Tisch Environmental, Inc.

Cascade Impactor Series 10-8XX
Viable (Microbial)
Particle Sizing Instruments

OPERATIONS MANUAL

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PREFACE

Tisch Environmental, Inc. is a third generation family owned business. The owners Wilbur J. Tisch and James P. Tisch have been involved in the High Volume Air Pollution field for the last 20 years. Started in March of 1998, they would like to welcome you to their company.

The intent of this manual is to instruct the user with unpacking, assembly, operating and calibration techniques. For information on air sampling principles, procedures and requirements please contact the local Environmental Protection Agency Office serving your area.

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INTRODUCTION

The study of the microbial content of the air has become increasingly more significant in the past decade as the need for “contamination free” environments have become more apparent.

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 used to collect and study aerobic species of bacteria and fungi. Even though many viable samplers, including the Tisch Environmental sampler, 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, first, the particle must be separated from the air for any viability study, and second, the ability to reproduce (viability) must be demonstrated.

The purpose of this manual is to outline proper methods for the study of biological aerosols using the Tisch Environmental Viable Particle Samplers.

SIX-STAGE VIABLE PARTICLE SAMPLER

The Tisch 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 ambient air. The 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.

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

a. Collection plates are prepared by aseptically pipetting 27ml of sterile bacteriological agar (45-50ºc) into each of six glass 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 flow is provided with a continuous-duty vacuum pump. Periodic calibration is recommended (See Calibration Section). 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. The user should calibrate this flow meter, using a Wet-Test or Dry-Test Gas Meter.

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 A (i.e., 1-1, 1-2, 1-3, etc.).

h. Place all agar plates, inverted to prevent condensation drip, in an incubator at 35ºc 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 20º to 25ºc for maximum recovery of fungi.

i. After incubation, the number of colonies on each plate is counted, using a standard bacterial colony counter.

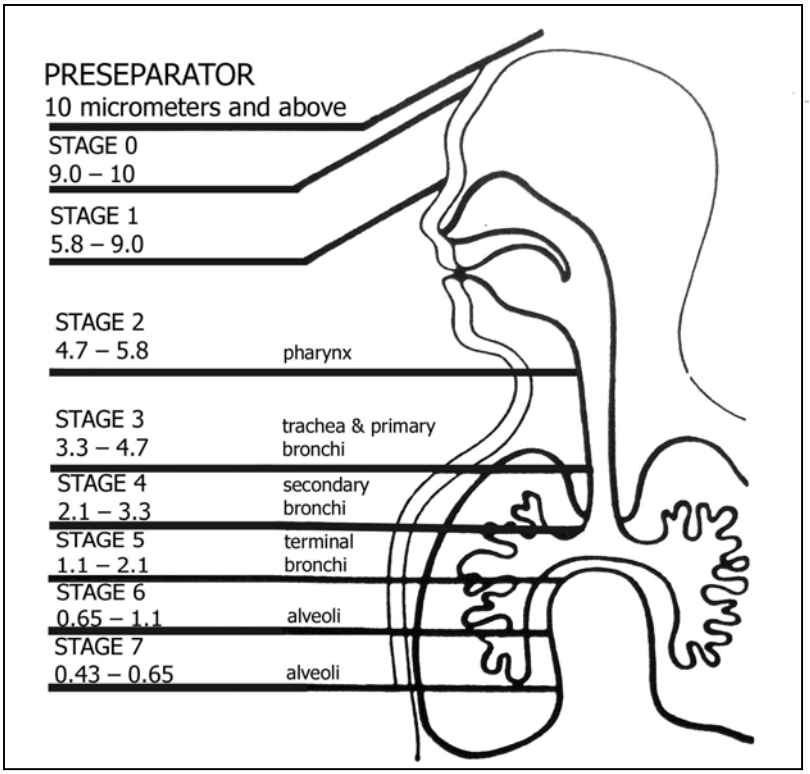
j. Knowing the air sample flow rate and the sampling time, the mean number of viable particles (aerobic bacteria and/or fungi) per unit volume of air can be calculated, and the percent or particles in each size range can be estimated.

AERODYNAMIC PARTICLE SIZING

The design concept of the Tisch Viable sampler evolved from the following information:

The human respiratory system 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) or 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 Tisch 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 that extent to which the sample would have penetrated the respiratory system. Figure 1 shows the deposition efficiencies in the nasal-pharyngeal, tracheo-bronchial and pulmonary regions of the human respiratory tract as a function of particle size. Large particles deposit primarily in the nasal-pharyngeal area, whereas particles of sub-micrometer size particles deposit mainly in the pulmonary area

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 sampler with 400 small 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 reintrained and lost in the exhaust air. This effect is minimized when a sticky agar surface is used as the collection medium.



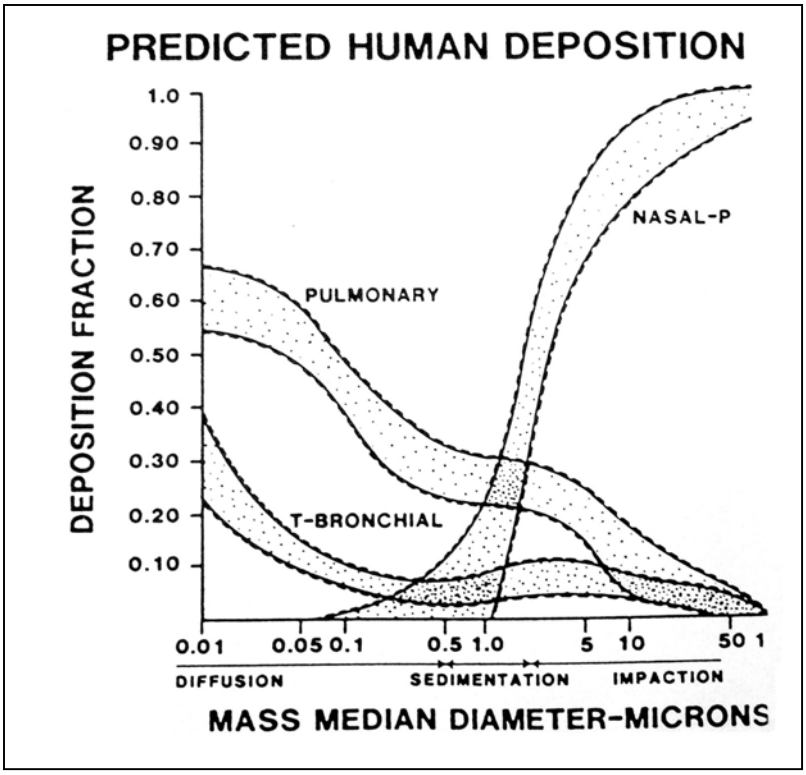


FIGURE 1. TISCH SAMPLER SIMULATES THE HUMAN RESPIRATORY SYSTEM

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) and is equivalent to:

$$\frac{C\rho U D_p^2}{18\mu D_c}$$

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 Cummingham slip correction factor.

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

The Cummingham slip correction factor (C) is equal to:1+0.16 x 10-4/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 modules, they tend to "slip" between gas modules 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 impacting 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 impacting devices, is minimized by design. Ranz and Wong 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 impacting surface. In contrast to other samplers, which have larger rectangular jets in each stage, the Tisch 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 also stated that round jets have sharper cutoffs than rectangular jets. The Tisch sampler, therefore, on a theoretical basis, has a sharper cutoff. Another inherent advantage of the Tisch Air Sampler 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).

SIX-STAGE VIABLE PARTICLE SAMPLER DESCRIPTION

The Tisch 1 ACFM Viable Particle sampler is constructed with six aluminum stages that are held together by three spring clamps and sealed with 0-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 1.18 mm on the first stage to 0.25 mm on the sixth stage. Each stage has a removable glass Petri dish with a glass or metal cover. The exhaust section of each stage is approximately 19mm larger in diameter than the Petri dish, which allows unimpacted particles to go around the dish into the next stage.

The Tisch Six-Stage Viable Particle Sampler and Vacuum Pump include their own carrying case for ease of portability.

A constant air sampler 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.

FIGURE 2. TISCH SIX-STAGE VIABLE SAMPLER

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

Stage	Orifice Diameter (mm)	Range of Particle Sizes (Microns)
1	1.18	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

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 (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 impactor 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 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 with the are used and 27 ml of agar 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.

SAMPLING

When ready to sample, the vacuum pump is turned on and a sample stream of 1 ACFM will flow through the sampler. Figure 3 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.

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 parallel or to remove the agar plates representing one-sample and insert fresh plates for a second sample.

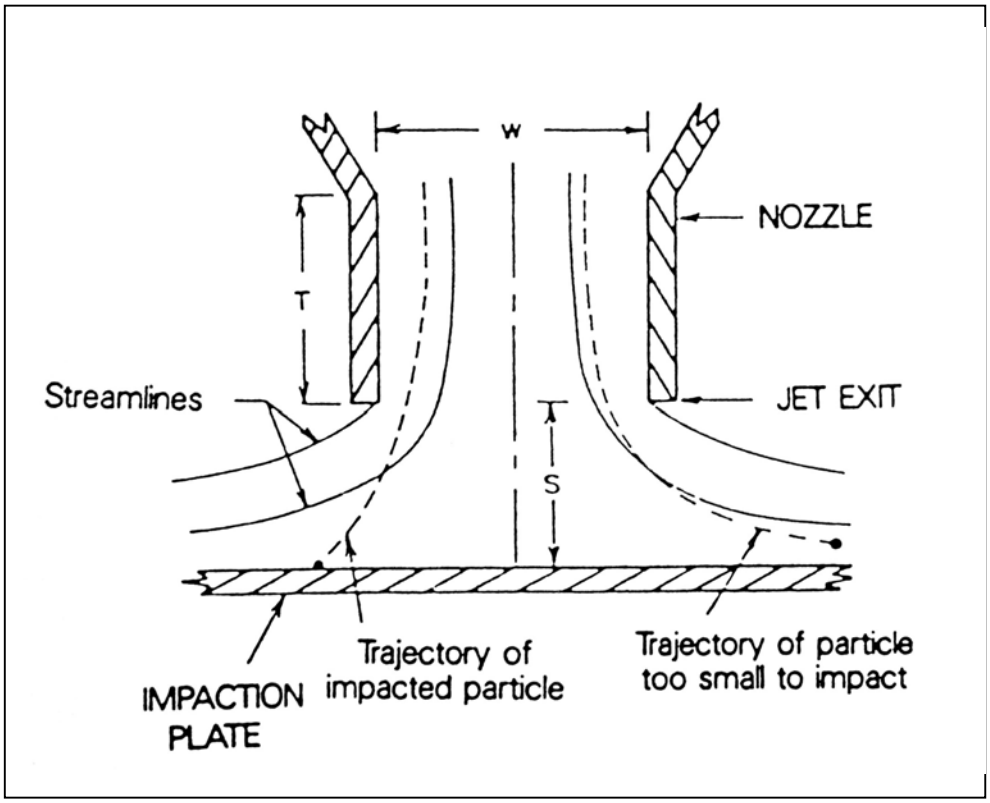


FIGURE 3. SCHEMATIC OF IMPACTOR STAGE

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

CALIBRATION

Since the orifice velocities determine the size fraction for each stage, 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 Tisch 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 Tisch pump-impactor assembly is calibrated before shipment to deliver 1ACFM at ambient temperature and pressure levels in Cleves, Ohio. 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 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 for DC pumps will vary with voltage. One ACFM can be drawn through the impactor if the voltage is maintained near 12 volts. AC pumps will not vary significantly on line voltage.