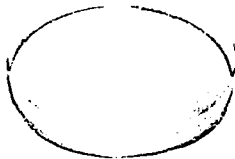
1. Stage 0



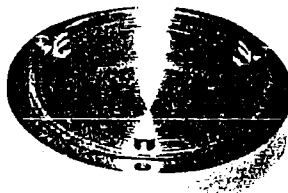
Size fraction

"Non"
Respirable"

2. Petri Dish (not included)



$< 8\mu m$

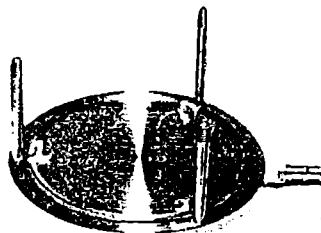


3. Stage 1



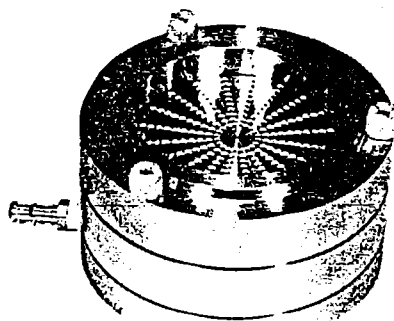
"Respirable"

4. Petri Dish



5. Base with brass nipple

EXPLODED VIEW



7
Figure 6. ASSEMBLED UNIT
Andersen Two-Stage Aluminum
Viable Particle Sampler

V. ANALYSIS AND INTERPRETATION OF DATA FROM
ANDERSEN VIABLE PARTICLE SAMPLERS

The number of viable aerobic 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 particular sample. Divide 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.

$$\frac{\text{Total Number of Colonies from all Sample Plates}}{\text{Total Sampling Time in Minutes (1 ACFM)}} = \text{Mean Number of Viable Particles per Cubic Foot of Air Sampled}$$

Note that 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.

The percentage of viable particles in each size range can be determined by dividing the number of colonies on a given stage by the total number of colonies on all the stages.

$$\frac{\text{Colonies on Stage I of the Six-Stage Sampler}}{\text{Total Number of Colonies from all Sample Plates}} \times 100 = \text{Percent of Viable Particles Over 7.0 Microns in Diameter}$$

The site of deposition of these particles in the human respiratory tract can be predicted from this data. The approximate settling rate in air of the

particles collected can also be calculated from the particle size data.

Settling Rates of Airborne Particles	
Diameter of Particles (microns)	Velocity of Settling (feet per minute)
0.8	0.005
1.0	0.007
4.0	0.095
10.0	0.59
40.0	9.5
100.0	59.2

Condensed from "Size and characteristics of airborne solids", W. G. Frank in the Smithsonian Meteorological Tables. Rates are for particles in the shape of spheres with a specific gravity of 1.0, settling in air at 70°F.

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

The variation in concentration of viable airborne particles with time can be determined by collecting intermittent samples at the same location.

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 plates and the conversion of this count to a particle count by use of 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 given plate increases, the probability of the next particle 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 the holes become positive, some holes will receive a number of particles. The values in the table were calculated from the basic formula (Feller, 1950):

$$P_r = N \left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \dots + \frac{1}{N-r+1} \right]$$

Where P_r 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 r th hole. Since, in the actual case of sampling, the flow of particles stops at random, the expected number of particles present if r positive holes are observed, would be equal to or greater than P_r but less than P_{r+1} and the average would be $(P_r + P_{r+1} - 1)/2$. 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. With this method, the number of viable particles per stage is determined after a short incubation period by counting, with the aid of a microscope, the micro-colonies in a number of deposit areas and calculating the total for the plate. A deposit area is that area which receives particles from one jet or hole. The micro-colonies must be counted before they merge and, if done at the right time, as many as 20 or 30 per deposit area can be

counted. By this method, total sampler counts as high as 40,000 or 50,000 can be made."²

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	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	209	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	331	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 (approx 2628 particles) is exceeded.

VI. INSTRUCTIONS FOR THE NEW ANDERSEN 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 Andersen 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 — turn screw in to increase the flow and out to decrease the flow.

It is important the unit always operate at 1 cfm. 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 1/4" 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 cfm over a three minute test period (determined by an accurate stop watch). 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 cfm 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 cfm.

12 VOLT PUMP OPERATION

Battery required: 12 volt automotive type, minimum
69 amp hour capacity

TO OPERATE

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

ANDERSEN

SAMPLERS INCORPORATED

FLOW CALIBRATION CERTIFICATION

Model # 10-709

Serial # 187

Tube Length (2 Feet Unless Specified Differently) _____

Temperature 79° F

Barometric Pressure 26.34 CM Hg

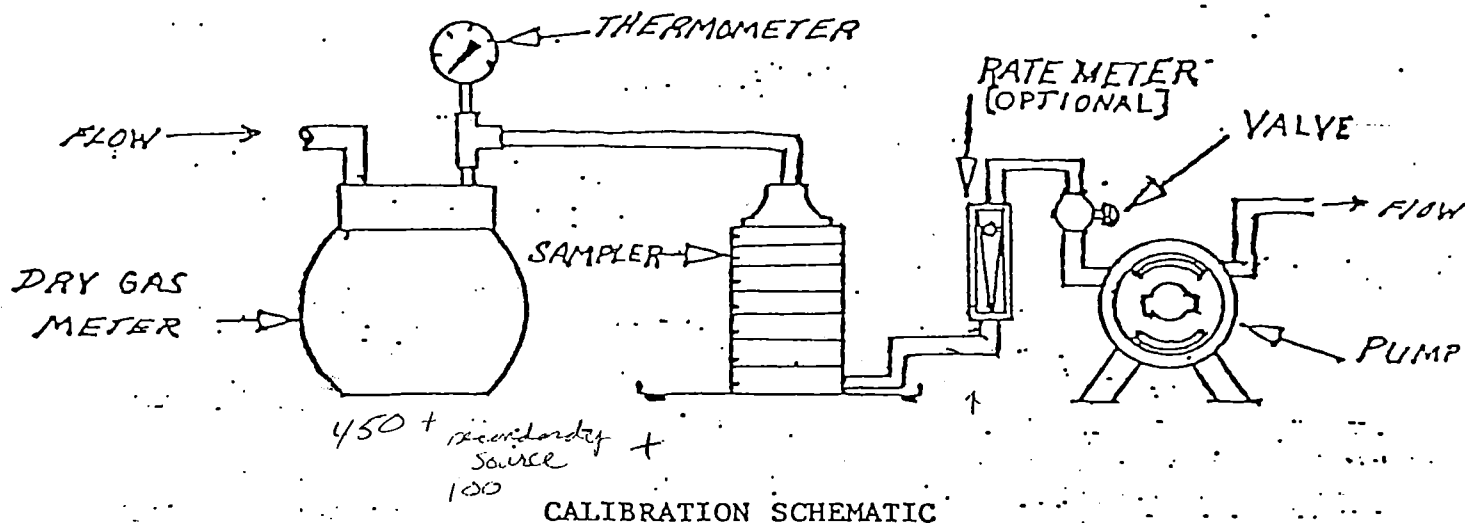
Date 5-29-85

Calibrated By CRC

CALIBRATION PROCEDURE (SEE FIGURE BELOW):

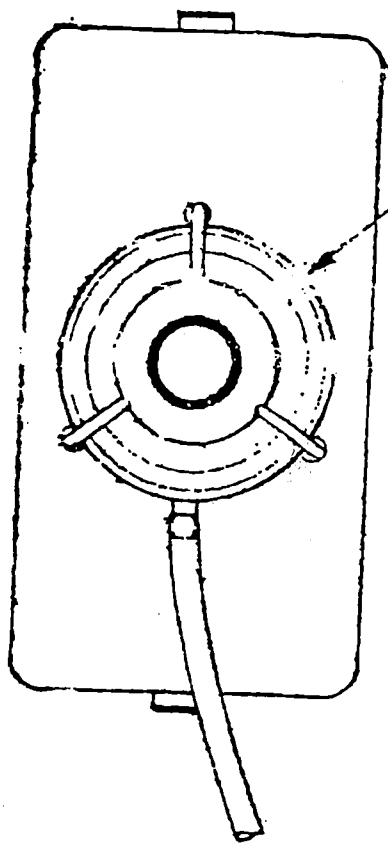
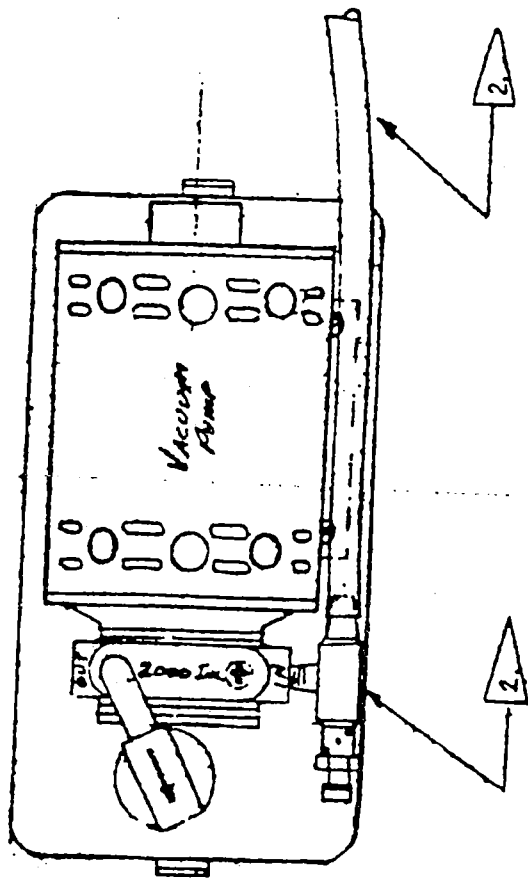
1. Assemble the impactor complete with collection substrates (petri dishes if Viable Sampler) and glass fiber backup filter (for Non-Viable Samplers Only).
2. Connect inlet of impactor to outlet of a calibrated dry gas meter.
3. Connect outlet of impactor to inlet of flow adjustment valve.
4. Connect outlet of flow adjustment valve to inlet of vacuum pump.
5. Turn on vacuum pump and allow to run for 15 minutes to stabilize flowrate.
6. Measure flow rate through the dry gas meter and open or close adjustment valve as necessary to achieve one actual cubic foot per minute (ACFM) flow rate. Once the desired flow rate is achieved, the flow rate through the dry gas meter should be measured for two separate two minute intervals to be certain that flow rate is $1.00 \pm .01$ ACFM.
7. Tighten the locknut on the flow adjustment valve to lock the flow adjustment valve at the proper flow.

2. Connect clip of black shielded wire to 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 manufacturer's instructions attached.
6. Pumping rate of the 12v D. C. unit will vary with voltage. Normal pump operation requires a current draw of approximately 11 amps. Continuous running in excess of 3 hours may result in reduced battery voltage and lower CFM through the Andersen sampler.
7. Fully recharge battery between uses.

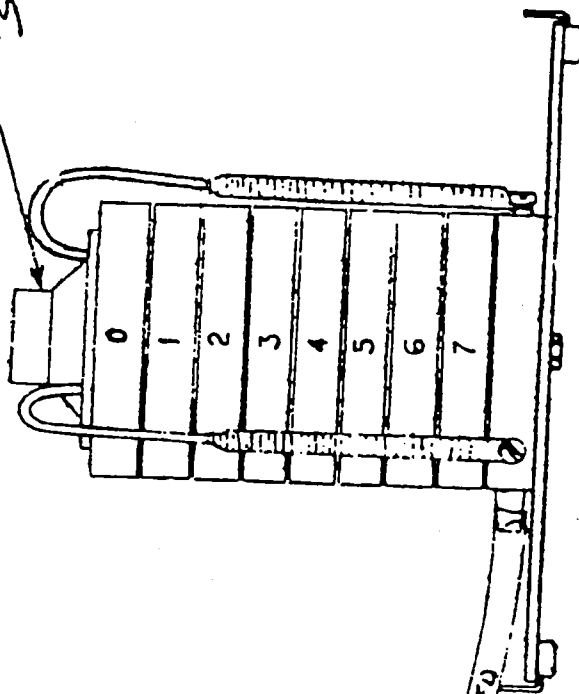


NOTES:

1. The Andersen Sampler flow rate has been calibrated at Atlanta, Georgia (elevation 840 ft) at Ambient temperature and pressure. If the pump is to be operated at elevations and or temperatures significantly different from those at which it was calibrated, the flowrate should be recalibrated for actual sampling conditions.
2. As a check to be certain the flowrate through the sampler is maintained, a ROTAMETER can be connected between the outlet of the impactor and the inlet of the adjustment valve. The premarked flow rates on the Rotameter will not be accurate at the reduced pressure downstream of the impactor and the 1 ACFM flow mark will have to be determined using the above Calibration Schematic.
3. There will not normally be any flowrate change during a sample because there is no pressure drop change during sampling. There is no backup filter associated with the Viable Sampler and normally less than 10 mg of particulate will be collected on the backup filter of the Non-Viable Sampler during a sampling period.



ANDERSEN
SAMPLER HEAD



Note: 1. Pump may be isolated from sampler up to 50 ft.

2. Flow regulator is adjusted at factory for 1 cfm through sampler with 2 ft. of tubing (supplied). Any change in length of tubing will require readjustment of flow rate.

ANDERSEN Air Samplers	
2000 INC.	
P. O. BOX 2074 2000 SULLIVAN RD. ATLANTA, GA 30320	
SUIT. CODE IDENT. NO.	DATE NO.
B	CHC-120111
	REV

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