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Research Report

AN IMPROVED MODEL OF THE VACUUM PROBE

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ABSTRACT

The need for a microbiological surface sampling device with the capability for sampling large areas that are lightly loaded with microorganisms motivated the development of the vacuum probe. The intended use of the instrument is to sample clean surfaces in laminar flow clean rooms, but the device could be utilized for sampling surfaces in other clean environments. Such a device was designed, fabricated, and tested at Sandia Laboratories, Albuquerque, New Mexico. In these tests the vacuum probe removed a mean of 89% and assayed a mean of 67% of bacterial spores approximately 1 μ in length settled on smooth surfaces which were free of viscous films. Detailed machine and assembly drawings and instructions are included.

Project No. 0064010

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ACKNOWLEDGMENT

We wish to express appreciation to

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AN IMPROVED MODEL OF THE VACUUM PROBE

Introduction

The recovery of microorganisms from surfaces has been studied by microbiologists since the early part of the century. During this period five basic methods have evolved for the microbiological examination of surfaces: the agar overlay method, the agar contact method, the swab-rinse method, the rinse method, and the agar-dip method.[14] Each method has individual advantages and disadvantages, but all were designed for sampling relatively large populations of microorganisms on small to moderately sized surfaces.

The planetary quarantine requirement that space vehicles landing on planets designated as biological preserves be sterilized[8] has imposed a requirement for the sampling of large surface areas with small amounts of microbial contamination.

The settling strip method[12] has been developed and used for estimating the viable contamination deposited on surfaces. With this method sterile stainless steel strips are placed in the same environment as the surface, and after a determined period of environmental exposure, the strips are assayed for microbial contamination. The criticisms of this method are that it is an indirect method and that it is inaccurate when the amount of microbial contamination is small.

The need for a microbiological surface sampling device with the capability for sampling large areas that are lightly loaded with microorganisms motivated the development of the vacuum probe sampler (Figure 1). The intended use of the instrument is to sample surfaces in laminar flow clean rooms.

For evaluation of the instrument a severe case was chosen, i. e., using settled-out aerosols of single Bacillus subtilis var. niger spores. In practice, however, few single spores or bacteria can be expected to settle out onto surfaces from the atmosphere. Nobel, et al[11] indicated that the majority of airborne microorganisms

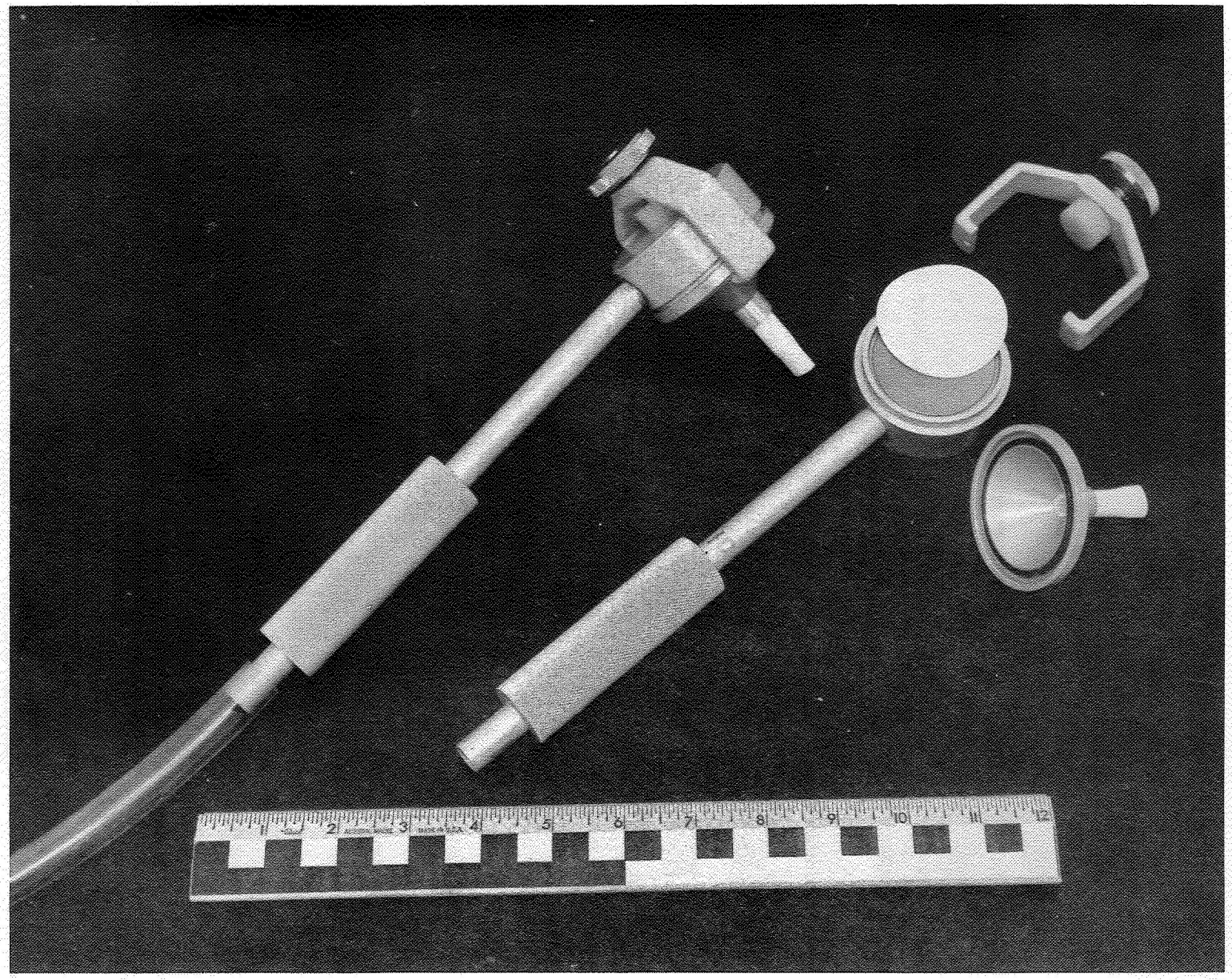


Figure 1. Assembled and Disassembled Vacuum Probes

in laboratory environments are carried on 4-20 μ diameter particles. Since the removal efficiency for particles remains consistently higher for larger particles[3,4], this evaluation was performed under the worst particle size conditions to be expected in practice.

Materials and Methods

Physical Description of Vacuum Probe

The vacuum probe (Figure 2) is an instrument that utilizes airflow through an orifice to remove particles from surfaces and a membrane filter to capture these particles. When the teflon tip (Figure 2 detail) contacts a surface, two orifices--one on the front side of the detail and one on the back--are formed. Since teflon has a low coefficient of friction with most materials, the tip is readily moved back-and-forth across surfaces for sampling. The cone is a machined and anodized aluminum-magnesium alloy casting which serves as a mechanical mount for the tip and holds an "O"-ring to seal the membrane filter to the filter backing screen. A two-inch-diameter membrane filter with a pore size of 0.45 μ or larger must be used or the filter limits the airflow excessively. A filter pore size should be selected, consistent with the particle size to be sampled. The filter backing screen is a circular piece of 100 mesh stainless steel screen clamped between an inner and outer ring. The filter backing screen is attached to the base with an autoclaveable epoxy adhesive (Shell Chemical Company, Epon 914). The base, which is also a machined and anodized aluminum-magnesium alloy casting, contains a plenum large enough to allow an even flow of air through the filter. The clamp holds the cone to the base, providing a rigid assembly and a good pressure seal. The anodized aluminum handle is sealed to the base using the same epoxy adhesive. The hollow handle serves to connect a vacuum system to the base during sampling.

Microbiological Procedures

All experimental procedures were carried out in a Class 100 laminar flow clean room[6]. This particular clean room[2,15] was well suited for such studies because of its size and airflow characteristics. Clean air flowing from one wall directly over the work surface to the floor allowed manipulations of contaminated test surfaces to be accomplished with minimal chance for additional contamination from personnel.

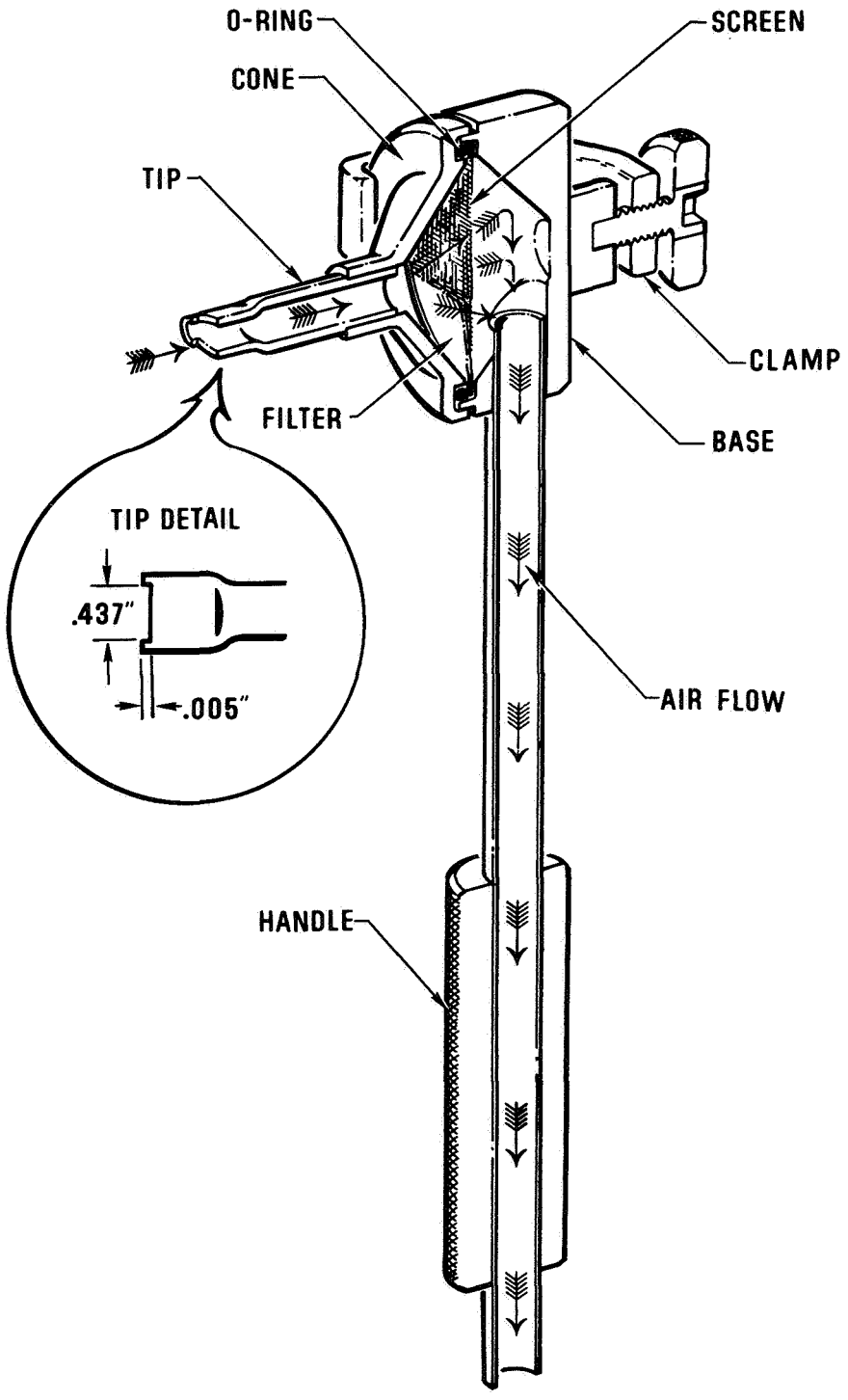


Figure 2. Cross Section of Vacuum Probe Showing Airflow and a Detail View of the Teflon Tip

The clean room was operated at all times, except during aerosolization and settling of particles. Just prior to aerosolization of bacterial spores, the clean room air circulation was turned off and spores were disseminated. After a settling period of 30 minutes, during which time the surfaces were contaminated with the test organisms, airflow was turned on and subsequent operations were performed in a clean air environment.

Bacillus subtilis var. niger spores suspended in 95% ethanol were disseminated with a DeVilbiss No. 40 nebulizer into a mixing fan which provided air circulation throughout the room. The fan was operated for one minute following aerosolization; then the spores were allowed to settle. The spore suspensions used were cleaned by insonation and differential centrifugation and were used in concentrations of 10^4 - 10^6 per ml. By microscopic observation the settled aerosol contained mostly single spores. By calculation, less than 1% of the spore-containing particles contained two spores and a negligible percentage contained more than two spores[7, 10]. For purposes of the experiment, the spores were free of ethanol on contact with the surface because the ethanol evaporated in a very short time compared to the settle-out time of the particles[7].

The vacuum probe was sterilized and dried to remove any moisture. Sterile technique was followed in the installation of the 0.8 μ pore size, two-inch diameter membrane filter. This pore size was suitable for filtering the spores. With the filter in place and the probe clamped together, the assembly was attached to a vacuum source. In normal use, a vacuum of 25 inches of mercury operating at a flow rate of approximately one cubic foot per minute was maintained. The probe tip was then placed in contact with and perpendicular to the surface to be sampled. A cross-hatched pattern was traced over the surface with the probe tip while maintaining the tip perpendicular to the sample surface. The crosshatched pattern was traced through twice, thus covering the sample surface two times in each direction. The teflon tip wears slowly and should be checked periodically. The rate of wear depends mainly on the force applied during sampling.

The probe was tested for efficiency of removal of spores from four different types of surfaces:

1. Glass — glass dishes 4" x 8-1/2" with a surface roughness height of 2.0 μ inches.

2. Plastic — 20 mm x 150 mm disposable petri dishes with a surface roughness height of 5.5 μ inches. A 4-inch square at the center of the plate was sampled.

3. Aluminum — 4" x 8-1/2" plates with a surface roughness height of 14.5 μ inches.

4. Stainless Steel — 4" x 8-1/2" plates with a surface roughness height of 15.0 μ inches.

Except for plastic, half of each surface was vacuumed with the other half serving as a control. Plastic dishes were vacuumed while adjacent dishes served as controls.

Surface contamination was assayed by overlaying the surface with sterile Trypticase Soy Agar (TSA;BBL), incubating at 32°C for 48-72 hr., and counting the colonies. To determine if the agar overlay procedure would move particles on the test surfaces, the sampled half of each surface was first crosshatched with molten TSA using a Cornwall syringe. After the TSA in the crosshatched portion had solidified, the entire surface was overlaid with more TSA. This crosshatching procedure prevented the possibility of washing viable particles from the sampled half to the control half of the test surface or vice versa. However, overlaying directly with agar had no noticeable effect on the spore distribution.

The metal surfaces were placed in sterile pans for vacuuming and for assay. After these pans were overlaid with TSA, they were left uncovered within the unoccupied, operating laminar flow clean room for approximately one hour to allow the surface of the TSA to dry. The pans were then covered with Saran Wrap and incubated at 32°C for 48-72 hr.

The cone and membrane filter were removed using sterile techniques. Spores collected on the membrane filter were assayed by overlaying the filter with TSA in a sterile petri dish, incubating at 32°C for 48-72 hr and counting the colonies developed.

According to N. L. Peterson's suggestion (U. S. P. H. S., N. C. D. C., Phoenix Field Station, Phoenix, Arizona; personal communication) the entire probe tip and cone were insonated. Insonation was performed for eight minutes in sterile 0.1% Tween 80, and the insonated fluid plated with TSA and incubated at 32°C for 48-72 hr. Later experiments, performed by vacuuming 5 μ fluorescent particles and examining the interior of the vacuum probe with ultraviolet light, showed considerable particle

deposition on the inside surface of the cone and on the teflon tip. These experiments showed that the cone and tip would have to be insonated to raise total assay percentages.

In testing for percent assay as a function of percent of critical flow (Figure 3), an aerosol of 3×10^5 spores was settled on aluminum surfaces. The flow rate of air was varied with a flow meter.

Results

Table I shows sampling efficiencies on four different types of surfaces exposed to different levels of contamination. Particle removal efficiencies were consistently in excess of 80%, with a mean removal efficiency of 89%. Removal efficiencies appear to be independent of surface contamination densities studied. Total assay efficiencies were much more variable, but achieved a mean of 67% with a mean loss of 26% of those spores removed. Table I, as well as the fluorescent particle studies, shows that there was considerable deposition of particles on the inside surface of the cone and on the tip.

Figure 3 shows the decrease of total assay efficiency when the vacuum probe was operated with subcritical airflow rates at the tip.

Discussion

Since we were sampling from surfaces on which the loading density varied between test surface and control surface, assays of over 100% sometimes occurred. For the same reason we encountered removal efficiencies of greater than 100%; consequently, percentages of over 100% should not be interpreted as incorrect data, but are within the bounds of statistical variation.

Spores or other micron-sized particles on a smooth surface are difficult to disturb because the boundary layer of air near the surface is difficult to move and the small particles lie in the lower regions of this boundary. The force of adhesion between small particles and smooth surfaces is large compared to the normal aerodynamic forces encountered.[3] The air entering the orifice when the tip touches a surface disturbs this boundary layer. The spores are dislodged and enter the moving airstream. The spores are then caught by a membrane filter.

TABLE I

Vacuum Probe Sampling Efficiencies
(Bacillus subtilis var. niger Spores)

Surface	Total Number of Spores Aerosolized	Unvacuumed Half	Vacuumed Half	Insonated Filter	Insonated Cone	% Removal[a]	Assay % Total[b]	Assay % Filter[c]	Assay % Cone[d]
Aluminum	3×10^5	89	5	51	8	94	66	57	9
		129	5	104	32	96	105	81	24
		149	13	76	27	91	69	51	18
		138	3	121	27	98	107	88	19
	Avg	126	6	88	24	95	89	70	19
	3×10^6	1550	76	430	368	95	52	28	24
		886	88	428	414	90	95	48	47
		1500	123	600	298	92	60	40	20
		1776	91	964	575	95	87	54	33
	Avg	1428	94	605	414	93	71	42	29
Stain. Steel	3×10^5	117	12	73	14	90	74	62	12
		142	26	30	84	82	80	21	59
		140	18	57	86	87	102	41	61
		150	16	86	34	89	80	57	23
	Avg	137	18	69	54	87	90	50	40
Stain. Steel	3×10^6	1400	154	393	207	89	43	28	15
		1340	120	262	161	91	32	20	12
		1600	145	516	414	91	58	32	26
		808	100	250	460	88	88	31	57
	Avg	1287	130	355	311	90	52	28	24
Plastic	3×10^4	17	5	7	9	71	94	41	53
		40	4	5	7	90	30	12	18
		34	5	2	9	85	32	6	26
		28	5	6	16	82	79	22	57
	Avg	30	5	5	10	84	50	17	33
Glass	3×10^6	828	130	245	138	84	46	30	16
		1010	95	189	104	91	29	19	10
		800	155	231	161	81	49	29	20
	Avg	879	127	222	134	86	41	25	16

a - % Removal = $\frac{\text{number on unvacuumed half of surface} - \text{number on vacuumed half of surface}}{\text{number on unvacuumed half of surface}} \times 100\%$

b - % Total = $\frac{\text{number on filter + number on cone}}{\text{number on unvacuumed half of surface}} \times 100\%$

c - % Filter = $\frac{\text{number on filter}}{\text{number on unvacuumed half of surface}} \times 100\%$

d - % Cone = $\frac{\text{number on cone and tip}}{\text{number on unvacuumed half of surface}} \times 100\%$

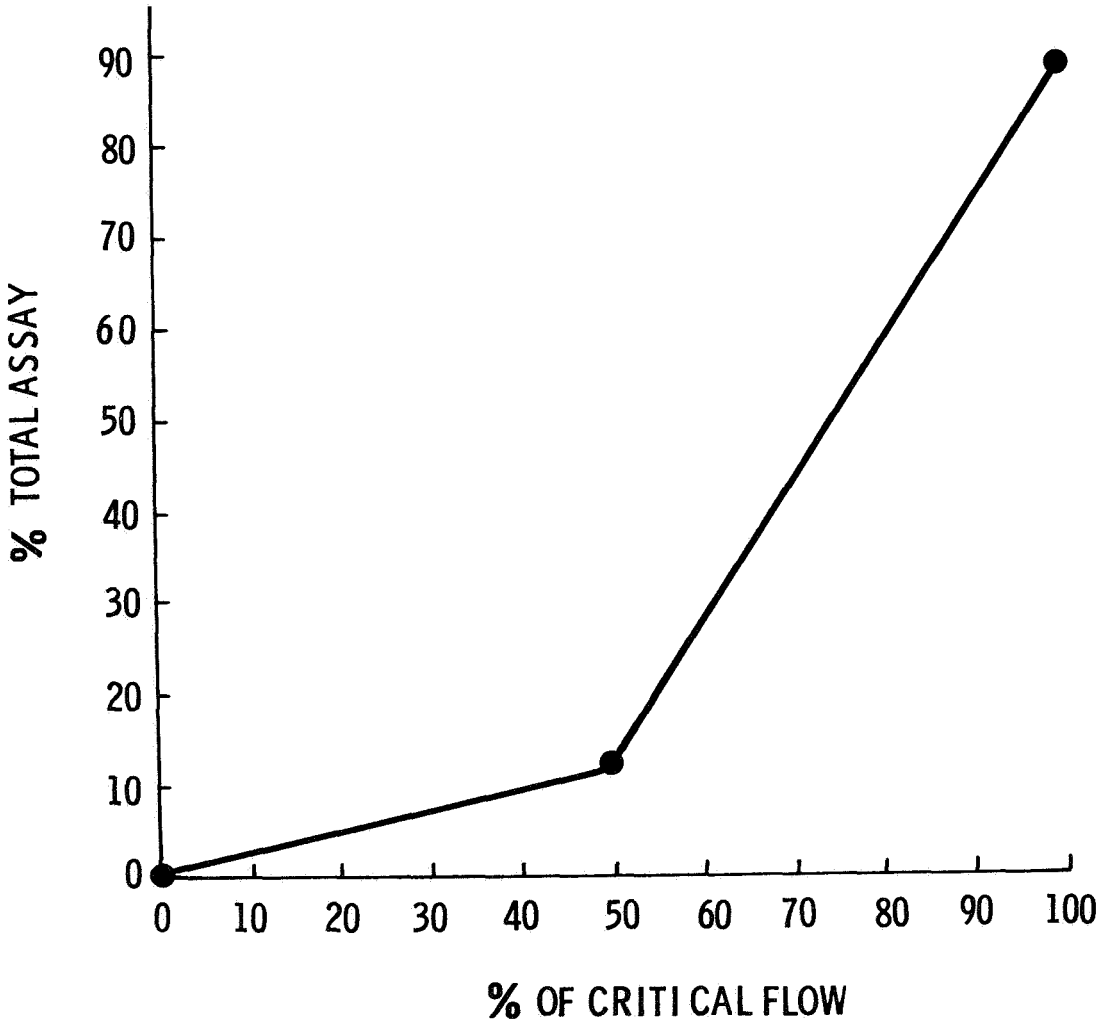


Figure 3. Effect of Airflow Rate Through Vacuum Probe on % Total Assay of Spores

A number of factors affect the removal and assay percentages in removing spores from different surfaces.

The most important criterion for high percentage removal and assay is that the two orifices in operation be at a critical flow rate. At critical flow, a shock wave is generated at the orifices. The air at the shock wave is very turbulent and tends to remove small particles effectively. The shock wave is generated if the ratio of the pressure inside the cone to atmospheric pressure is less than or equal to 0.528.[13] All calibrations should be made with the filter in place. These values can be measured directly with a mercury manometer using a cone with a pressure fitting in the side. Figure 3 shows that critical flow must be reached to achieve high removal and assay percentages.

One anticipated problem was that particles were being overlaid with teflon from the tip during the sampling procedure. Studies with fluorescent powders 5 μ in diameter indicated that very few particles were overlaid with teflon. The fluorescent particles were placed on an aluminum surface and vacuumed under ultraviolet light. Traces of the powder showed where the teflon tip had passed, but the quantity was very small compared to the original loading. To further support these results, the experiment was repeated with a probe using a metal tip to eliminate teflon overlay. Removal percentages did not change significantly.

Another factor affecting removal percentages is particle size. Experimentation showed that the larger particles were easier to remove, so Bacillus subtilis var. niger spores approximately 1 μ in length were selected as the most difficult particle to remove.[3,4]

At the present time we have done very little work using spores overlaid with thin films such as oily handprints. Limited experimentation suggested that these particles were more difficult to remove.[3,4]

Electrostatic charges affected some experiments, especially those on glass and plastic surfaces, but the effects were not measured quantitatively. In some instances, very strong fields--strong enough to push the membrane filter from the bottom to the top of the petri dish in which it was laying before overlay--were generated on the filters and petri dishes. In general, aerosols generated by blasting particles with air have a fairly high density charge distribution;[9] therefore, part of the variation in assay percentages is possibly a result of interacting electrostatic forces along with aerodynamic forces. The electrostatic forces may explain the lower assay percentages on plastic.

Three other effects of interest are relative humidity, surface roughness, and the natural adhesiveness between particular pairs of materials. Although all of this work was done at 45% relative humidity, Corn[3] reported that particle adhesiveness generally increases as humidity increases. The effect of surface roughness was poorly defined in our experiments; however, Corn[3] indicated that adhesion decreases as surface roughness increases. The surface roughness heights[1] of the surfaces used are given. Adhesive forces vary considerably for different pairs of materials.[3]

We have modified the vacuum probe to provide a clean air sheath so that the device can be used to sample surfaces in environments other than those provided by

laminar flow rooms. We are also developing a method to impinge the particles directly onto agar nutrient rather than onto a filter which is then plated in agar nutrient.

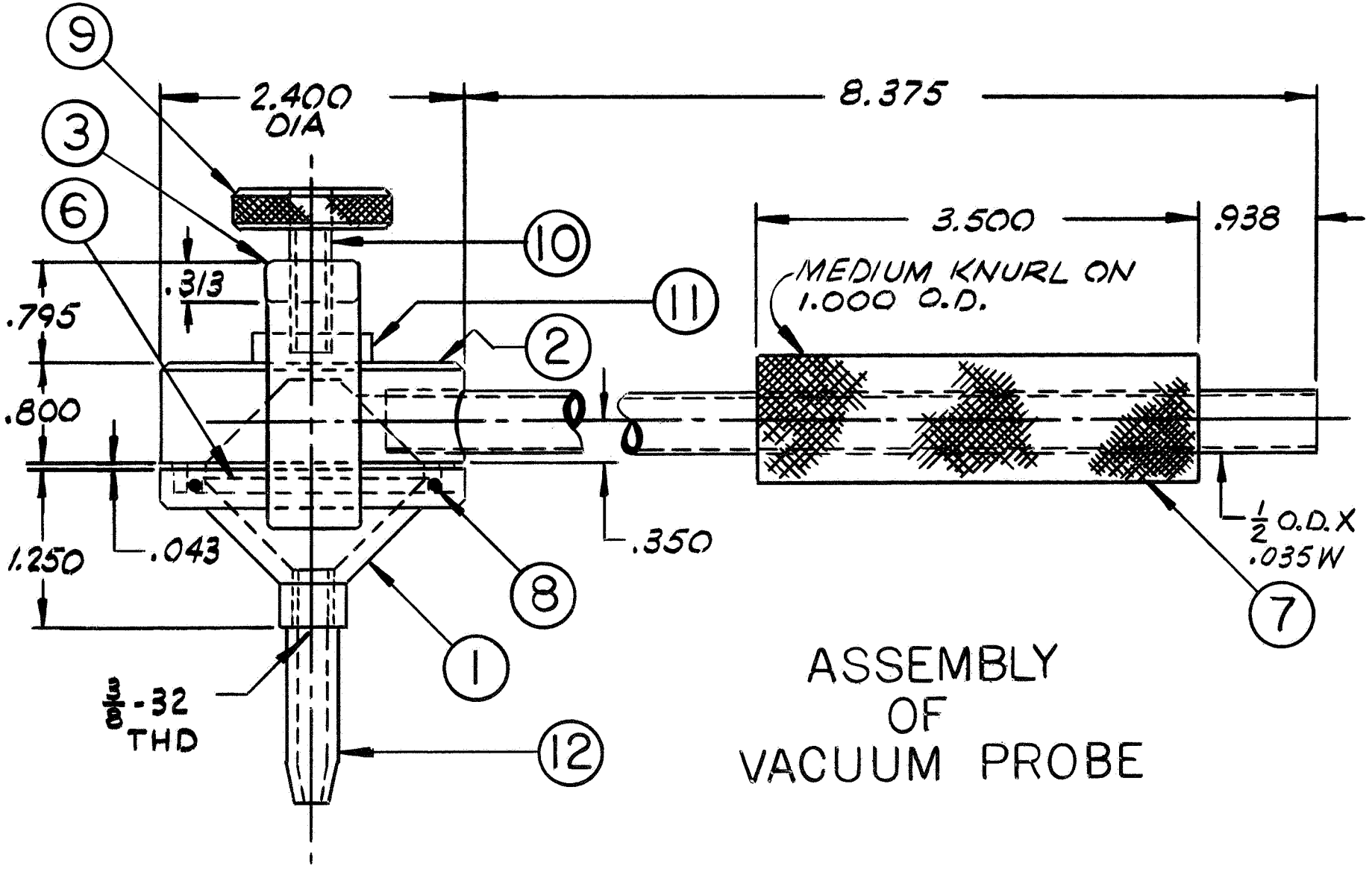
Machine and Assembly Drawings and Instructions

The teflon tip was machined from commercially available teflon tubing with nominal dimensions of 0.400 inch outside diameter by 0.214 inch inside diameter. After machining the threads and tubing to length 0.400 inch of the teflon was placed in a vise and clamped flat. The tip was then placed in a cone, used as a machining jig. The slot in the tip was machined using a 7/16-inch bit in an end mill. A first pass with the tool was made to cut the tip flat, and a second pass was made to cut the slot.

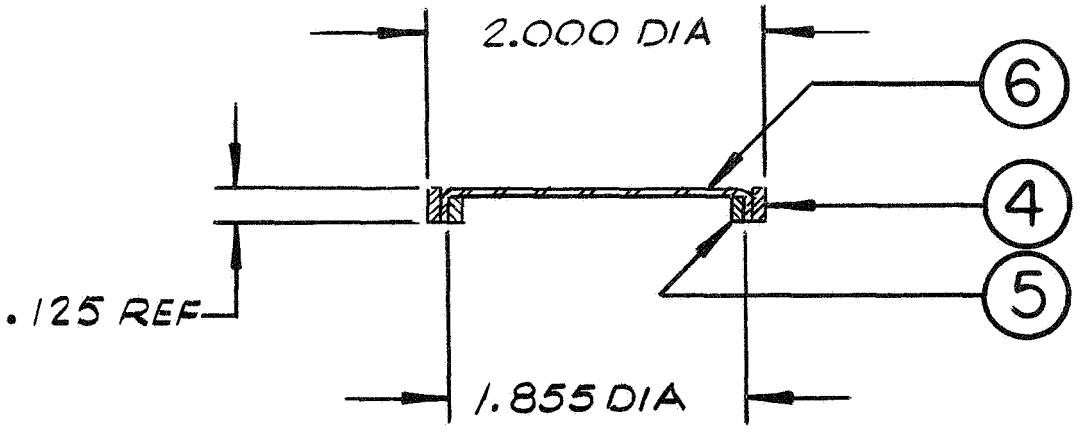
The inner and outer screen ring and the screen were assembled with a press.

An epoxy glue (Shell Chemical Company, Epon 914) was used to attach both the screen assembly and the handle to the base. The epoxy was cured at 350°F for two hours.

Item and assembly drawings follow.



ASSEMBLY
OF
VACUUM PROBE



SCREEN ASSEMBLY

NO REQD	DESIGN AGENCY NUMBER	DESCRIPTION	NOTE	SHEET ZONE	ITEM
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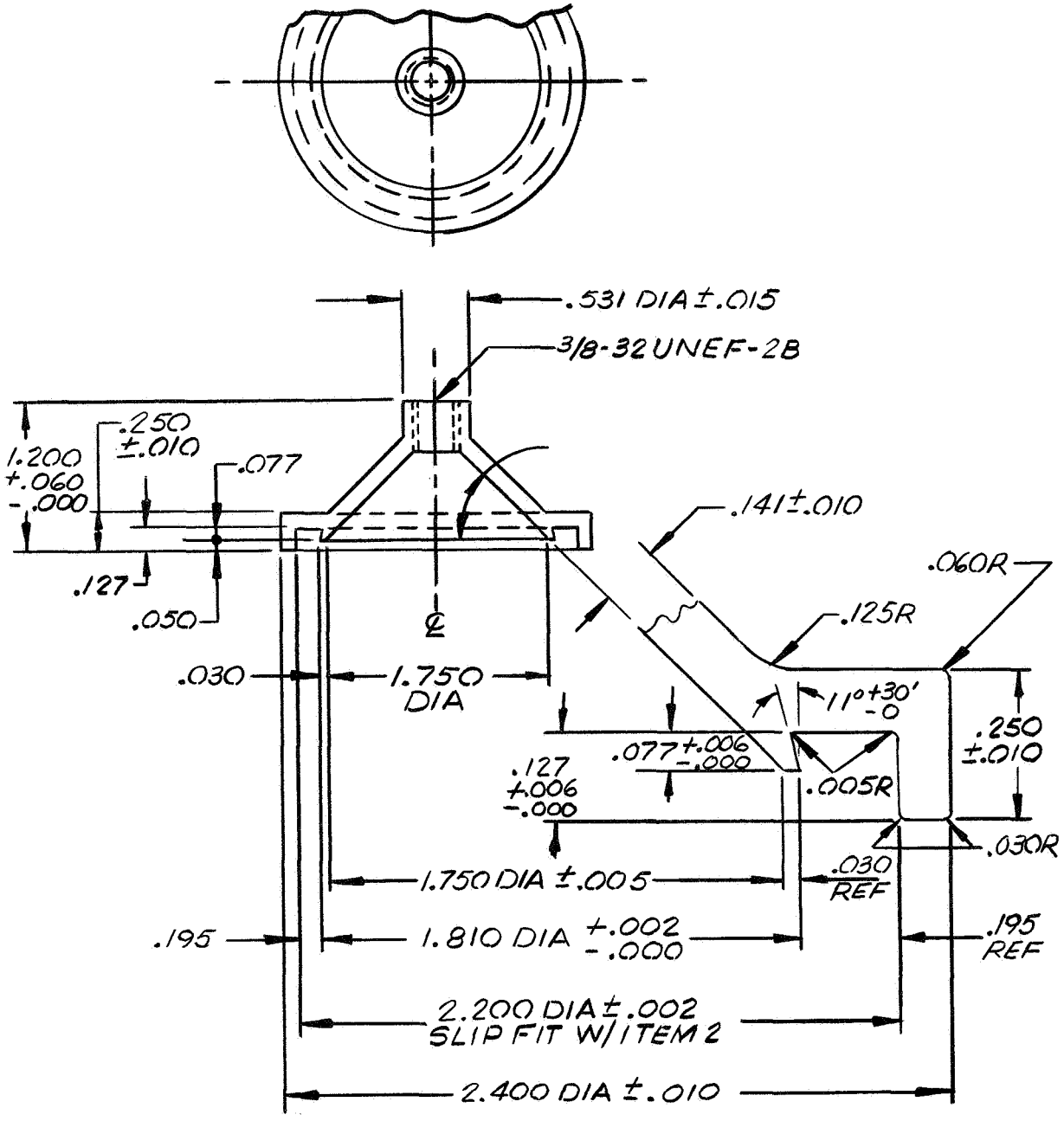
1		BASE			2
1		CLAMP			3
1		OUTER SCREEN RING			4
1		INNER SCREEN RING			5
1		SCREEN			6
1		HANDLE			7
1		O-RING, PARKER #2-133, BUNA-N, 70 DUROMETER			8
1		THUMBWHEEL			9
1		THUMBSCREW			10
1		NYLON PAD			11
1		TEFLON TIP			12

X DENOTES DOCUMENTS
AR DENOTES AS REQD

LIST OF MATERIAL



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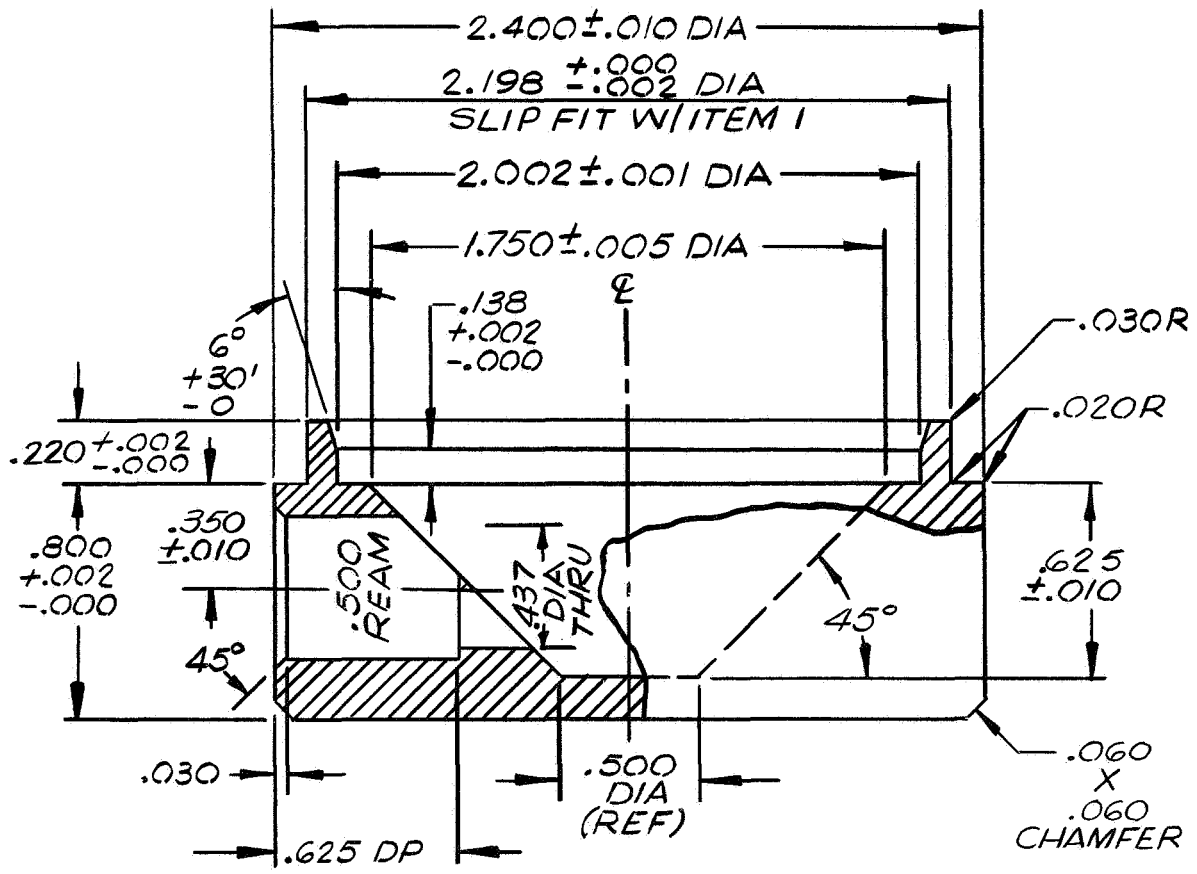

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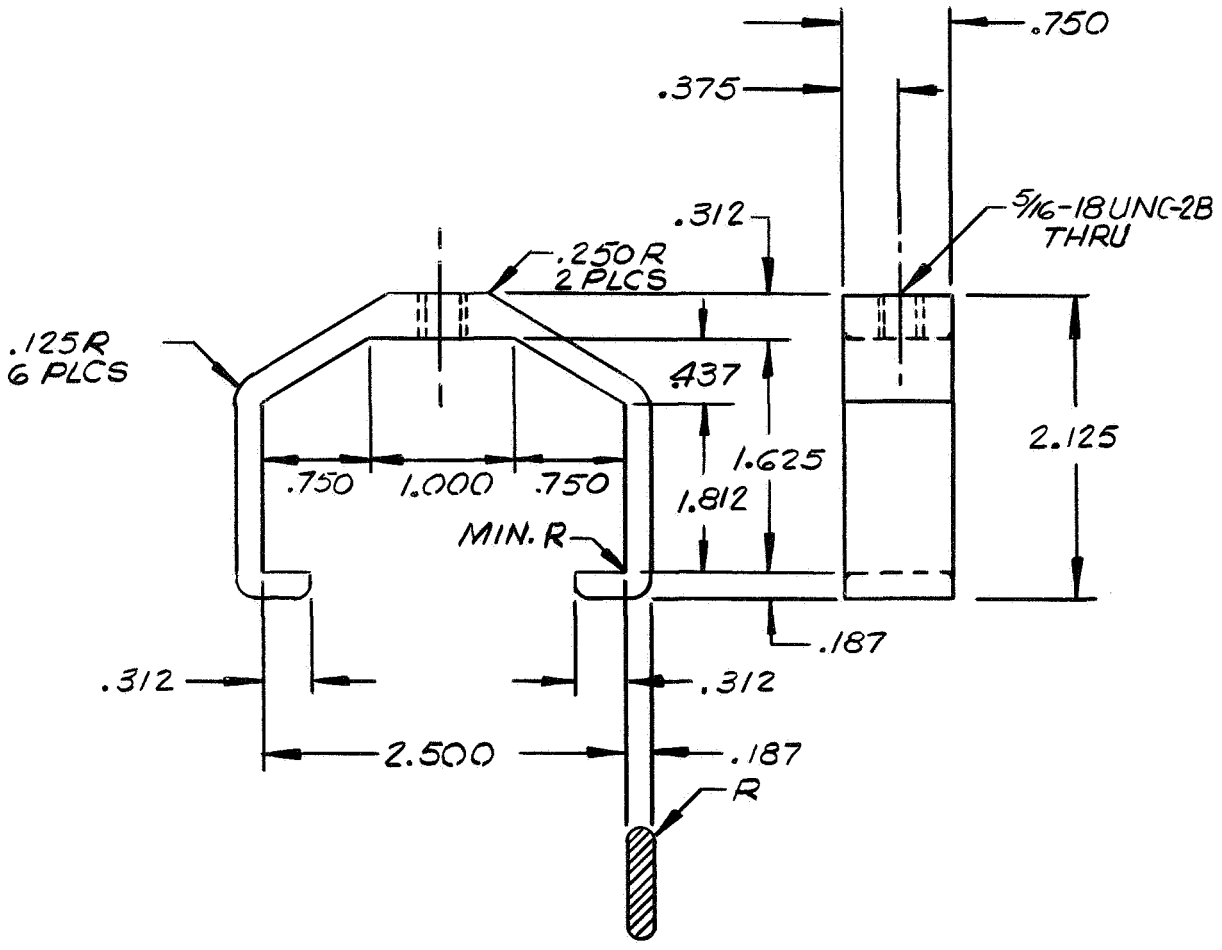

MATERIAL: ALMAG CASTING

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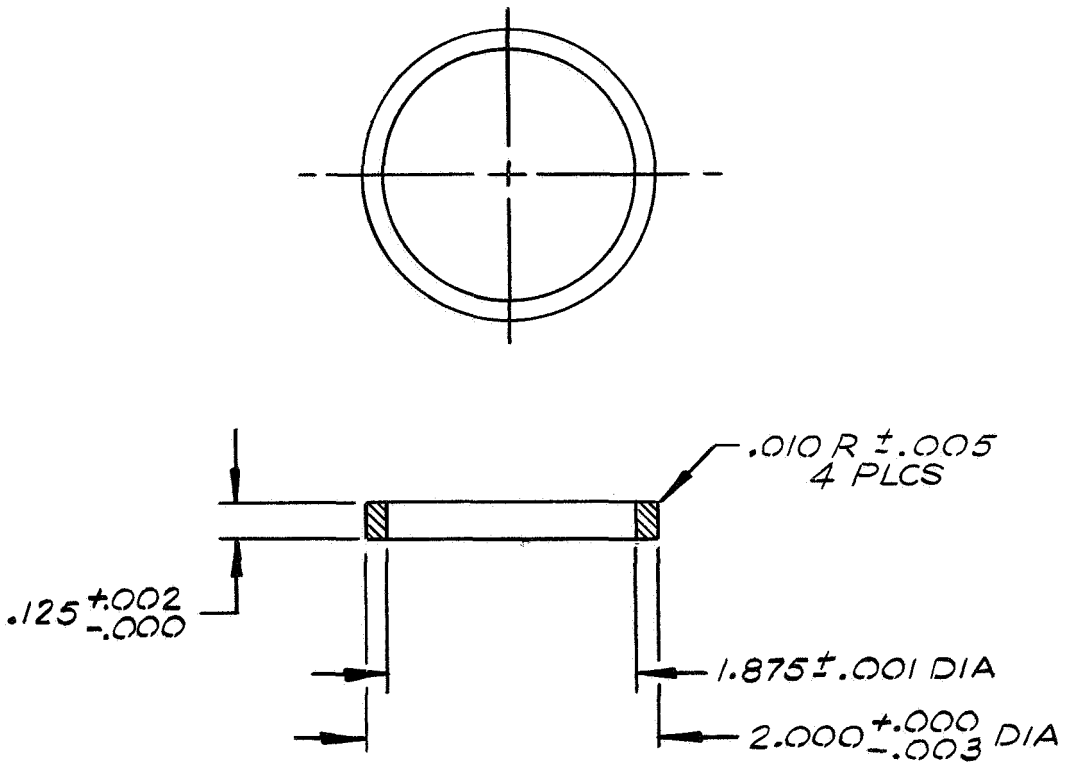

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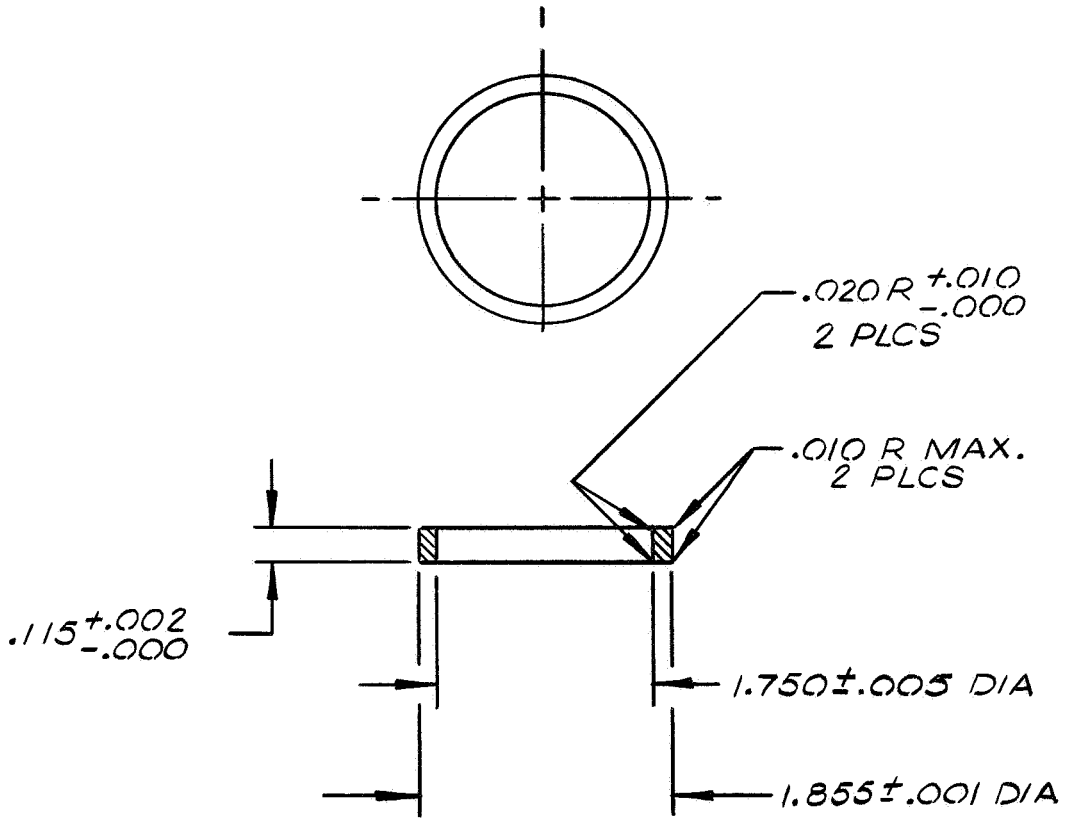

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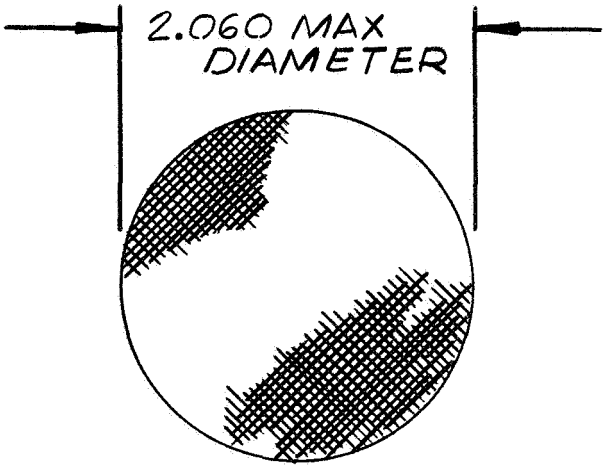
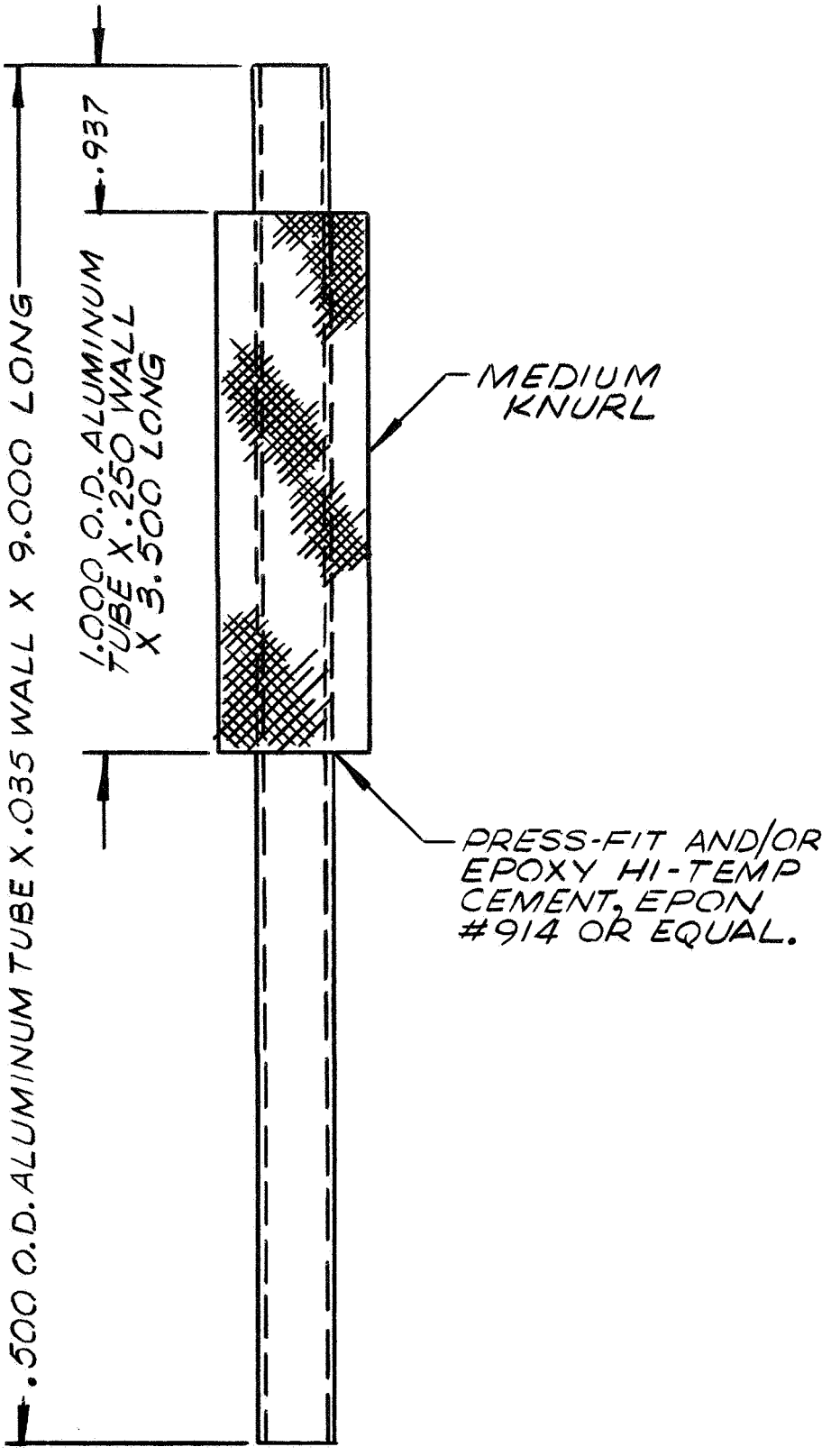


MATERIAL: 100 MESH STAINLESS STEEL SCREEN

ITEM 6


.937
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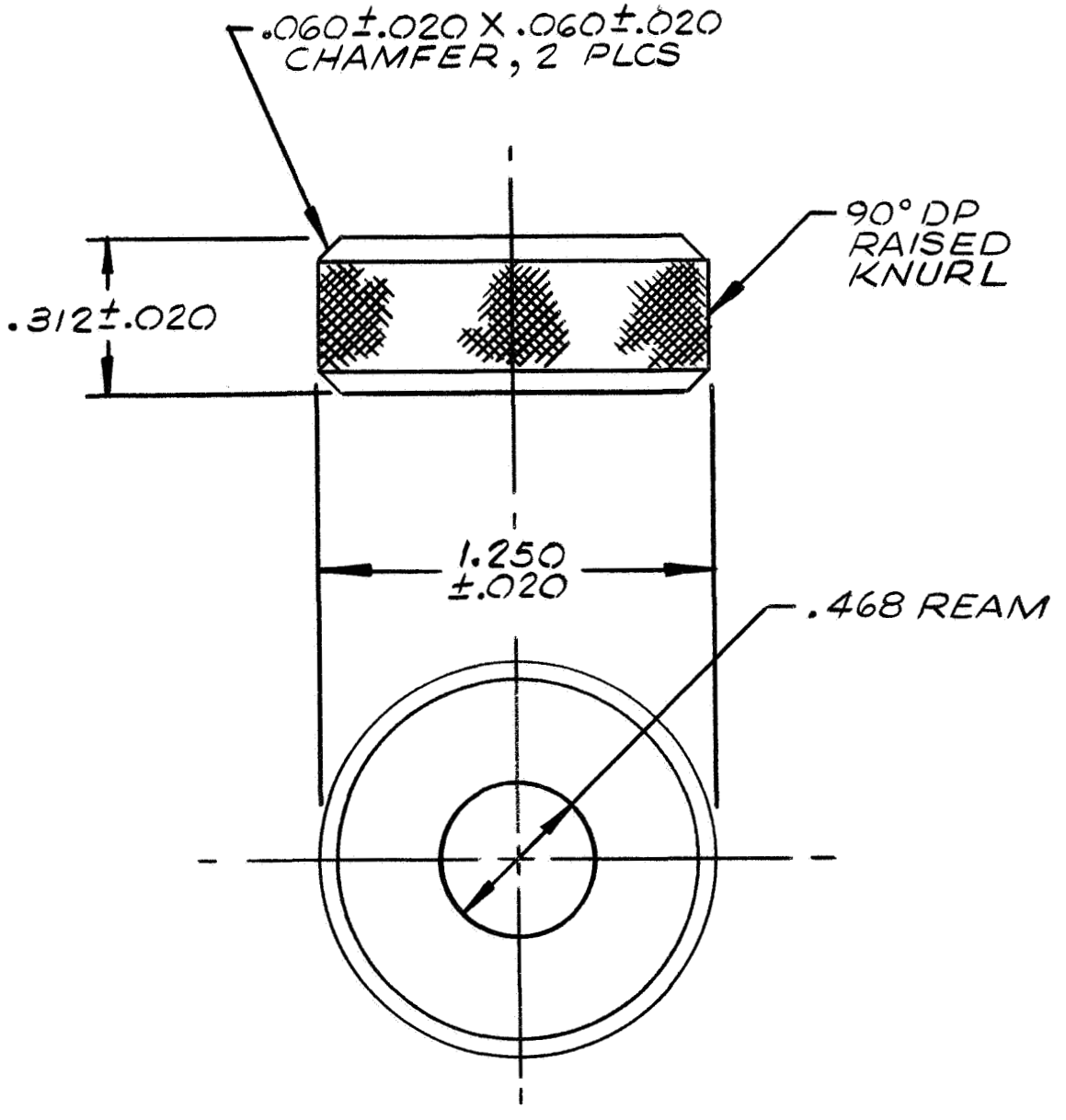
.500 O.D. ALUMINUM TUBE X .035 WALL X 9.000 LONG
MEDIUM KNURL
PRESS-FIT AND/OR EPOXY HI-TEMP CEMENT, EPON #914 OR EQUAL.

ITEM 7



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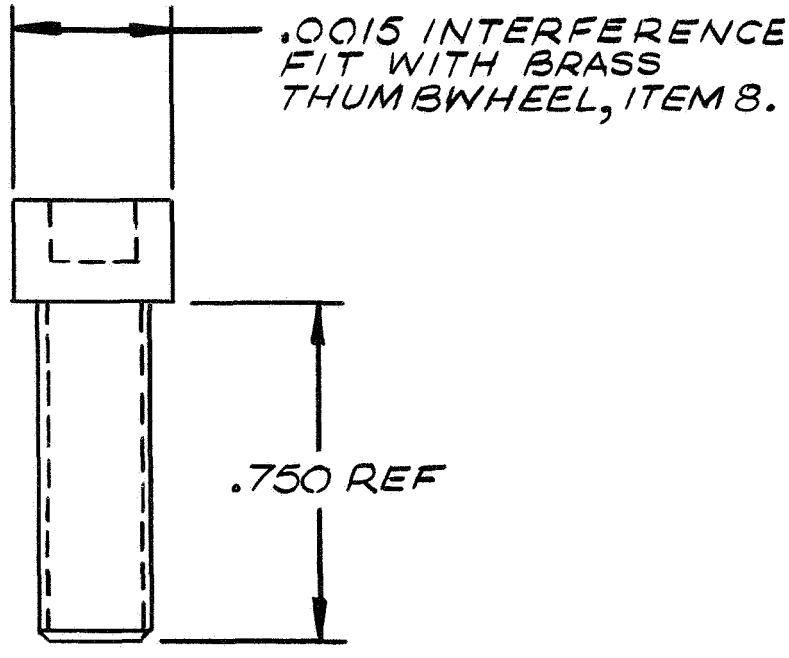
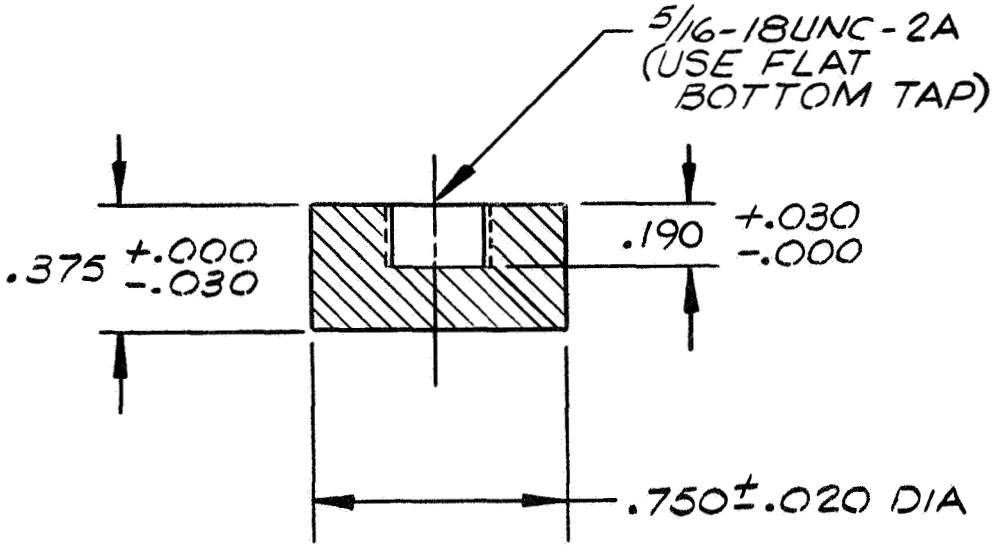


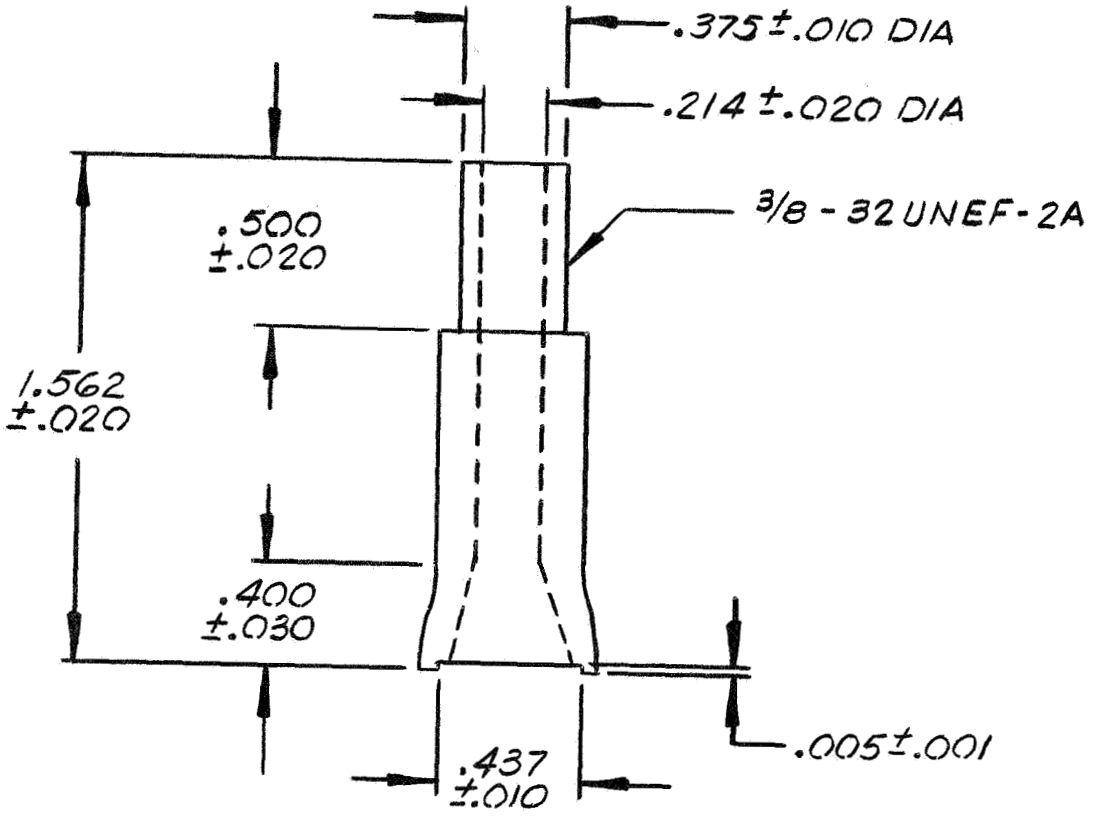
MATERIAL: SOCKET HD CAP SCREW, 5/16-18 UNC X 3/4 LG.

ITEM 10



MATERIAL: NYLON ROD

ITEM 11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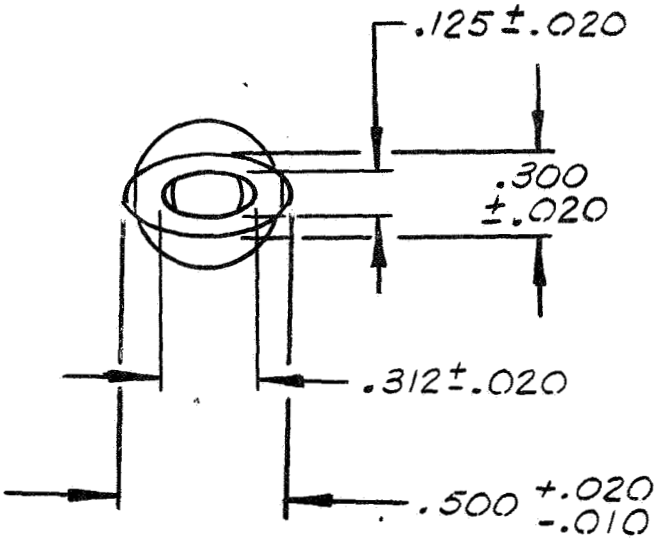
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ITEM 12

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