Series 10-800

Instruction Manual

Six and Two Stage Viable Samplers
Part Number 100072-00
29Oct2009

Thermo SCIENTIFIC

Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Thermo Fisher Scientific
Air Quality Instruments
27 Forge Parkway
Franklin, MA 02038
1-508-520-0430
www.thermo.com/aqi

WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. It is marked with the following symbol:

Thermo Fisher Scientific has contracted with one or more recycling/disposal companies in each EU Member State, and this product should be disposed of or recycled through them. Further information on Thermo Fisher Scientific's compliance with these Directives, the recyclers in your country, and information on Thermo Fisher Scientific products which may assist the detection of substances subject to the RoHS Directive are available at: www.thermo.com/WEEERoHS.

Warranty

Seller warrants that the Products will operate or perform substantially in conformance with Seller's published specifications and be free from defects in material and workmanship, when subjected to normal, proper and intended usage by properly trained personnel, for the period of time set forth in the product documentation, published specifications or package inserts. If a period of time is not specified in Seller's product documentation, published specifications or package inserts, the warranty period shall be one (1) year from the date of shipment to Buyer for equipment and ninety (90) days for all other products (the "Warranty Period"). Seller agrees during the Warranty Period, to repair or replace, at Seller's option, defective Products so as to cause the same to operate in substantial conformance with said published specifications; provided that (a) Buyer shall promptly notify Seller in writing upon the discovery of any defect, which notice shall include the product model and serial number (if applicable) and details of the warranty claim; (b) after Seller's review, Seller will provide Buyer with service data and/or a Return Material Authorization ("RMA"), which may include biohazard decontamination procedures and other product-specific handling instructions; and (c) then, if applicable, Buyer may return the defective Products to Seller with all costs prepaid by Buyer. Replacement parts may be new or refurbished, at the election of Seller. All replaced parts shall become the property of Seller. Shipment to Buyer of repaired or replacement Products shall be made in accordance with the Delivery provisions of the Seller's Terms and Conditions of Sale. Consumables, including but not limited to lamps, fuses, batteries, bulbs and other such expendable items, are expressly excluded from the warranty under this warranty.

Notwithstanding the foregoing, Products supplied by Seller that are obtained by Seller from an original manufacturer or third party supplier are not warranted by Seller, but Seller agrees to assign to Buyer any warranty rights in such Product that Seller may have from the original manufacturer or third party supplier, to the extent such assignment is allowed by such original manufacturer or third party supplier.

In no event shall Seller have any obligation to make repairs, replacements or corrections required, in whole or in part, as the result of (i) normal wear and tear, (ii) accident, disaster or event of force majeure, (iii) misuse, fault or negligence of or by Buyer, (iv) use of the Products in a manner for which they were not designed, (v) causes external to the Products such as, but not limited to, power failure or electrical power surges, (vi) improper storage and handling of the Products or (vii) use of the Products in combination with equipment or software not supplied by Seller. If Seller determines that Products for which Buyer has requested warranty services are not

covered by the warranty hereunder, Buyer shall pay or reimburse Seller for all costs of investigating and responding to such request at Seller's then prevailing time and materials rates. If Seller provides repair services or replacement parts that are not covered by the warranty provided in this warranty, Buyer shall pay Seller therefor at Seller's then prevailing time and materials rates. ANY INSTALLATION, MAINTENANCE, REPAIR, SERVICE, RELOCATION OR ALTERATION TO OR OF, OR OTHER TAMPERING WITH, THE PRODUCTS PERFORMED BY ANY PERSON OR ENTITY OTHER THAN SELLER WITHOUT SELLER'S PRIOR WRITTEN APPROVAL, OR ANY USE OF REPLACEMENT PARTS NOT SUPPLIED BY SELLER, SHALL IMMEDIATELY VOID AND CANCEL ALL WARRANTIES WITH RESPECT TO THE AFFECTED PRODUCTS.

THE OBLIGATIONS CREATED BY THIS WARRANTY STATEMENT TO REPAIR OR REPLACE A DEFECTIVE PRODUCT SHALL BE THE SOLE REMEDY OF BUYER IN THE EVENT OF A DEFECTIVE PRODUCT. EXCEPT AS EXPRESSLY PROVIDED IN THIS WARRANTY STATEMENT, SELLER DISCLAIMS ALL OTHER WARRANTIES, WHETHER EXPRESS OR IMPLIED, ORAL OR WRITTEN, WITH RESPECT TO THE PRODUCTS, INCLUDING WITHOUT LIMITATION ALL IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE. SELLER DOES NOT WARRANT THAT THE PRODUCTS ARE ERROR-FREE OR WILL ACCOMPLISH ANY PARTICULAR RESULT.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 Thermo Fisher Scientific Andersen Viable Cascade Impactor (ACI), 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 Viable Cascade Impactor (ACI).

I I . INTRODUCTION

SIX-STAGE VIABLE CASCADE IMPACTOR

The Six-Stage ACI 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 intramural 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 colony 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 CASCADE IMPACTOR

The Two-Stage ACI 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 impactor separates viable particles into two size ranges with the 50% cut-off diameter of Stage 1 at 8.0 microns for spherical particles of unit density. The impactor 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 27m1 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. Plastic Petri dishes should not be used because the static charge generated reduces the collection efficiency.

   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.

   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.

e. Viable particles are retained on the agar plates, and the exhaust air is carried-through the vacuum hose to the vacuum source (pump or in-house vacuum system).

f. For maximum collection efficiency, a constant air sampler flow of 1 ACFM must be provided. This constant flow is provided with a continuous-duty, carbon vane vacuum pump, and is controlled by an adjustable valve on the pump. Periodic calibration is recommended (See Section VI). Another method of assuring a constant flow would be to insert an airflow meter (not provided), with a minimum capacity of 1 ACFM (28.3 liters/min.) in the vacuum hose between the Sampler and the vacuum source. This flowmeter should be properly calibrated.

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, 1-3, etc.).

h. Place all 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° to 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 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 100xl5mm 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.

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.

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 ACI 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 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 dimensions (Figure 1). Because the lung penetrability of unit density particles is known and since the particle sizes that are collected on each stage of the ACI 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 impactors. Thus, the Six-Stage ACI with 400 small round jets per stage and the Two-Stage ACI 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 reintrained and lost in the exhaust air. This effect is minimized when a sticky agar surface is used as the collection medium.

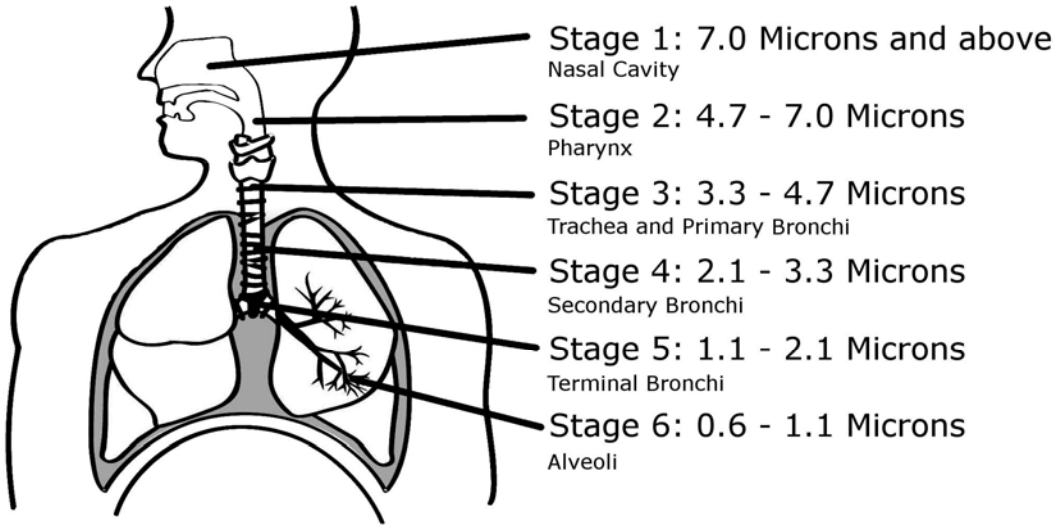

Figure 1. Six-Stage ACI Sampler Simulates Human Respiratory System

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

$$K = \frac{CpUDp^2}{18\mu D_c}$$

Where U is the relative velocity, p is the particle density, Dp is the particle diameter, μ 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 ACI, 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:

$C = 1 + 0.16 \times 10^{-4}/D_p$ 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 these 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 ACI 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 ACI sampler, therefore, on a theoretical basis, should have a sharper cutoff.

Another inherent advantage of the ACI 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 CASCADE IMPACTOR

1. Description

The Six-Stage ACI is constructed with six aluminum stages that are held together by three spring clamps and sealed with O-ring gaskets (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 within each stage, but are 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 ACI 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 ¼ 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. TFS ACI Six-Stage Viable Sampler

2. Assembly

The orifice stages should be cleaned and disinfected each time the instrument is used. A mild pH neutral 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 is effective in cleaning them. Just before use, wipe all surfaces with 70% isopropyl alcohol using a lint free 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 for sealing. These O-rings should be checked regularly and replaced when they no longer provide an airtight seal.

The assembly of the Six-Stage ACI begins by placing an agar collection plate, uncovered, on the base plate so that the Petri dish rest on three raised metal pins. Insert Stage 6 over the Petri dish. Place a second Petri dish on the top of Stage 6 and continue this manner until all six agar collection plates have been positioned in the ACI. 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 ACI 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 collected. 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.

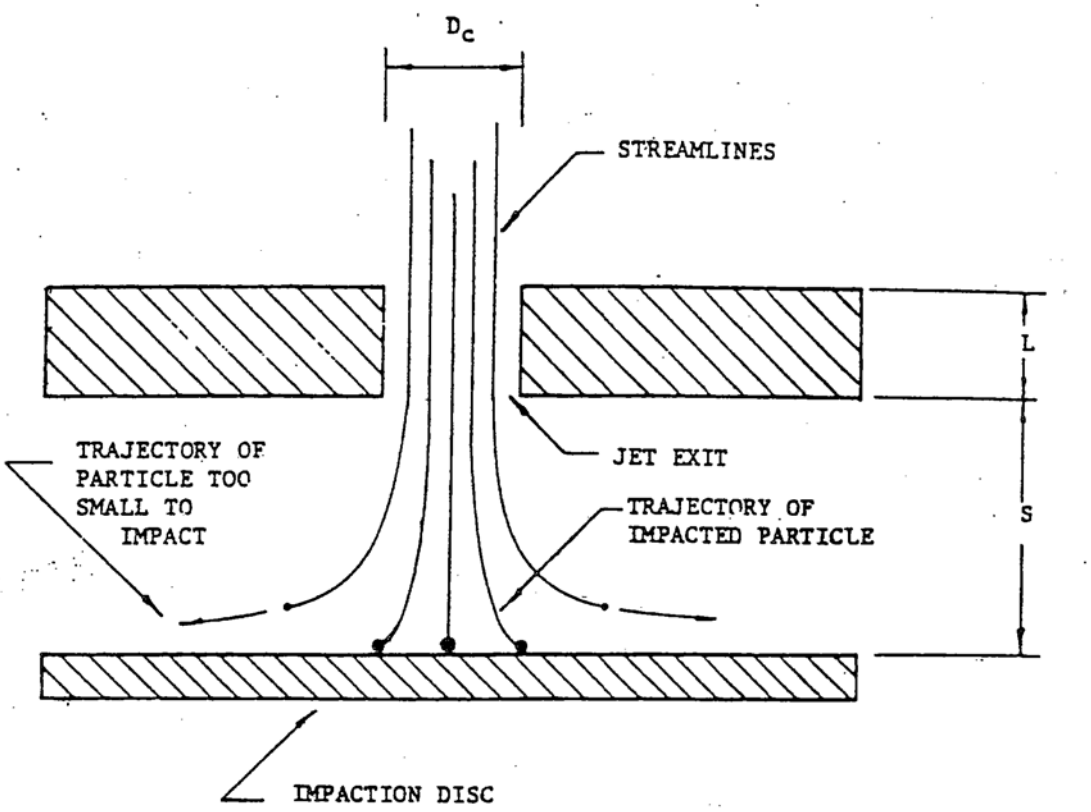

Figure 5. Schematic of Impactor Stage

After the sampling has been completed, the ACI 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 ACI 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 ACI 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 ACI pump impactor assembly is calibrated before shipment to deliver 1 ACFM at ambient temperature and pressure levels in Franklin, MA. In order to recalibrate at your sampling environment, the following procedure is recommended:

> Place a calibrated flow meter upstream from the ACI. 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 flow meter. Adjust the pump valve until you are pulling l 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 ACI.

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 DC 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 12 Volt Pump" for detailed 12 volt DC pump operation.

B. TWO-STAGE ALUMINUM VIABLE CASCADE IMPACTOR

1. Description

The ACI 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 ACI, 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, carbon vane vacuum pump is available which will provide a constant sample flow of 1 ACFM.

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). 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 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.) must be maintained. 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. 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

Since the size fraction for each stage is determined by the orifice velocities, it is important that the ACI 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 ACI 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 ACI pump impactor assembly is calibrated before shipment to deliver 1 ACFM at ambient temperature and pressure levels in Franklin, MA. In order to recalibrate at your sampling environment, the following procedure is recommended:

> Place a calibrated flow meter upstream from the ACI. 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 flow meter. Adjust the pump valve until you are pulling l 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 ACI.

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 DC 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 12 Volt Pump" for detailed 12 volt DC pump operation.

V. ANALYSIS AND INTERPRETATION OF DATA

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.

$$\frac{\text{Colonies on Stage 1 of the Six-Stage Sampler}}{\text{Total Number of Colonies from all Sample Plates}} \times 100 = \text{Percent of Viable Particles Over 7.0 Microns In Diameter}$$

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 Six and Two Stage Viable Cascade Impactors.

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

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

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 rth 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 1,200 or 1,500 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-

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

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	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*

TABLE II

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

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

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

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

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

VI. INSTRUCTIONS FOR 12 VOLT 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 - 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. 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 flow 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 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.

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 DC 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 ACI.

7. Fully recharge battery between uses.

SERVICE LOCATIONS

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

Toll Free U.S. only 1-866-282-0430

U.S., Latin America, and Canada 1-508-520-0430

Europe +31 76 579 5555

China +86 10 8419 3588

Asia Pacific +91 22 27781102