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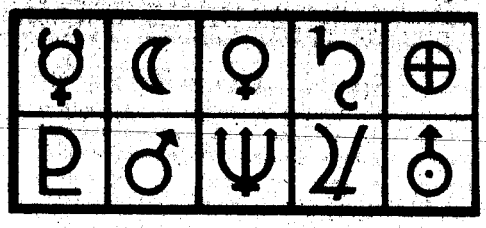

PLANETARY QUARANTINE

PRINCIPLES OF OPERATION OF THE VACUUM PROBE MICROBIOLOGICAL SAMPLER

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PRINCIPLES OF OPERATION OF THE VACUUM PROBE MICROBIOLOGICAL SAMPLER

by

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Albuquerque, New Mexico

August 1967

ABSTRACT

This paper contains the detail drawings which describe the physical design of the vacuum probe, which has been designated the "hand probe" or the "filter probe", and a suggested protocol for the repeated use of this instrument. Also included are a discussion of how the probe's critical orifice varies with atmospheric pressure and vacuum pressure and how small particles may be influenced by the sonic noise which is produced by the operation of the orifice.

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TABLE OF CONTENTS

INTRODUCTION

The need for a microbiological surface sampling device with the capability for sampling relatively large areas which possess light loading densities of microorganisms has prompted the development of the device known as the vacuum probe. This device has demonstrated the ability to repeatably remove in excess of 90% of the settled microbiological foci, which are in the one micron size range or larger, from a smooth surface[1] without harming the surface. This capability can be appreciated when complexes such as spacecraft must be sampled in ultra-clean environments.

This report has been written to present the detail engineering drawings of the combination of the vacuum probe and a membrane filter holder known as the "filter probe" and to discuss two interesting facets of the operation of the vacuum probe. The first of these facets is how the critical condition of the probe's orifice is varied with changes in ambient atmospheric pressure and changes in the vacuum pressure used to operate the probe. The second topic includes a discussion of the sonic noise produced by the operation of the device and of the possible effect of this acoustical energy on the removal of bacteria-sized particles from a surface. This report is a supplement to a previous report: "A New Approach to the Microbiological Sampling of Surfaces: The Vacuum Probe Sampler", SC-RR-67-114, March, 1967.

PHYSICAL DESCRIPTION OF "FILTER PROBE"

A complete assembly drawing of the "filter probe" is shown in Figure 1. This device is a hybrid unit consisting of (1) a special design for the probe tip, the conical section of the filter housing, and the handle and (2) standard

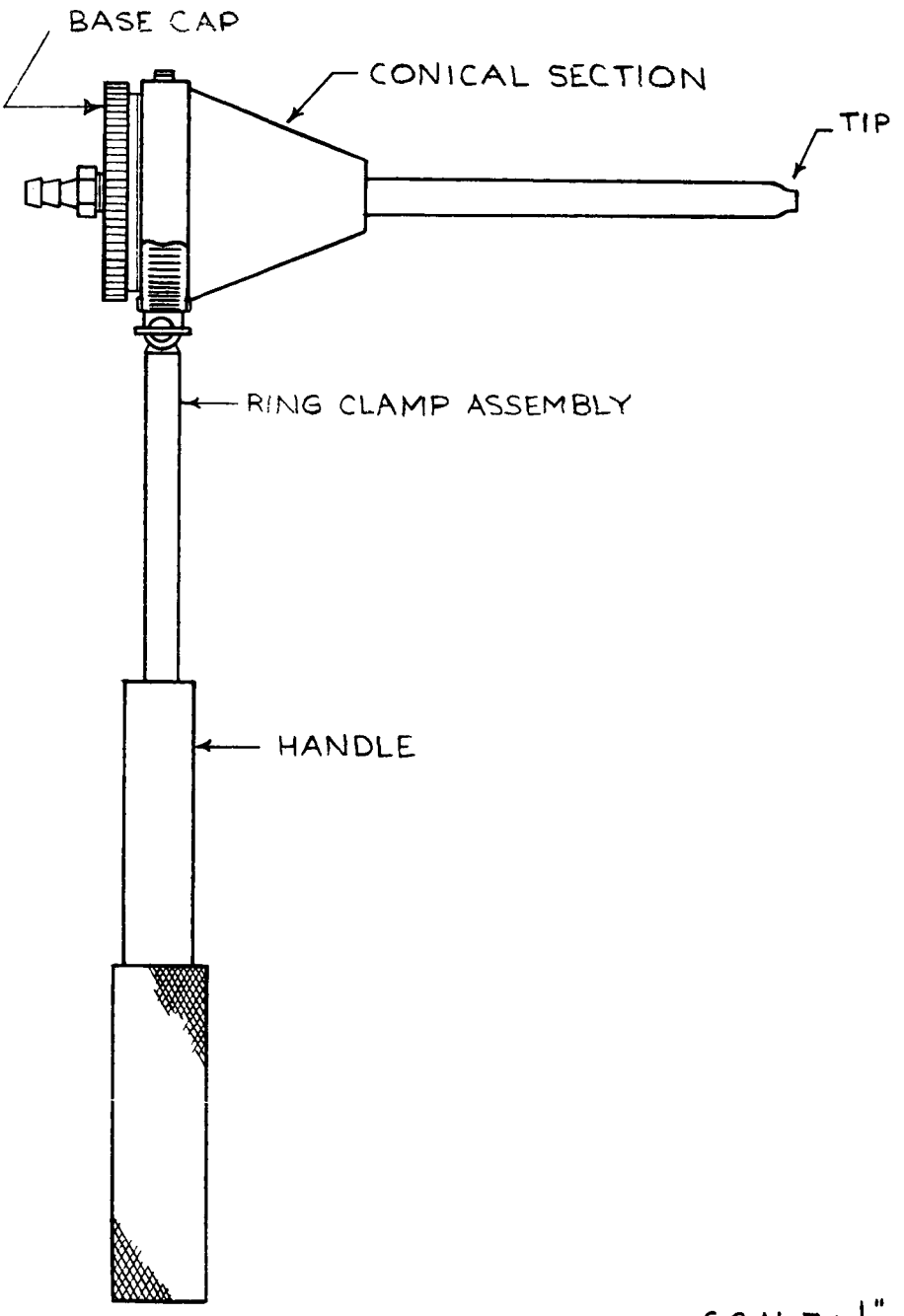


FIGURE 1

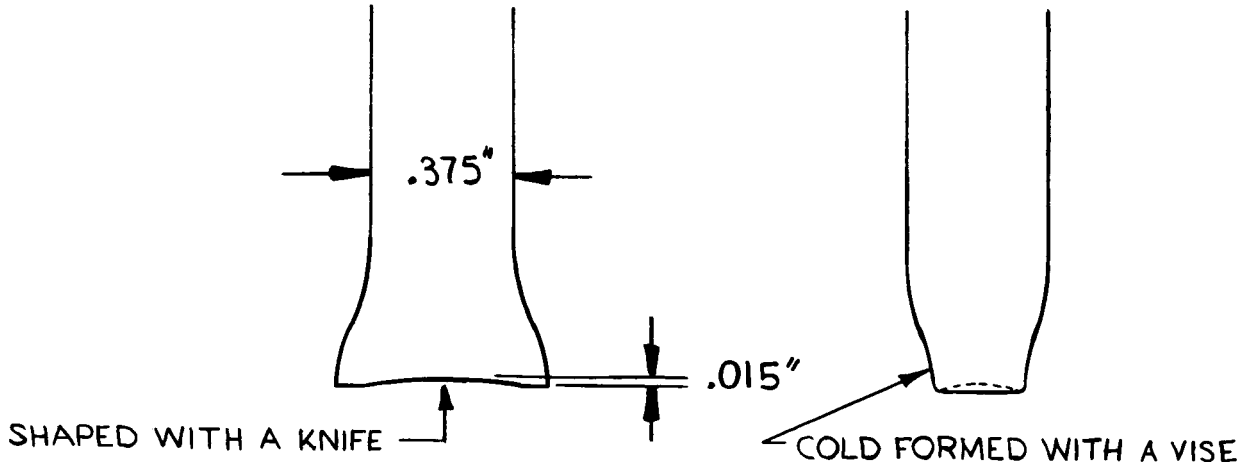
FILTER PROBE ASSEMBLY

commercial units for the ring clamp attachment and the base cap assembly of the filter housing. The base cap assembly consists of the base cap from a Gelman[2] two inch stainless steel filter holder* (No. 2214) along with the copper mesh backing pad and the circular filter retaining ring. The ring clamp which holds the filter housing and provides the handle attachment is a Nester glassware clamp* obtained from Van Waters and Rodgers, Incorporated (Catalog No. 21657).

Figure 2 shows a detail drawing of the probe tip. This tip can be constructed from a number of different materials, but Teflon has been found to be the most suitable since it offers a coefficient of friction of only 0.04 on metal or plastic surfaces. The orifice can most easily be made by forming the end of a section of circular cross section Teflon tubing.

A detail drawing of the conical section of the filter holder is shown in Figure 3. The design of this section of the filter holder was selected to allow the air entering the conical section to expand as evenly as possible while keeping the interior surface area at a minimum. The height of the conical section must be large enough to prevent the air stream from having a velocity at the face of the membrane filter great enough to blow particles off the filter and onto the housing walls. However, the height must not be so great that the internal surface area is excessively large, since this encourages greater impingement of particles on the walls rather than on the membrane filter. The design shown in Figure 3 was found to be most nearly optimum for a critical flow rate of 1.5 to 2.0 standard cubic feet per minute.

*This commercial unit was used because it was available and because it filled the need. Any other commercial equipment with similar properties would be completely acceptable.



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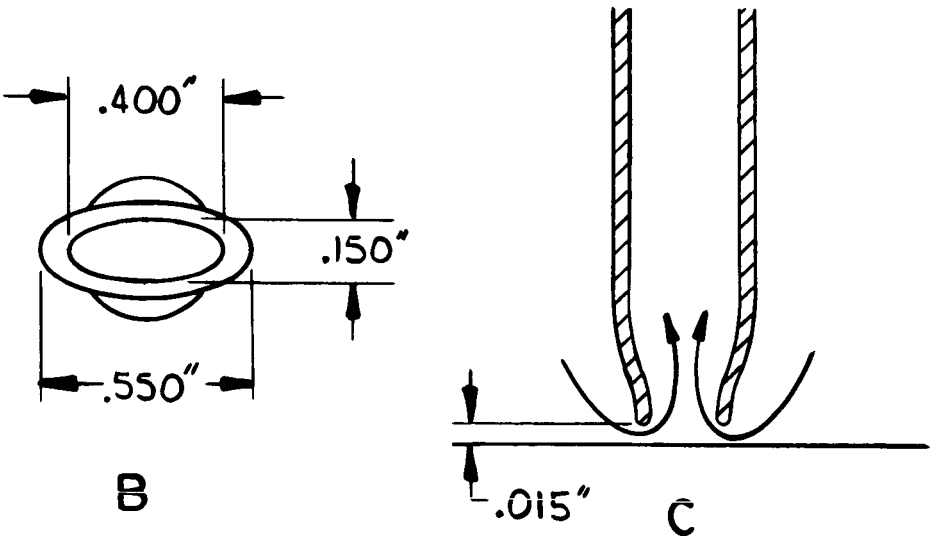

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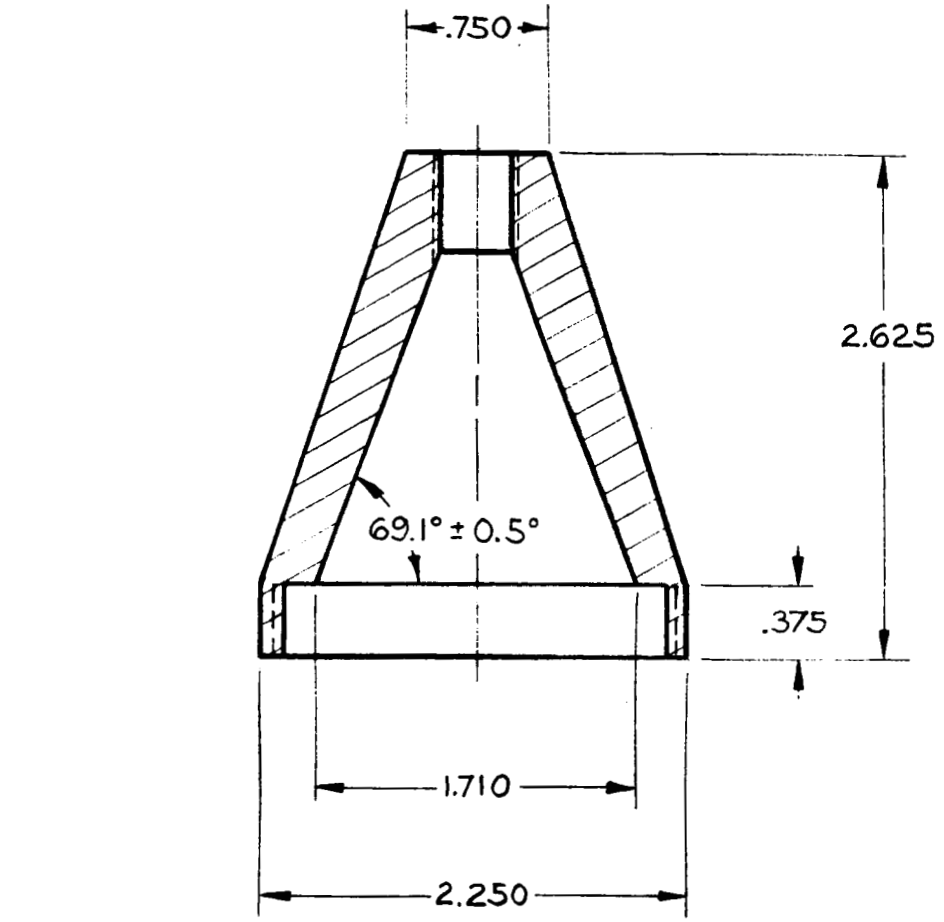
VACUUM PROBE SCHEMATIC DRAWING

FIGURE 2



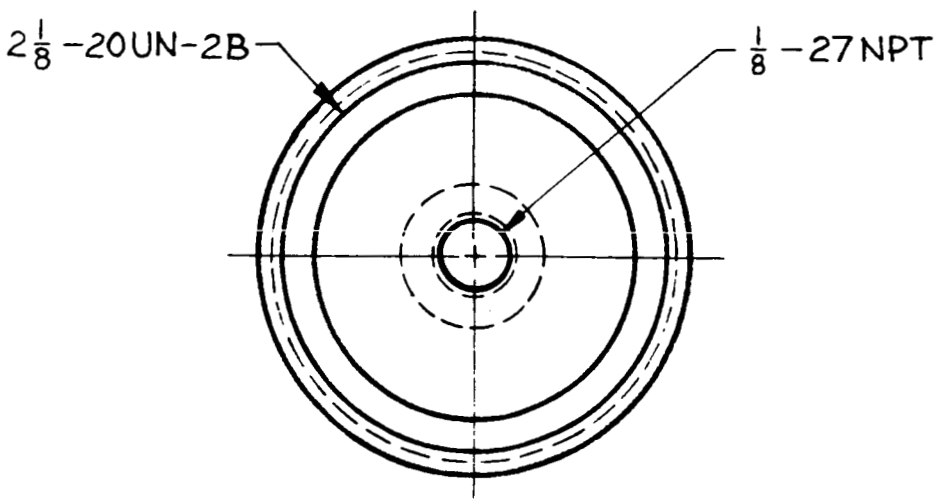


FIGURE 3
FILTER HOUSING

ALL DIMENSIONS IN INCHES
MATERIAL: 2024-T4 ALUM.
SCALE: 1"=1"
ALL DECIMAL DIMENSIONS ±.060

Figure 4 shows a detail drawing of a simple handle attachment which fits onto the filter housing clamp.

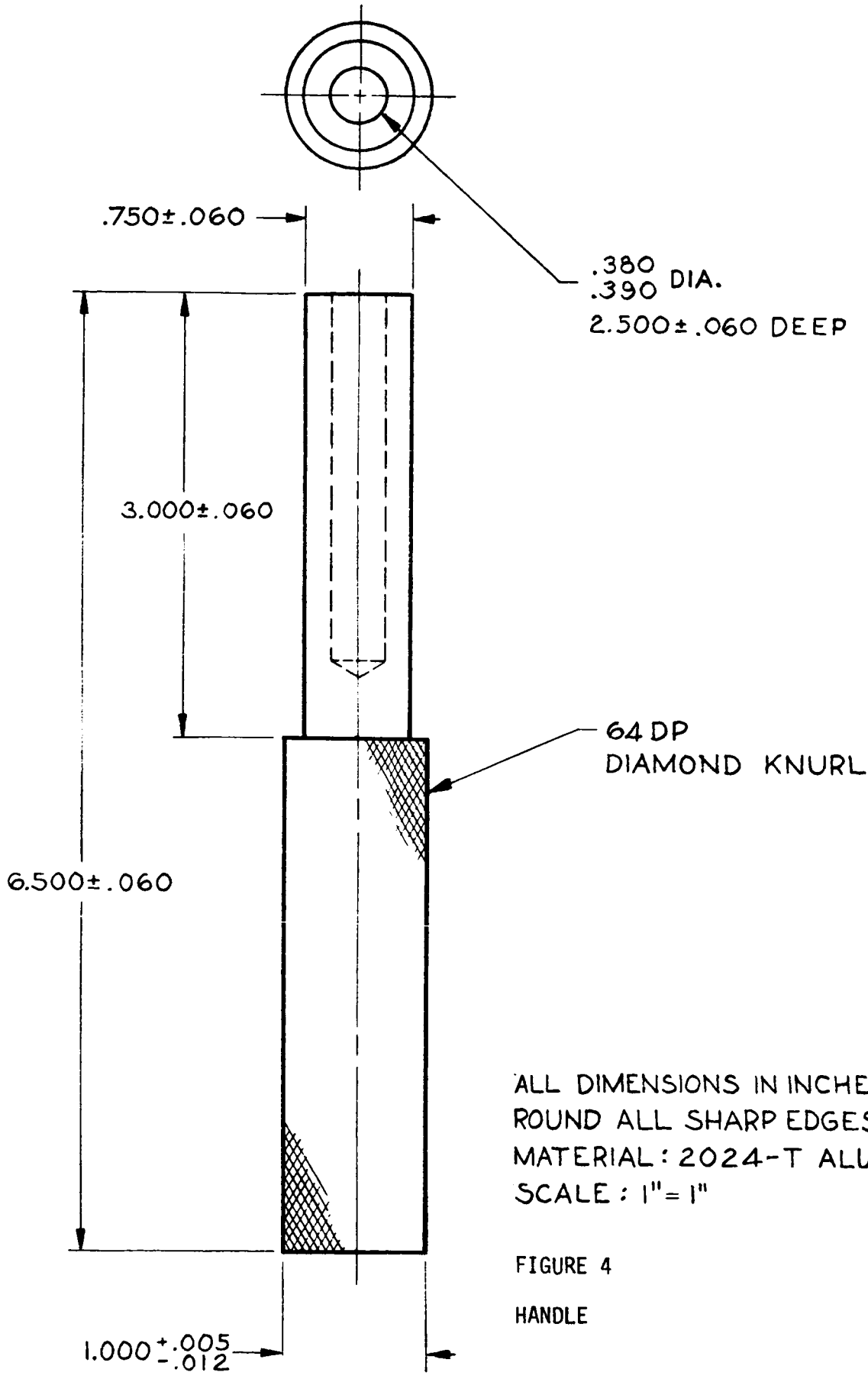
PROBE OPERATION PROTOCOL

Listed below is a suggested series of steps to follow when using the "filter probe".

I. Sterilization of the metal-teflon probe assembly with openings covered with aluminum foil.
   A. Use dry heat of 160°C for two hours and allow to cool.
   B. Or autoclave at 121°C for 20 minutes and allow time to dry.

II. Filter loading techniques in a laminar flow clean room facility.
   A. Unscrew base cap of filter assembly and place cap on some sterile fixture such as an open mouth flask with the open face up.
   B. Using a pair of sterile forceps, place a sterile, 2" diameter, 0.45 micron membrane filter on top of the copper mesh backing pad which lies within the base cap. Use of a colored membrane filter aids when colony counts are to be made.
   C. Place the round filter retaining ring on top of the membrane filter with the flat side of the ring against the filter.
   D. Reassemble the filter housing by placing the conical section on top of the base cap, which remains on top of the holding fixture, and screw the two together.

III. Use of the filter probe as a microbiological sampling instrument within a laminar flow clean room or clean bench.
   A. Connect the exhaust terminal of the filter housing to a vacuum source capable of 2.5 cubic feet per minute under a vacuum of 20 inches of mercury. To be assured of having a sufficient vacuum



ALL DIMENSIONS IN INCHES
ROUND ALL SHARP EDGES
MATERIAL: 2024-T ALUM.
SCALE: 1"=1"

FIGURE 4

HANDLE

capacity to operate the probe's orifice in the critical range, the following test may be used: Drill a small hole (1/16 inch diameter) in the conical section of the filter holder on the upstream side of the membrane filter. Insert a tubular fitting in this hole such that a manometer may be attached. Measure the pressure with the probe tip applied perpendicular to a smooth surface and with the vacuum source applied. If the pressure indicated is one-half atmosphere or less, then the orifice is in the critical range and the vacuum source is sufficient. Plug hole before use.

B. Pass the probe tip over the surface to be sampled approximately four times being careful to keep the tip perpendicular to the surface and making sure that each point of the surface is covered.

IV. Evaluation of probe data in a filtered laminar flow environment.

A. Place the probe against the holding fixture (flask) with the base cap held flat against the fixture. Unscrew the base cap and remove the conical section of the filter housing.

B. Using sterile forceps remove the retaining ring. Then, with the same forceps remove the contaminated membrane filter and place it on the agar surface of a partially filled petri dish with the contaminated side up.

C. After the filter pad has become wet due to its contact with the agar, pour enough molten agar into the dish to just cover the filter and cover the petri dish.

D. Place the petri dish containing the filter into an incubator and incubate for 72 hours at $32^{o}C$, making counts at each 24 hour interval, if necessary. (Other incubation conditions may be desirable for some specific type of test.)

E. After the incubation period is complete, remove the dish and cover the surface of the agar with a room temperature solution of 0.05% 2,3,5 triphenyl tetrazolium chloride. This is a dye which colors the colonies red and makes them easy to recognize and count. Allow the dye to remain on the agar surface for approximately 20 minutes (or until the colonies are stained) before beginning to count the colonies.

F. Count the colonies and record the number along with the area, position, and type of surface which was sampled.

V. Assay of interior walls of the filter housing. Since a few microorganisms may impinge on the walls of the filter housing, good practice may suggest that the interior surfaces be sampled. One method which may be used is the swab-rinse technique.

A. After finishing the sampling procedure and processing the filter, swab one-half the interior wall with one wetted swab and the other half with another. Use cotton or calcium alginate swabs.

B. Place cotton swabs in 50 ml. of 1% peptone water and the calcium alginate swabs in a 50 ml. solution of 1% sodium citrate in 1% peptone.

C. Shake until the cotton is fluffed or the calcium alginate is dissolved.

D. Plate out appropriate aliquots of these suspensions and incubate along with the overlaid filter.

VI. Care for probe tip. Since the teflon tip will wear down and since the size of the tip's orifice is very important, the size of the orifice should be checked with feeler gauges after every 2-3 uses of the device.

DEPENDENCE OF THE VACUUM PROBE'S CRITICAL ORIFICE UPON VACUUM PRESSURE AND ATMOSPHERIC PRESSURE

As was stated in the previous report on the vacuum probe, the efficient operation of the probe is dependent upon the tip's orifice reaching the critical condition at the desired airflow rate. The condition called a critical orifice represents the limiting case in which the velocity of the air passing through the constriction ceases to increase with further decreases in the downstream orifice pressure provided the upstream orifice pressure is held constant. In the case of the vacuum probe the upstream pressure is simply the atmospheric pressure for the area in which the instrument is being used. Since changes in atmospheric pressure do effect the flow rate at which the orifice reaches the critical condition and since this may have an effect on vacuum pump requirements, a series of tests were performed to determine the actual dependence involved. The test setup which was used is shown in Figure 5. The entire series of tests was run without the membrane filter in place.

As depicted in Figure 5 the technique used in these tests was to pressurize (or partially evacuate) a sealed aluminum container in which the vacuum probe tip was mounted perpendicular to a smooth stainless steel plate. The pressure of the air in the container could be closely controlled by adjusting the regulated air supply to some constant flow rate; and therefore, any desired atmospheric pressure could be simulated. Since the entire system was sealed, with the exception of the input at the air supply and the output at the vacuum pump, the airflow through the probe's orifice could be measured by monitoring the steady-state flow into the sealed container. The Brook Flow-Meter used to monitor the airflow is a certified primary standard with $\pm$ 3% accuracy and

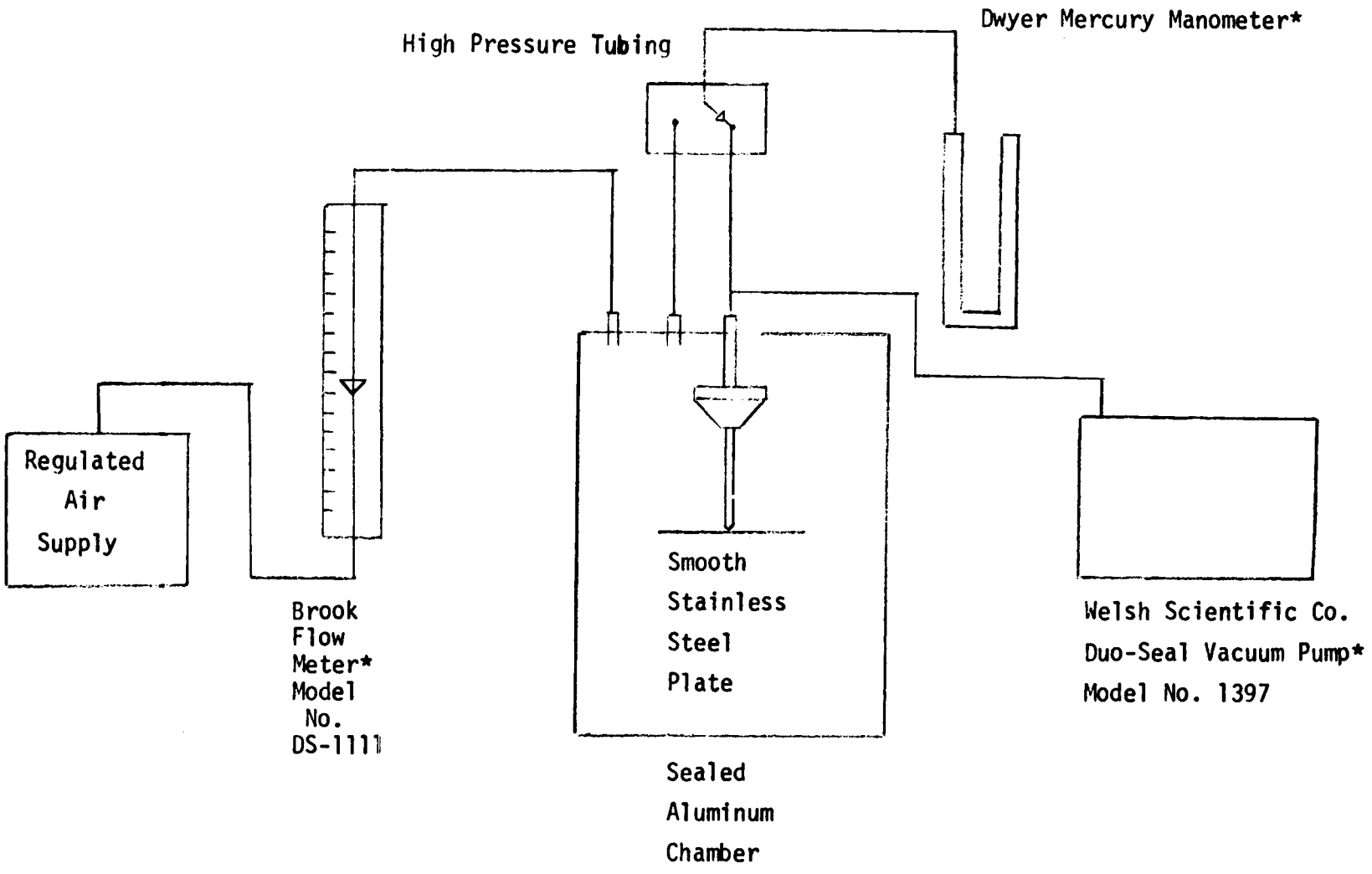


FIGURE 5

TEST SETUP TO DETERMINE THE DEPENDENCE OF
THE VACUUM PROBE CRITICAL ORIFICE ON OPERATING PRESSURES

has calibration tables[3] with conversion charts for operation at points other than standard temperature and pressure. The Dwyer Mercury Manometer was used to measure the operating container pressure and the downstream probe orifice pressure relative to the atmospheric pressure of Albuquerque, New Mexico. (24.6 inches of Mercury).

With the experimental setup which has been described a series of curves were empirically determined by varying the downstream orifice pressure for several different container pressures and several sizes of orifices. Figure 6 shows how the air flow rate through a critical orifice, such as shown in Figure 2, varies with changes in the orifice's downstream absolute pressure for a series of six different upstream or chamber pressures. The height of this orifice was 0.012 inches. As noticed from this set of curves, the saturation point of the airflow occurs at a downstream orifice pressure which is approximately one-half the upstream orifice pressure, and the flow rate at which saturation occurs becomes increasingly larger for increases in the upstream pressure. Figure 7 describes how the magnitude of the critical flow rate will change with changes in the chamber pressure (atmospheric pressure) for four orifice sizes. For this set of curves the downstream orifice pressure was kept at a value which was much less than one-half the lowest chamber pressure. This guarantees that operation is well into the saturated portion of the curve. From these curves the fact that the critical flow rate has very nearly a linear dependence upon the container pressure (atmospheric pressure) may be seen. Also, for the smaller size orifices, the dependence of the flow rate upon the orifice height dimension is approximately linear at any given atmospheric pressure.

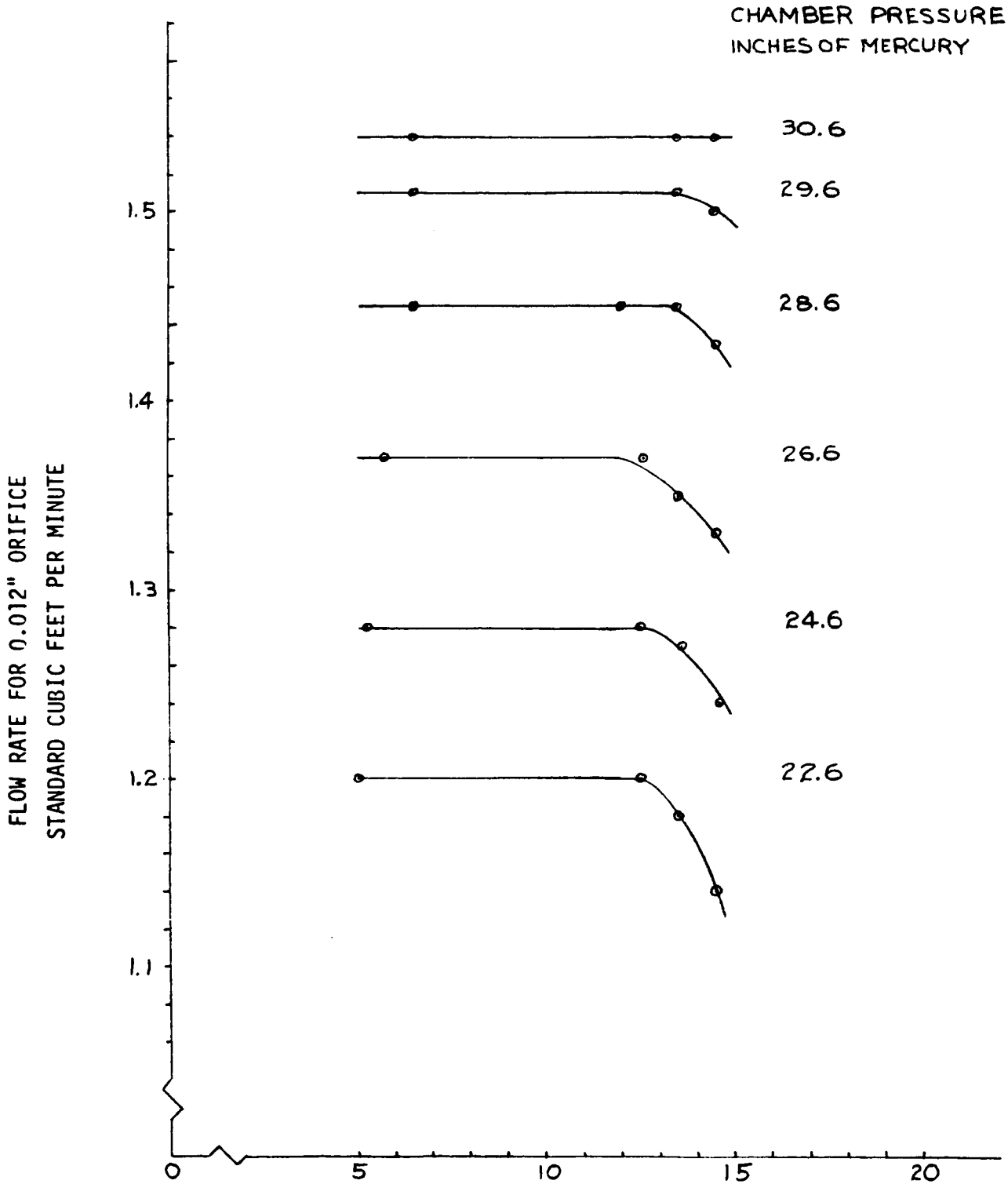


FIGURE 6

DEPENDENCE OF A CRITICAL ORIFICE UPON THE DOWNSTREAM ABSOLUTE PRESSURE

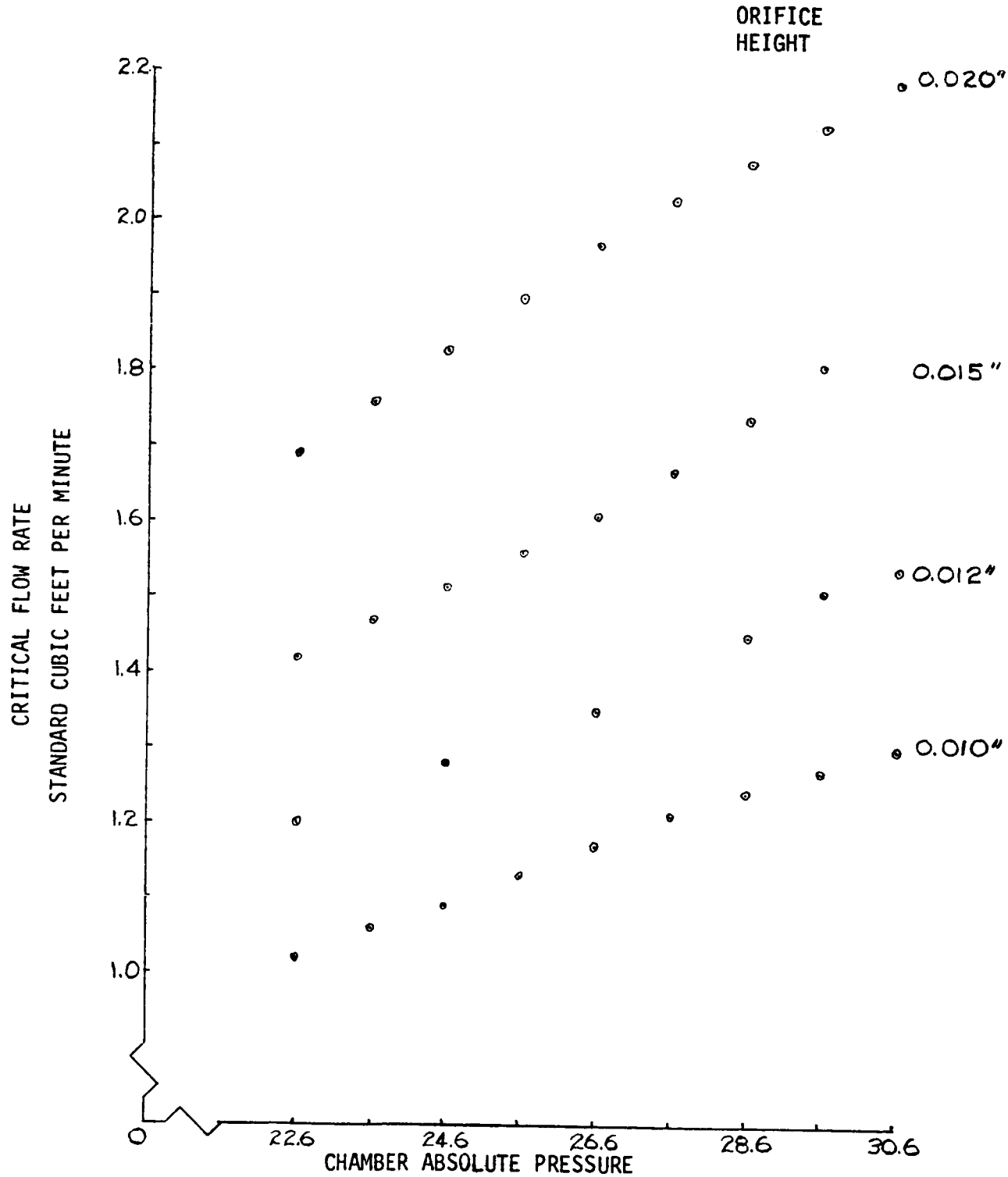


FIGURE 7

VARIATIONS OF CRITICAL FLOW WITH CHANGES IN ATMOSPHERIC PRESSURE FOR DIFFERENT ORIFICE DIMENSIONS

SONIC NOISE EXPERIMENTS

With the vacuum probe's orifice operating in the critical condition the air molecules are accelerated very rapidly from a slowly moving laminar state just outside the tip to a very rapidly moving turbulent state just inside the tip. This rapid transition from a laminar flow state to a turbulent flow state generates some amount of ultrasonic energy. Since this energy release is right at the orifice, there is a distinct possibility that it could have some effect on the dislodging and removing of small particles from a surface. The first step in investigating this problem was to attempt to obtain a frequency spectrum of the relative sound pressure levels produced by the operating orifice. The test setup used for this study is shown in Figure 8. Due to the size of the microphone system and the fact that the probe tip must rest against a surface while operating, the transducer could only be placed as close as 1.6 cm. from the tip-surface interface. The probe used had the dimensions shown in Figure 2, and a critical flow rate of 1.55 standard cubic feet per minute (SCFM) was used.

The test setup shown in Figure 8 was mounted in an acoustically shielded room with a very low background noise level above 135 Hertz. The vacuum pumps used were in another chamber which was well insulated from the test room.

The results of these tests, shown as a bandpass analysis of the vacuum probe's sonic noise, are presented in Figure 9. The abscissa of the graph is graduated with a series of third octave bandpasses, and the ordinate is graduated in decibels (db) with a reference of zero db equal to 2×10^{-4} dynes per square centimeter. Therefore, the ordinate represents a relative sound pressure level (SPL) with the reference $P_0 = 2 \times 10^{-4}$ dynes/cm^2.

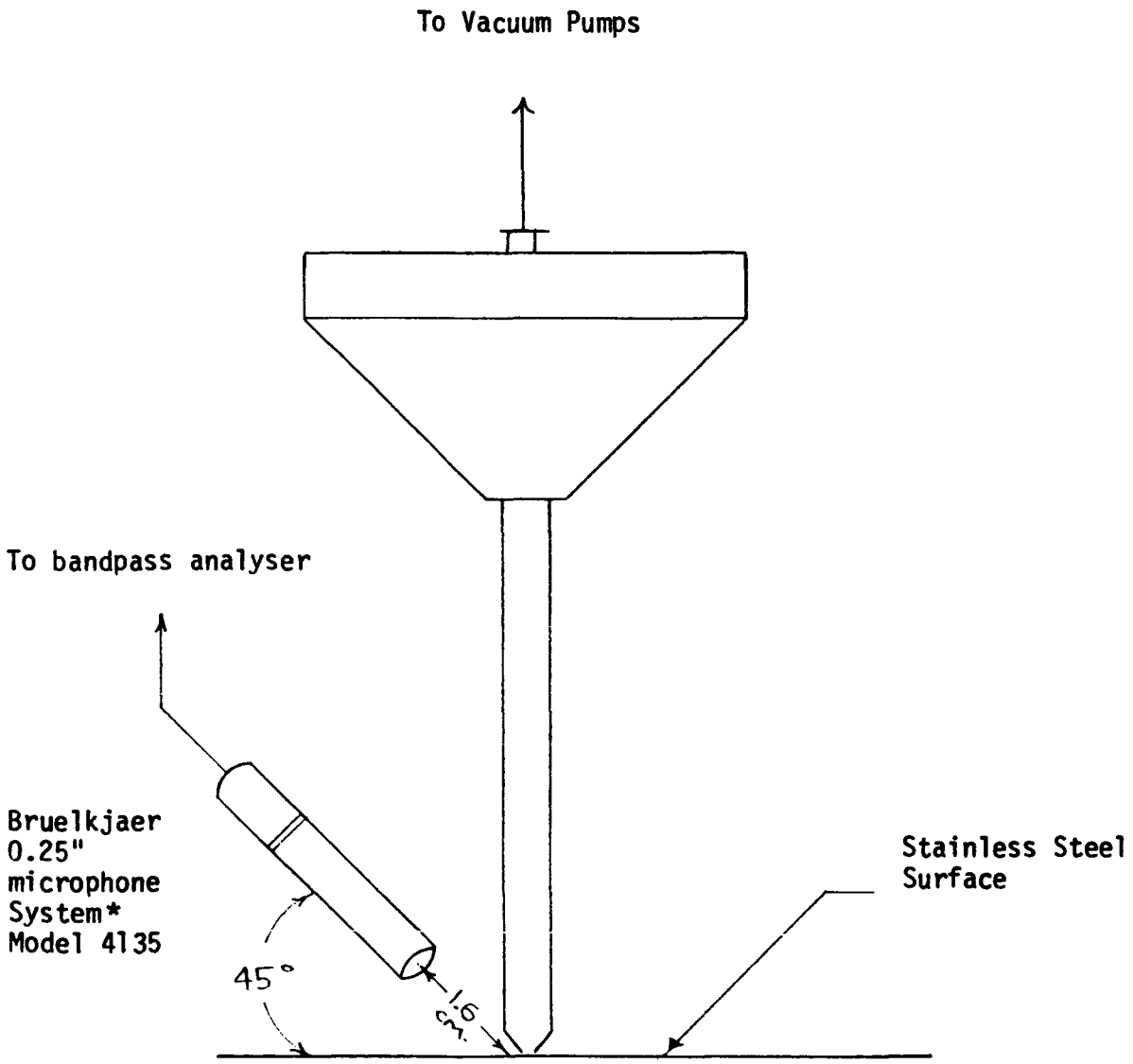


FIGURE 8

TEST SETUP FOR BANDPASS ANALYSIS OF
SONIC NOISE PRODUCED BY VACUUM PROBE

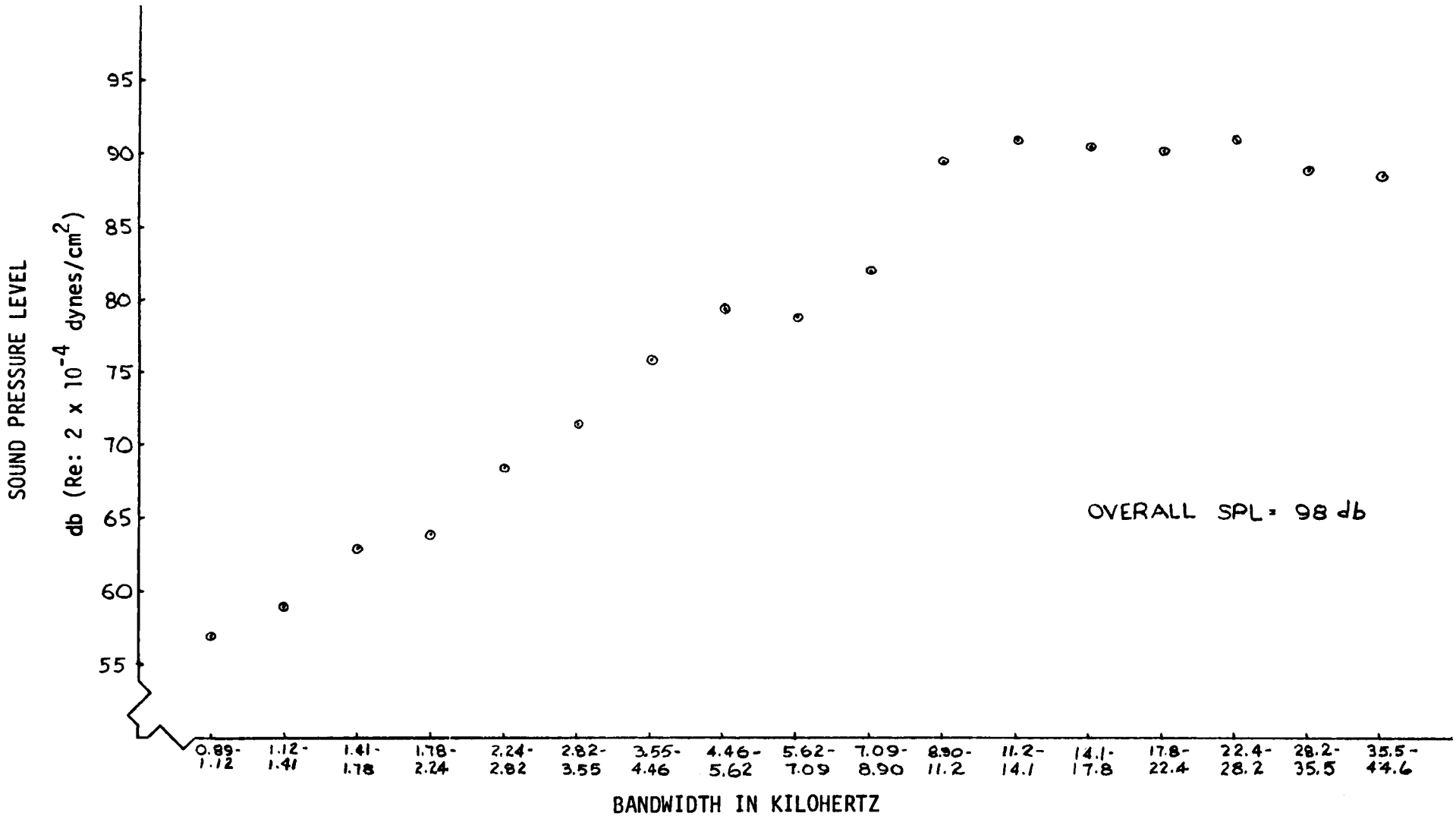


FIGURE 9 - BANDPASS ANALYSIS OF SONIC NOISE MEASURED 1.6 CENTIMETERS FROM PROBE TIP

If one considers the bandpass from 35.5KHz to 44.6KHz a SPL of approximately 88 db is present at the point 1.6 cm from the probe tip. The sound pressure P may be calculated as follows[4]:

$$\beta(db) = 10 \log \frac{I}{I_o} \text{ with } I \text{ in } \frac{ergs}{sec.cm^2}$$

$$I = \frac{P^2}{2\rho c} \text{ where}$$

P is in dynes/cm.^{2} and is the sound pressure

ρ is in gm/cm.^{3}

c is in cm./sec.

$$\alpha(db) = 10 \log \frac{P^2/2\rho c}{P_o^2/2\rho c} = 10 \log \left(\frac{P}{P_o}\right)^2 = 20 \log \frac{P}{P_o}$$

$$88 \text{ db} = 20 \log \frac{P}{2 \times 10^{-4}}$$

$$P = 5.02 \frac{dynes}{cm.^2}$$

To obtain a relative feel for the effect which this sound pressure may have upon a microorganism resting on a surface, several assumptions must be made. First, the microorganism is most probably affected the greatest when the probe tip is very near. If the source of the sonic noise behaves somewhat like a point source, then the level will be proportional to the inverse square of the distance from the point source. For the purpose of this example a distance of 0.2 cm. from the center of the noise source has been selected.

At a distance this close to the source the far field assumption of a point source is probably invalid, but it still provides an estimate. Extrapolating in accordance with this square law to the distance of 0.2 cm., the sound pressure would be:

$$P = \left(\frac{1.6}{0.2}\right)^2 (5.02) = 322 \text{ dynes/cm.}^2$$

Assuming that a bacterial spore is spheroidal with a diameter of one micron (10^{-6} meters), the cross-sectional area A would represent the area on which the pressure wave would be applied and would be:

$$\text{Area} = \pi r^2 = 0.785 \times 10^{-12} \text{ m.}^2 = 0.785 \times 10^{-8} \text{ cm.}^2$$

The force on one bacterium could then be:

$$F_T = P \times A = 322 \frac{\text{dynes}}{\text{cm.}^2} \times 0.785 \times 10^{-8} \text{ cm.}^2 = 2.52 \times 10^{-6} \text{ dynes}$$

Further assuming that the spore is lying on an incremental surface area which is smooth relative to the size of the spore and that the vectorial force is applied at some 45 degree angle to the horizontal surface increment, the resultant forces on the cell are as shown in Figure 10.

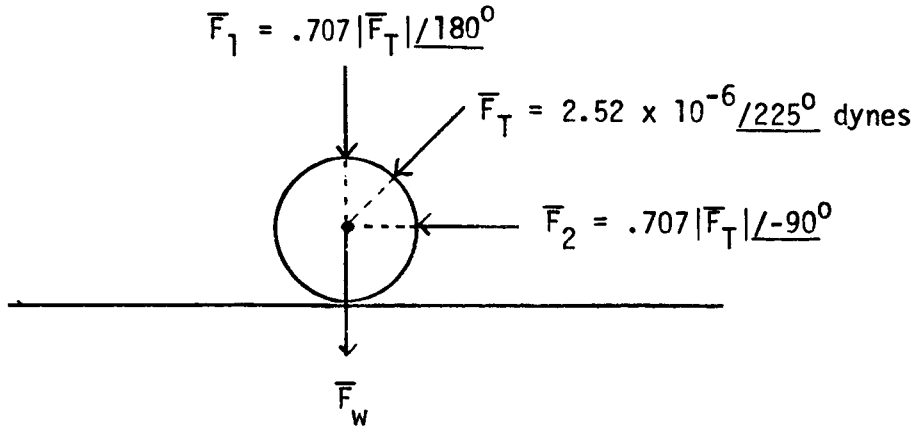


FIGURE 10

The mass of a typical spore is approximately 10^{-12} grams[5]; therefore, the force due to gravity is:

$$|\overline{F}_w| = 10^{-12} \text{ grams} \times 980 \text{ cm/sec.} = 9.8 \times 10^{-10} \text{ dynes}$$

Then,

$$|\overline{F}_o| = |\overline{F}_1| + |\overline{F}_w| = 1.78 \times 10^{-6} \text{ dynes}$$

Allowing a representative coefficient of friction of 0.5, the total restraining force would be

$$|\overline{F}_R| = 0.5 \times 1.78 \times 10^{-6} \text{ dynes} = 0.891 \times 10^{-6} \text{ dynes.}$$

The magnitude of the force F_2 would be

$$|\overline{F}_2| = .707 \ |\overline{F}_T| = 1.78 \times 10^{-6} \text{ dynes,}$$

and the magnitude of the resulting accelerating force would be:

$$|\overline{F}_a| = |\overline{F}_2| - |\overline{F}_R| = 8.91 \times 10^{-7} \text{ dynes}$$

If this accelerating force is approximately constant for one-half the period of a wave with a frequency of 40KHz, the displacement at the end of the half period would be

$$d = \frac{1}{2} a t^2$$

$$= 0.5 \times \frac{8.91 \times 10^{-7}}{10^{-12}} \times 1.56 \times 10^{-10}$$

$$= 6.95 \times 10^{-5} \text{ cm.} = 0.695 \text{ microns}$$

Considering a 10KHz pressure wave which shows a SPL of also very nearly 88 db,

$$d = 0.5 \times \frac{8.91 \times 10^{-7}}{10^{-12}} \times 2.5 \times 10^{-9} = 1.11 \times 10^{-3} \text{ cm.} = 11.1 \text{ microns}$$

These very simplified classical calculations could be in error to either extreme since such things as absorption and reflections of energies have not been considered. However, this elementary routine was presented hopefully to give some insight into the effect that this energy might have on the vacuum probe's removal of microorganisms from a surface. From the calculations presented there is definite evidence that the microorganism could be at least dislodged, and possibly this result is a conservative one since an overall sound pressure level of 98 db was recorded for the entire frequency spectrum.

This could conceivably add an order of magnitude to the displacements calculated, as could other factors such as the angle of the incident energy and the distance of the particle from the probe tip.

CONCLUSION

The material in this report was presented to give the detail drawings of the "filter probe" so that they could be manufactured by other groups having the necessary facilities, to give a suggested protocol for the probe's use, and to present the findings of two experimental procedures.

A study designed to determine the dependence of the vacuum probe critical orifice on operating pressures showed that the saturation point of the airflow through the orifice occurs at a downstream orifice pressure which is approximately one-half the upstream orifice pressure. Also seen was the fact that the orifice critical airflow rate has approximately a linear dependence upon the atmospheric pressure and the orifice height dimension.

A second study designed to evaluate the levels of acoustical energy generated by the operating critical orifice has provided evidence of the possibility of the energy being great enough to dislodge bacteria-sized particles from a smooth surface.

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