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DEVELOPMENT OF DESIGN PARAMETERS AND CONCEPTUAL DRAWING  
FOR A PLASMA ETCHER TO CLEAN AND STERILIZE  
SURGICAL INSTRUMENTS

FINAL, PHASE I REPORT

ROBERT W. BARR

FEBRUARY 15, 1989

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Anatech Ltd.  
5510 Vine Street  
Alexandria, Virginia 22310

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# INDEX

|                                                                | PAGE |
|----------------------------------------------------------------|------|
| Section I<br>Project Objective                                 | 1    |
| Section II<br>Progress August 15, 1988 to September 15, 1988   | 2    |
| Section III<br>Progress September 15, 1988 to October 15, 1988 | 4    |
| Section IV<br>Progress October 15, 1988 to November 15, 1988   | 8    |
| Section V<br>Progress November 15, 1988 to December 15, 1988   | 16   |
| Section VI<br>Progress December 15, 1988 to January 15, 1989   | 23   |
| Section VII<br>Summary August 15, 1988 to February 15, 1989    | 29   |
| Section VIII<br>Design Parameters                              | 36   |
| Section IX<br>Gantt Chart                                      | 39   |

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## Section I - Project Objective

The proposed process will use accelerated ions to remove organic matter by physically desorbing molecular fragments and by reacting chemically to produce volatile, non-toxic gases such as CO<sub>2</sub>. Studies with related equipment have demonstrated feasibility. Phase I research is to determine the optimal size of the process chamber and how surgical instruments can most effectively be arranged in it; the optimal air pressure inside the chamber; the required power density; and evaluate alternative process generated by direct current, alternating current and radio frequency. The result of Phase I will be a set of design parameters and a conceptual drawing of the proposed etcher.

## Section II - Progress August 15, 1988 to September 15, 1988

### A. Use of Plasmas Produced by Direct Current

During this period most of our effort has been focused on evaluating the use of plasma which was produced by a direct current power source. Studies have been carried out with a modified Polaron E5100 sputter coater which produces DC ion plasmas. Our experiments with this technology show that DC-based ion plasmas can be successfully employed as the basis for operation of the plasmaclave. Such plasmas are able to clean and sterilize in remote and inaccessible regions of hemostats and scissors. A wide variety of bacteria and bacterial endospores are killed and removed from stainless steel surfaces under conditions where instruments are not significantly heated. Cleaning and sterilizing can be accomplished after 5 to 10 minutes of exposure to the ion plasma and 10 to 20 average sized surgical tools can be treated at once. A fully functional instrument could be sturdy and light enough in weight to be contained in a suitcase and carried by one individual.

It is usual practice that surgical tools are individually wrapped for the cleaning and sterilizing process. After sterilization, such wrapped instruments could be removed from the plasmaclave chamber and stored in a sterile condition until they are needed. In our recent work on the plasmaclave we have attempted to design it so that wrapped instruments can be cleaned and sterilized.

### B. Studies with Wrapped Instruments: Use of RF Ion Plasmas

Studies with wrapped instruments satisfied us that ion plasmas produced by radio frequency power sources were superior to those produced by DC. Plasma occurred beneath the wrappings with RF power sources, but did not occur with DC. Most of our studies with wrapped instruments have, therefore, been carried out with RF discharges. Further experiments will be performed using a modified IG50 plasma source and DC power supply.

Experiments have been performed with the Anatech PA-200 plasma etcher and with a Harrick PDC3XG plasma cleaner. Tests of a particular cleaning and sterilization regimen were carried out in two stages.

1. First, a felt tipped pen (Sharpie) was used to mark on aluminum foil or on glass objects. These are then wrapped and exposed to the RF ion plasma. If ink was removed satisfactorily under the test conditions, then we proceeded to the second stage of testing.
2. In the second stage, stainless steel tweezers, scissors and scalpels were deliberately contaminated with bacteria, wrapped and exposed to the test plasma. The efficiency of sterilization was then tested by unwrapping the instruments and incubating them in bacteriological media. Growth of bacteria was considered as evidence that sterilization by the plasma was not effective. Two

species of bacteria were employed for each test. They were: (1) a sporulating culture of *Bacillus cereus* and (2) *Staphylococcus aureus*.

During the present reporting period we have carried out tests with several potential wrapping materials. Below is a list of the materials tested and a comment about the effectiveness of each.

1. Paper - not satisfactory. The RF ion plasma erodes and weakens paper to an unacceptable extent.
2. Cloth - not satisfactory. Cloth is weakened to an unacceptable extent by the RF ion plasma.
3. Aluminum foil - satisfactory, but the time required to sterilize foil-wrapped instruments is increased significantly.
4. Glass - stainless steel instruments are contained in screw-capped glass tubes. Satisfactory plasma is effective inside the glass tube and the glass container is not adversely affected by the ion plasma
5. Plastic (polycarbonate) - not satisfactory. Plasma takes place inside plastic wrapping, but plastic becomes opaque and brittle during the etching process. Other kinds of plastic wrappings should be tested.

Studies have been carried out to determine the time required to clean and sterilize contaminated tweezers contained in glass tubes. These tests were performed at the maximum power setting (30W-50W) in Harrick PDC3XG plasma cleaner. The results are given below.

Time of Etching

Tweezers Sterile

|        |     |
|--------|-----|
| 0      | No  |
| 1 hr.  | No  |
| 2 hr.  | No  |
| 8 hr.  | Yes |
| 16 hr. | Yes |

### Section III - Progress September 15, 1988 to October 15, 1988

During the past month most of our effort has been devoted to studying cleaning and sterilizing by direct current ion plasmas. Experiments have been performed with the modified Polaron E5100 sputter coater, and they have been aimed at studying two parameters, (1) the power density required for killing bacterial endospores and (2) the time required to kill vegetative bacterial cells. The two studies are described separately below.

#### Power Density.

Studies of the power density have been carried out with spores of *Bacillus cereus*. A Suspension of spores in water was used to coat circular (0.5 cm diameter) discs of aluminum foil. Spores were dried on the discs which were then exposed to an ion plasma at constant air pressure (100 mTorr), but different current (2 mA, 10 mA, and 20 mA). The foil discs were etched on both sides. After etching, the discs were introduced into 2 ml of bacterial medium (L.broth) and allowed to incubate overnight at 37 degrees C. Tubes (containing foil disc and medium) were scored either as having bacterial growth or no growth. Samples of representative tubes showing growth were also examined microscopically to verify that the growth was *Bacillus cereus*. Tubes were expected to contain no bacterial growth only if all spores had been removed from the foil disc by the etching process.

Table 1 shows the results of our experiments carried out at 100 mTorr. They indicate that the etching time required to sterilize foil discs was not particularly sensitive to the power density over the range tested (2 mA to 20 mA). Ten minutes of etching was found to be sufficient to sterilize foil discs at all currents tested. Five minutes was sufficient in many cases, but one minute of etching never produced sterilization in any case tested. Qualitative tests (by feel) of representative discs showed that they were not noticeably warmed after 10 minutes of exposure to the ion plasma at any current examined.

#### Time Required to Kill Vegetative Bacterial Cells.

Tests were carried out to measure the rate at which vegetative *Bacillus subtilis* cells were killed in the type of ion plasma we expect to use in the plasmaclave. Plasmas were produced in the Polaron E5100 operated at 10 mA and an air pressure of 100 mTorr. Experiments were performed with live *B. subtilis* cells that were spread on 5cm x 5cm squares of aluminum or stainless steel foil. Foil squares containing dried cells were exposed to the ion plasma for various periods of time and then rinsed thoroughly in 5ml of sterile L.broth. An aliquot (0.1 ml) of the L.broth was then spread evenly on a bacteriological plate of L agar (agar containing L.broth) and incubated overnight at 37 degrees C. Bacterial colonies present on the agar plate were then counted. The counts provided a measure of the number of viable bacteria remaining on foil squares after etching. The aliquot size (0.1 ml) was chosen so that 200-300 colonies would grow out from the rinse of unetched foil squares.

The results, as shown in Figure 1, demonstrated that vegetative cells were killed with a half-time of approximately 12 seconds. No viable cells were detected after 120 seconds of exposure to the ion plasma. Neither aluminum foil nor stainless steel foil squares were found (by feel) to be detectably heated after 120 seconds of exposure to the ion plasma. It is most unlikely, therefore, that sterilization was produced by heating.



**Table 1: Use of Ion Plasmas to Sterilize Aluminum Foil Discs Coated with Bacillus cereus Spores - Power Density Requirement**

**Test 1: 100 mTorr, 20 mA**

| Etching<br>Time (min.) | Bacterial Growth in Experiment No. |   |   |   |   |
|------------------------|------------------------------------|---|---|---|---|
|                        | 1                                  | 2 | 3 | 4 | 5 |
| 0                      | +                                  | + | + | + | + |
| 1                      | +                                  | + | + | + | + |
| 5                      | -                                  | + | - | - | - |
| 10                     | -                                  | - | - | - | + |

**Test 2: 100 mTorr, 10 mA**

| Etching<br>Time (min.) | Bacterial Growth in Experiment No. |   |   |   |   |
|------------------------|------------------------------------|---|---|---|---|
|                        | 1                                  | 2 | 3 | 4 | 5 |
| 0                      | +                                  | + | + | + | + |
| 1                      | +                                  | - | + | + | + |
| 5                      | +                                  | - | + | - | + |
| 10                     | -                                  | - | - | - | - |

**Test 3: 100 mTorr, 2 mA**

| Etching<br>Time (min.) | Bacterial Growth in Experiment No. |   |   |
|------------------------|------------------------------------|---|---|
|                        | 1                                  | 2 | 3 |
| 0                      | +                                  | + | + |
| 5                      | +                                  | - | - |
| 10                     | -                                  | - | - |
| 30                     | -                                  | - | - |

**Experimental Conditions:** 0.5 cm diameter aluminum foil discs coated with Bacillus cereus endospores were exposed to an ion plasma for the indicated times. The ion plasma was produced between two circular aluminum electrodes (14 cm in diameter) 6 cm apart at an air pressure of 100 mTorr. After exposure to the ion plasma, foil discs were incubated overnight in 2 ml L broth at 37°C. Tubes containing L broth and foil disc were scored "+" if they had bacterial growth and "-" if they did not.

# Figure 1

Etching Conditions:

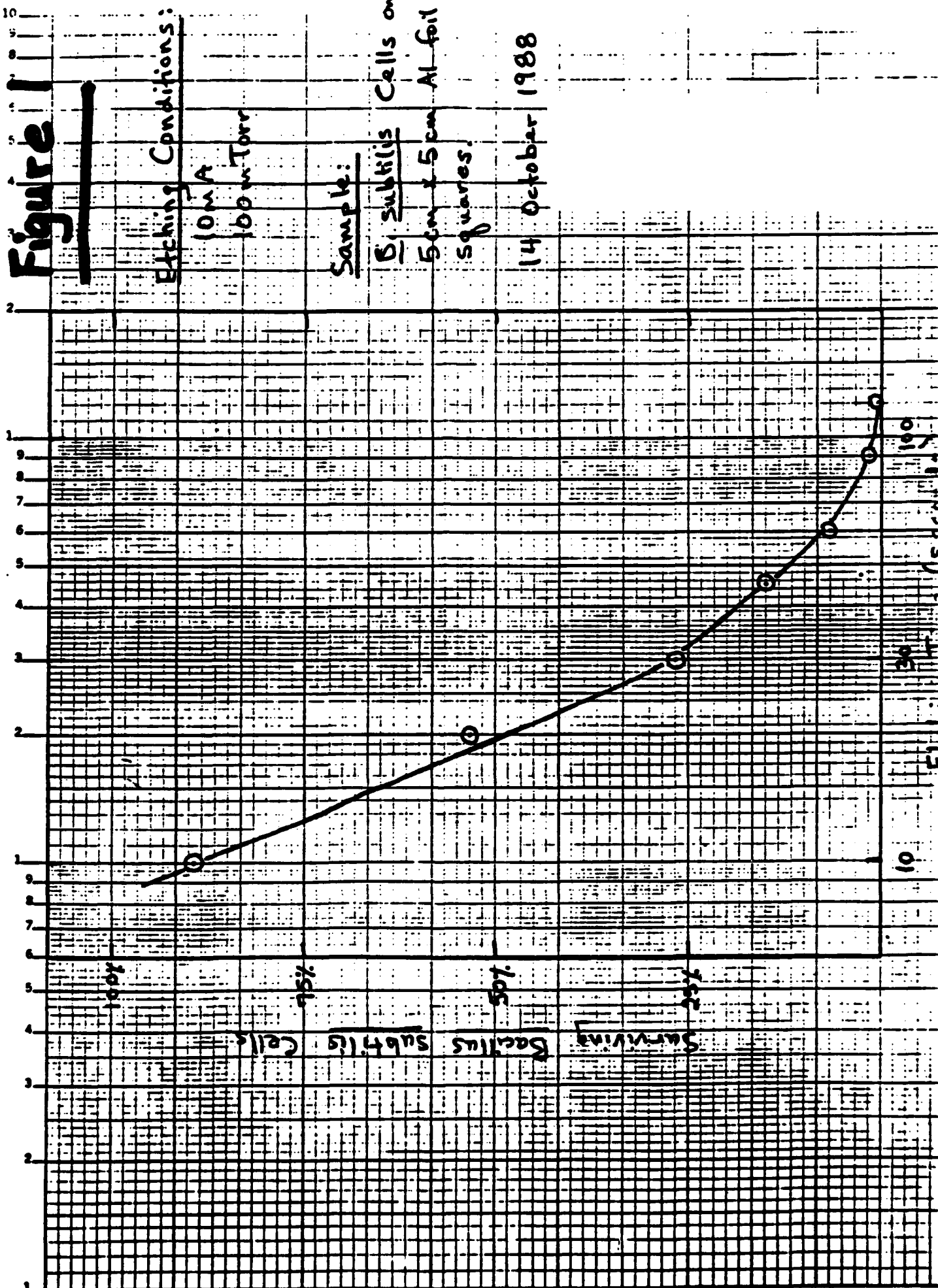
10 mA

100 m Torr

Sample:

*B. subtilis* Cells on  
5 cm x 5 cm Al foil  
squares.

14 October 1988



#### Section IV - Progress October 15, 1988 to November 15, 1988

Studies during the current reporting period were devoted to a thorough analysis of cleaning and sterilization by a radio frequency (RF; 13.56MHz) glow discharge. The specific goals were to evaluate the effects of: (1) power density, (2) air pressure and (3) orientation of the specimen in the etching chamber.

All experiments (except as noted in Table 1) were carried out in the Anatech PA-200 radio frequency plasma cleaner in which the total power delivered can be adjusted continuously from 20W to 160W. Target materials were prepared by spreading a turbid suspension of *Bacillus subtilis* spores more or less evenly on 2cm x 2cm squares of aluminum foil. After air drying these were subject to glow discharges as required for the specific experiment as described below. Squares were then removed from the etching chamber in a sterile manner and transferred to tubes containing 2ml of sterile Luria broth. (L.broth). A standard bacteriological medium known to support the growth of *Bacillus subtilis*.) Tubes were then incubated at 37 degrees for 30 hrs - 36 hrs and observed visually and microscopically for the presence of bacterial growth. Growth of *B. subtilis* was considered as evidence that the experimental treatment had failed to sterilize the foil square.

- (a) **Power Density:** Tests of the power density requirement were performed with glow discharges maintained at 30 watts, 100 watts and 160 watts. The results, as shown in Table 1, indicate that sterilization was more rapid at 100 watts than at 30 watts and more rapid at 160 watts than 100 watts. For example, all targets etched for 5 min or 10 min at 100 watts or 160 watts were sterilized while they were not at 30 watts. More targets were sterilized after 2 minutes of etching at 160 than at 100 watts. The results are shown graphically in Figure 1. Together they suggest that the power density and etching time are very much related. The same degree of sterilization can be accomplished by a short etching period at a high power or by a longer etching time at lower power. In the future, therefore, it might be most useful to employ a parameter such as watt minutes or kW minutes to describe the degree of etching administered.
- (b) **Air Pressure:** The effect of air pressure on sterilization rate was examined over the range of 0.2 to 2.0 Torr. All experiments were performed at 160 watts in the Anatech PA-200 instrument. The results are shown in Table 2 and Figures 2 and 3. They demonstrate that the rate of spore killing was maximal at around 1 Torr and less at both higher and lower air pressures. This effect is particularly clear if one examines the percent of targets sterilized after two minutes in the plasma at various air pressures (Figure 2). This result was quite different from what we expected. We supposed that the higher the gas pressure the higher would be the number of accelerated ions and other atomic species present, and therefore the higher would be the observed rate of killing. The experimental results suggest that there is some particularly active atomic or molecular species (e.g. ozone, O<sub>2</sub> or OH radical) whose concentration in the plasma is highest at

the air pressure found optimal for killing.

A significant rate of killing is observed at air pressures both above and below the optimal one. In fact, if one increases the time of exposure to the ion plasma, then any desired level of sterilization can be obtained at non-optimal air pressures. This can be seen clearly by comparing the results shown in Figure 2 (2 minutes etching) with Figure 3 (5 minutes of etching).

- (c) Specimen Orientation: Since all surfaces of surgical instruments will need to be cleaned and sterilized, it was important for us to determine whether orientation in the etching chamber can affect the rate at which surface erosion takes place. At the outset, RF discharges were expected to be well-suited to fulfill this requirement because RF plasmas are not highly directional in character. All surfaces accessible to gas molecules were expected to be cleaned in RF discharges.

Tests of specimen orientation involved comparing the rate at which foil squares were sterilized when the spore-containing side was facing up with the rate observed with spores facing down. Experiments were performed in the Anatech PA-200 operated at 160W and 1.5 Torr air pressure. The results, as shown in Table 3, show no significant difference in sterilization rate due to specimen orientation. All foil squares were sterilized after 5 minutes or more of exposure to the ion plasma in both specimen orientations.

**Table 1: Effect of Power Density on the Ability of Ion Plasmas to Sterilize *Bacillus subtilis* Spores.\***

**(a) Etching Conditions: 30 Watts; 1 Torr air pressure (Harrick PDC - 3<sup>G</sup>)**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |    |
|-----------------------|------------------------------------|---|---|---|---|----|
|                       | 1                                  | 2 | 3 | 4 | 5 | 6  |
| 0                     | +                                  | + | + | + | + | +  |
| 2                     | +                                  | + | - | + | + | ND |
| 5                     | -                                  | + | - | + | + | +  |
| 10                    | -                                  | - | + | - | - | -  |

**(b) Etching Conditions: 100 Watts; 1 Torr Air pressure (Anatech PA - 200)**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 | 6 |
| 0                     | +                                  | + | + | + | + | + |
| 2                     | +                                  | - | - | + | - | - |
| 5                     | -                                  | - | - | - | - | - |
| 10                    | -                                  | - | - | - | - | - |

**(c) Etching Conditions: 160 Watts; 1 Torr air pressure (Anatech PA - 200)**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 | 6 |
| 0                     | +                                  | + | + | + | + | + |
| 2                     | +                                  | - | - | - | - | - |
| 5                     | -                                  | - | - | - | - | - |
| 10                    | -                                  | - | - | - | - | - |

\* All experiments were performed with *B subtilis* spores which were spread on 2cm x 2cm squares of aluminum foil. After exposure to the ion plasma as described in the table, foil squares were incubated overnight in 2ml Luria Broth at 37°C. Tubes containing broth and foil squares were scored "+" if they had bacterial growth and "-" if they did not.

ND = not determined

**Table 2: Effect of air pressure on the ability of Ion Plasmas to Sterilize *Bacillus subtilis* Spores \***

**(a) Etching Conditions: 160 Watts; 200m Torr air pressure**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 | 6 |
| 0                     | +                                  | + | + | + | + | + |
| 2                     | +                                  | + | + | + | + | + |
| 5                     | +                                  | + | - | + | - | - |
| 10                    | -                                  | + | - | - | - | - |

**(b) Etching Conditions: 160 Watts; 500m Torr air pressure**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 |
| 0                     | +                                  | + | + | + | + |
| 2                     | -                                  | - | - | + | + |
| 5                     | -                                  | - | - | - | - |
| 10                    | -                                  | - | - | - | - |

**(c) Etching Conditions: 160 Watts; 1 Torr air pressure**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 |
| 0                     | +                                  | + | + | + | + |
| 2                     | -                                  | - | - | - | - |
| 5                     | -                                  | - | - | - | - |
| 10                    | -                                  | - | - | - | - |

**(d) Etching Conditions: 160 Watts; 1 Torr air Pressure**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 | 6 |
| 0                     | +                                  | + | + | + | + | + |
| 2                     | -                                  | - | - | - | + | - |
| 5                     | -                                  | - | - | - | - | - |
| 10                    | -                                  | - | - | - | - | - |

**(e) Etching Conditions: 160 Watts; 2.0 Torr air pressure**

| Etching<br>Time (min) | Bacterial Growth in Experiment No. |   |   |   |   |
|-----------------------|------------------------------------|---|---|---|---|
|                       | 1                                  | 2 | 3 | 4 | 5 |
| 0                     | +                                  | + | + | + | + |
| 2                     | +                                  | + | + | - | + |
| 5                     | -                                  | - | - | - | - |
| 10                    | -                                  | - | - | - | - |

\* Experiments were carried out as described in Table 1. All experiments were performed in the Anatech PA-200 radio frequency (13.56 MHz) plasma cleaner.

**Table 3: Effect of Target Orientation on Plasma Sterilization of *Bacillus subtilis* spores.\***

**(a) Etching Conditions: Foil square facing up.  
160 Watts; 1.5m Torr air pressure**

| <u>Etching<br/>Time (min)</u> | <u>Bacterial Growth in Experiment No.</u> |          |          |          |          |
|-------------------------------|-------------------------------------------|----------|----------|----------|----------|
|                               | <u>1</u>                                  | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> |
| 0                             | +                                         | +        | +        | +        | +        |
| 2                             | +                                         | +        | -        | -        | ND       |
| 5                             | -                                         | -        | -        | -        | -        |
| 10                            | -                                         | -        | -        | -        | -        |

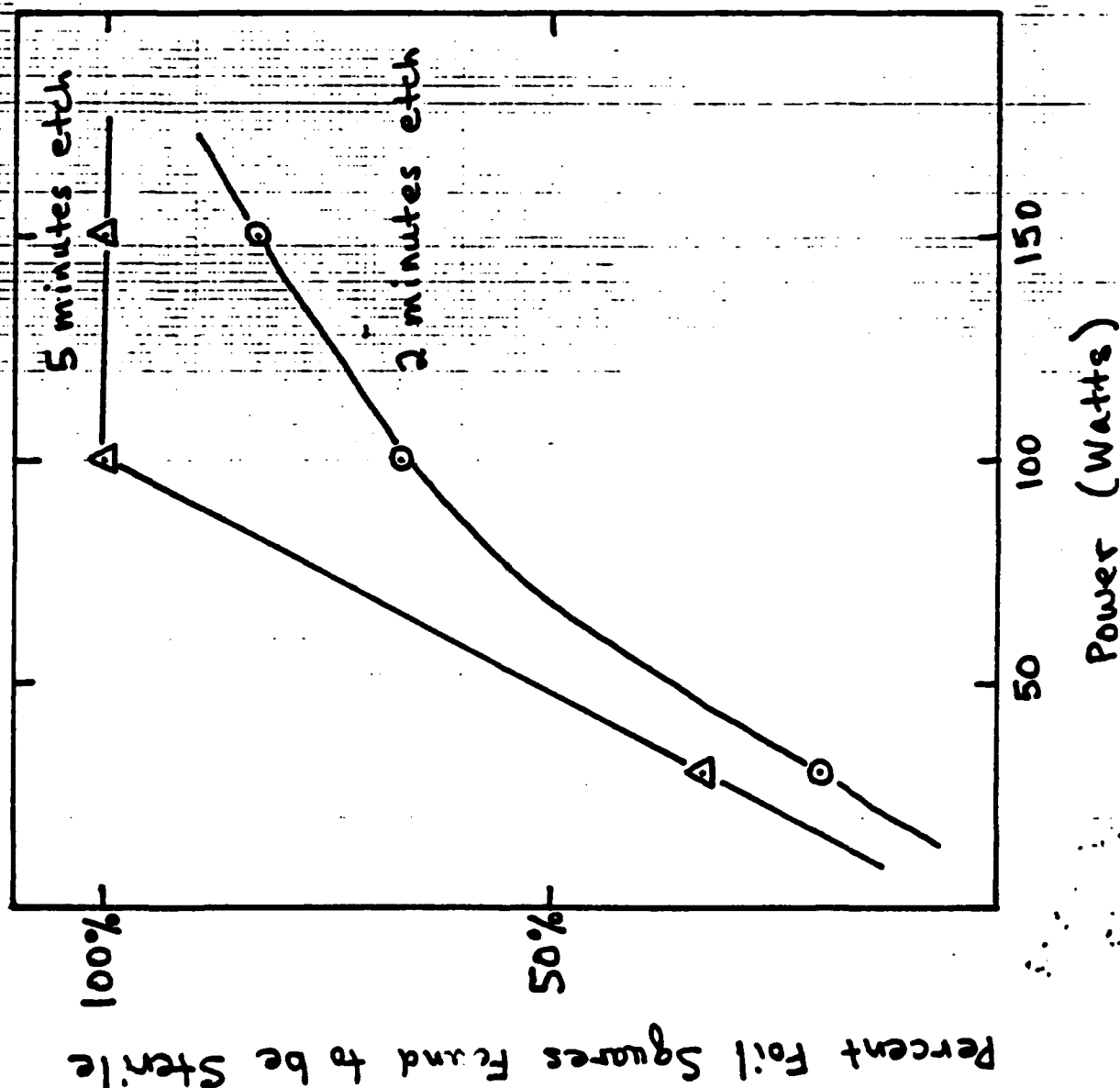
**(b) Etching Conditions: Foil sample facing down.  
160 Watts; 1.5m Torr air pressure**

| <u>Etching<br/>Time (min)</u> | <u>Bacterial Growth in Experiment No.</u> |          |          |          |          |
|-------------------------------|-------------------------------------------|----------|----------|----------|----------|
|                               | <u>1</u>                                  | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> |
| 0                             | +                                         | +        | +        | +        | +        |
| 2                             | -                                         | -        | -        | -        | -        |
| 10                            | ND                                        | ND       | ND       | ND       | ND       |
| 30                            | -                                         | -        | -        | -        | -        |

\* Experiments were performed as described in Table 1 in the Anatech PA-200 plasma cleaner. 2cm x 2cm aluminium foil squares containing spores were mounted on a wire screen facing either up (a) or down (b) in the etching chamber.

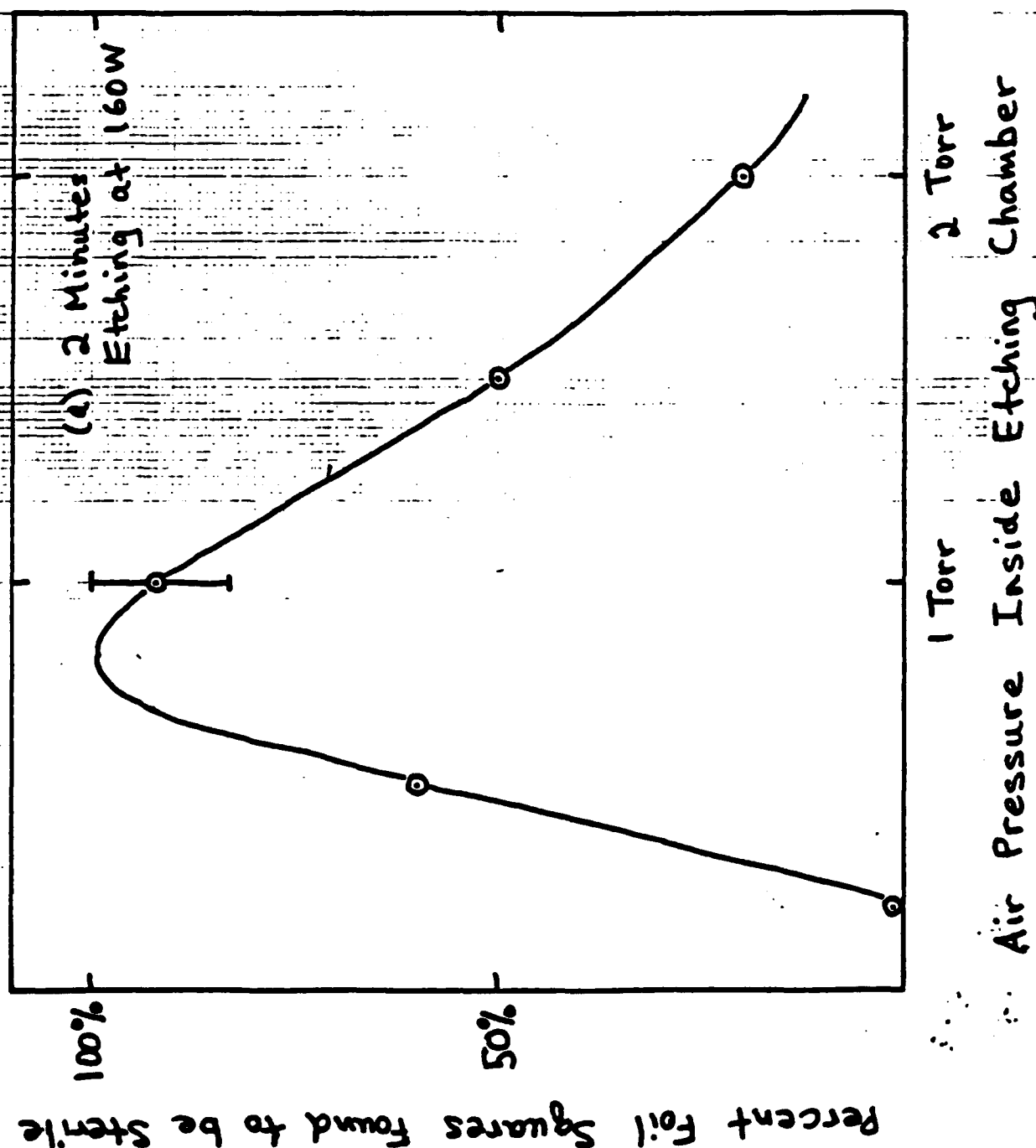
ND = not determined

**Figure 1:** Effect of power density on the ion plasma sterilization of *B. subtilis* spores. Experiments were carried out as described in Table 1. in a cylindrical etching chamber 8cm in diameter X 14cm long. The air pressure was 1 Torr.

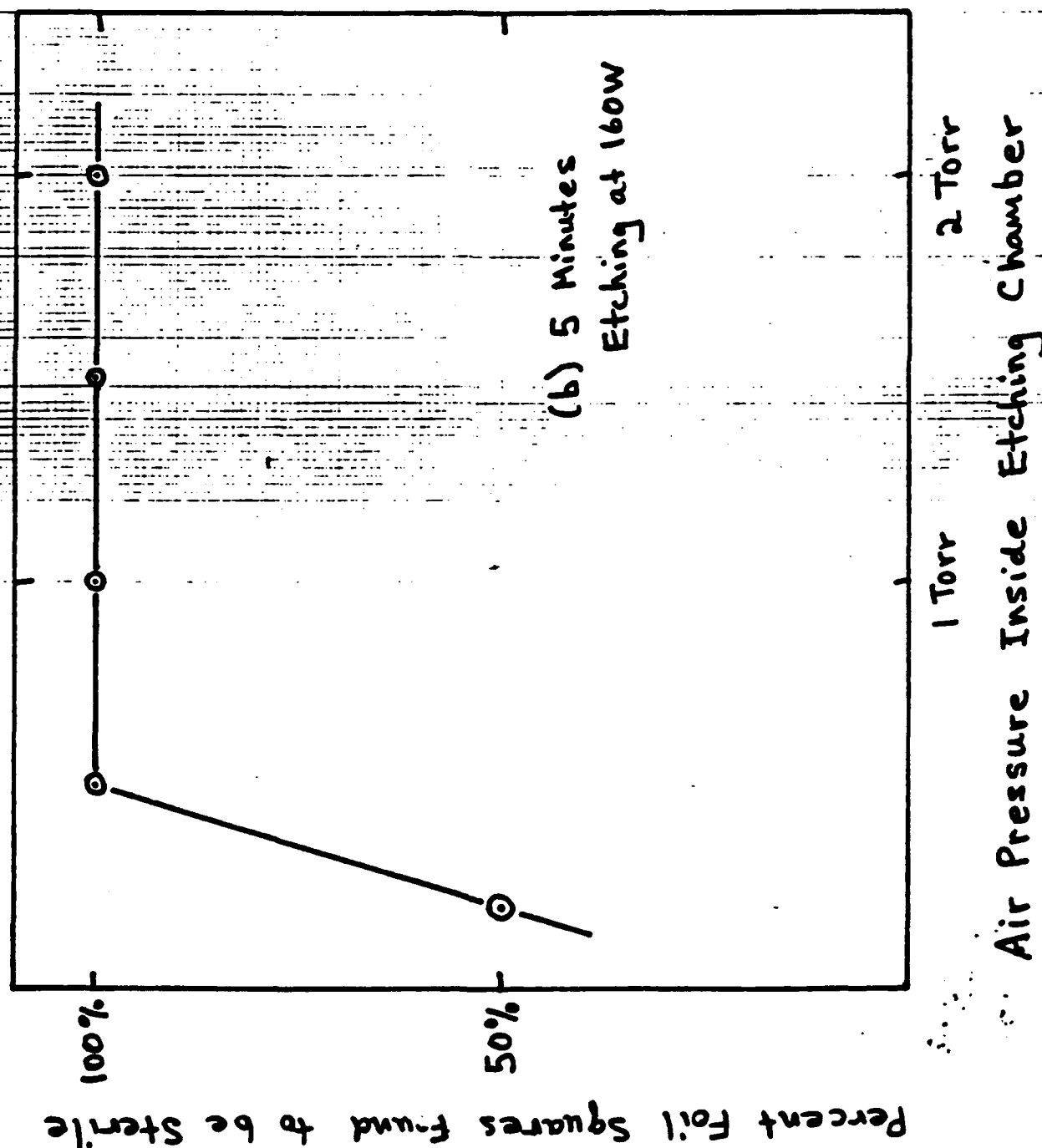




**Figure 2:** Effect of air pressure on the ion plasma sterilization of *B. subtilis* spores. Experiments were carried out in air at 160W as described in Table 2. All etching times were 2 minutes.



**Figure 3:** Effect of air pressure on the ion plasma sterilization of B. subtilis spores. Experiments were carried out in air at 160W as described in Table 2. All etching times were 5 minutes.



## Section V - Progress November 15, 1988 to December 15, 1988

Studies during the current reporting period were devoted to: (a) determining whether RF plasmas are able to clean and sterilize in remote and protected areas of stainless steel instruments and (2) comparing high frequency (2.45 GHz) with RF ion plasmas in the above properties.

### A. Ability of RF Ion Plasmas to Clean and Sterilize in Remote Areas of Stainless Steel Instruments.

Previous studies of RF ion plasmas (see Technical Progress Report No. 3) have demonstrated that RF ion plasmas of 160 Watts are able to clean ink and bacterial spores from exposed surfaces in a short time (5-10 minutes). The experiments performed during this reporting period were designed to test whether cleaning and sterilization would take place in protected areas such as the threads of screws and the lumens of hypodermic needles. Two kinds of experiments were carried out. In the first, bacterial spores were spread and dried on the threads of small (1cm in length) stainless steel screws. A nut was then fitted on and the combination was exposed to the RF ion plasma (160 Watts in the Anatech PA-200 plasma etcher) for 1-30 minutes. Bolt and nut were then separated in a sterile manner and incubated together in L.broth to determine whether spores had been killed. The results, as shown in Table 1, demonstrated that spores were killed after 10 minutes of exposure to the ion plasma at 1.5 Torr and 160 Watts. After 30 minutes of exposure, nut and bolt combinations were found to be sterile, but they were also quite hot. Sterilization could have been due to heating rather than to the direct effects of the ion plasma. Since sterilization, but not heating, was observed after 10 minutes of etching, we believe that this shorter time is preferable for our intended application.

The second test involved the use of stainless steel hypodermic syringe needles. Bacterial spores were spread and dried along the lumens of 1cm and 2cm needles which were then exposed for various times (up to 30 minutes) to the RF ion plasma. Irradiated needles were tested for the presence of viable spores by incubation in L.broth as previously described. The results (Table 2) showed that all 2cm needles were sterilized after 10 minutes of irradiation and 4 of 5 1cm needles were also sterile. All of the control, non-irradiated needles were found to contain viable spores. As in the case of experiments with nut and bolt combinations, we conclude that 10 minutes of irradiation in the RF plasma is sufficient to sterilize the protected surfaces of syringe needles.

We have spent some time considering how the RF ion plasma may be able to clean and sterilize in remote areas such as those described above. At first it seemed quite remarkable to us that cleaning and sterilization should be so efficient and quick. We feel that one of two basic explanations must apply. Either an ion plasma is being generated in the small crevices such as those in screw threads, or ions (or other lethal species) must be generated in open spaces and diffuse into the more inaccessible areas. We do not know which of the two possibilities applies. At the present time, however, we favor the

view that plasmas are generated in remote areas because cleaning and sterilization are so rapid at these sites.

#### B. Cleaning and Sterilization in a High Frequency (2.45 GHz) Ion Plasma

Evaluation of high frequency ion plasmas was an important goal for Phase I development of our proposed plasma cleaner. Studies to examine this technology involved use of the Plasma-Preen II manufactured by Plasmatic Systems, Inc. (1327 Aaron Road, New Brunswick, N.J. 08902; phone 201-297-9107). This instrument is about the size of a small microwave oven with a water-cooled chamber approximately the size of a loaf of bread. It operates at 2.45 GHz with a maximum power of 550 Watts which was used in all our experiments. Air pressure cannot be determined precisely in the Plasma-Preen II, but we estimate (from the amount of pumping we employed) that the air pressure inside the chamber was in the range of 5 Torr to 10 Torr.

Two types of experiments were carried out to test the effectiveness of high frequency ion plasmas. In the first, bacterial (*Bacillus cereus*) spores were spread and dried on 1 cm x 1cm square of aluminum foil which were then exposed to the ion plasma for varying periods of time up to five minutes. After irradiation, foil squares were tested for the presence of viable spores by incubating them in a suitable liquid bacteriological medium (L.broth). The results (Table 3) showed that sterilization depended significantly on whether the specimen was resting on the floor of the chamber or was raised up slightly (supported on a wire screen). Samples supported on the screen were sterilized in 0.5 min. or less while those resting on the floor of the chamber required 0.75 min. - 5.0 min. for complete sterilization. Examination of irradiated specimens in the scanning electron microscope (Figure 1) showed that spores could be rendered non-viable (hence sterilized) without being removed entirely from the foil target. Significant spore fragments remained even after 5 min. of etching (on a screen support) when the specimen had been rendered sterile. It is quite significant to note here that sterilization by the high frequency ion plasma was more rapid than sterilization by the RF or direct current ion plasmas as described in Technical Progress Reports No.3 and No.2, respectively. For example, sterilization of foil squares required approximately 5 min. in the RF ion plasma, but 0.5 min. or less in the high frequency instrument. A part of this difference may be due to the higher power employed in the Plasma-Preen II (550 W vs 160 W in the RF ion plasma). It is possible, however, that the high frequency of the GHz plasma also confers an advantage.

The second test of high frequency ion plasma involved the use of nut and bolt combinations as described in Table 1 for RF plasmas. As in the previous study, the goal was to test the ability of GHz discharges to clean and sterilize in remote and protected regions of surgical instruments. Experiments were carried out using the same protocol as described in Table 1 except that irradiation was performed in the Plasma-Preen II. The results (Table 4) showed that all nut and bolt combinations were sterilized after 10 minutes of etching. At this time, however, nuts and bolts were very hot, so hot in fact that

sterilization could possibly have been due only to heating. At shorter exposure times (e.g. 5 minutes) when nuts and bolts were not significantly heated, they were also not thoroughly sterilized. For instance, 2 of 5 bolts were not sterile after five minutes of exposure to the high frequency plasma.

**Table 1: Sterilization of Nut and Bolt Combinations by a Radio Frequency (13.56 MHz) Ion Plasma**

**Etching Conditions: 1.5 Torr; 160 Watts**

| <u>Time (min)</u> | <u>Bacterial Growth in Experiment No.</u> |          |          |          |          |                 |
|-------------------|-------------------------------------------|----------|----------|----------|----------|-----------------|
|                   | <u>1</u>                                  | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> | <u>6</u>        |
| 0                 | +                                         | +        | +        | ND       | ND       | ND <sup>c</sup> |
| 10                | -                                         | -        | -        | -        | -        | -               |
| 30                | -                                         | -        | -        | -        | -        | -               |

**Etching Conditions: 1.0 Torr; 160 Watts**

| <u>Time (min)</u> | <u>Bacterial Growth in Experiment No.</u> |          |          |          |                |
|-------------------|-------------------------------------------|----------|----------|----------|----------------|
|                   | <u>1</u>                                  | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u>       |
| 0                 | +                                         | +        | +        | ND       | ND             |
| 10                | -                                         | -        | +        | -        | - <sup>b</sup> |
| 30                | -                                         | -        | -        | -        | -              |

<sup>a</sup> Experiments were carried out with 1cm (3mm diameter) stainless steel bolts which were deliberately contaminated with 2ml of a suspension of *Bacillus cereus* spores. A stainless steel nut was then screwed into place and the combination was exposed to the RF plasma as described in the Table. Ion plasmas were developed in air at 1.0 Torr or 1.5 Torr. After irradiation, the nut and bolt were separated and incubated together, overnight in 2ml Luria broth (L. broth) at 37°C. Tubes were scored as "+" if they contained bacterial growth and "-" if they did not.

<sup>b</sup> Nut and bolt combinations were quite hot (too hot to touch) after 30 minutes of exposure to the ion plasma.

<sup>c</sup> ND= not determined.

**Table 2: Use of Radio Frequency (13.56 MHz) Ion Plasma to Sterilize the Lumens of Hypodermic Syringe Needles**

**Experimental Conditions: 1cm needle; 1.0 Torr; 160 Watts**

| <u>Time (min)</u> | Bacterial Growth in Experiment No. |          |          |          |          |
|-------------------|------------------------------------|----------|----------|----------|----------|
|                   | <u>1</u>                           | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> |
| 0                 | +                                  | +        | +        | +        | +        |
| 10                | -                                  | -        | -        | +        | -        |
| 30                | -                                  | -        | -        | -        | -        |

**Experimental Conditions: 2cm needle; 1.0 Torr; 160 Watts**

| <u>Time (min)</u> | Bacterial Growth in Experiment No. |          |          |          |          |
|-------------------|------------------------------------|----------|----------|----------|----------|
|                   | <u>1</u>                           | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> |
| 0                 | +                                  | +        | +        | +        | +        |
| 10                | -                                  | -        | -        | -        | -        |
| 30                | -                                  | -        | -        | -        | +        |

<sup>a</sup> Experiments were carried out with 19 gauge stainless steel hypodermic syringe needles that were deliberately contaminated in the lumen with 1 $\mu$ l of a suspension of *Bacillus cereus* spores. These were then exposed to the RF plasma for the time indicated in an Anatech PA200 plasma etcher operated in air at 1.0 Torr and 160 Watts. After exposure to the plasma, needles were incubated overnight in 2ml L. broth at 37°C. Tubes were scored as "+" if they contained bacterial growth and "-" if they did not. Needles not deliberately contaminated with spores were tested and found to be sterile.

**Table 3: Effect of a High Frequency (2.45GHz) Ion Plasma on the Viability of *Bacillus cereus* Spores.<sup>a</sup>**

**Experimental Conditions: 2.45GHz; 550 Watts; no screen**

| <u>Time (min)</u> | Bacterial Growth in Experiment No. |          |          |          |          |
|-------------------|------------------------------------|----------|----------|----------|----------|
|                   | <u>1</u>                           | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> |
| 0                 | +                                  | +        | +        | +        | +        |
| 0.50              | -                                  | +        | -        | +        | -        |
| 0.75              | -                                  | +        | +        | -        | -        |
| 5.00              | -                                  | -        | -        | -        | -        |

**Experimental Conditions: 2.45GHz; 550 Watts; specimen supported on wire screen.**

| <u>Time (min)</u> | Bacterial Growth in Experiment No. |          |          |          |                 |
|-------------------|------------------------------------|----------|----------|----------|-----------------|
|                   | <u>1</u>                           | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u>        |
| 0                 | +                                  | +        | +        | ND       | ND <sup>b</sup> |
| 0.50              | -                                  | -        | -        | -        | -               |
| 0.75              | -                                  | -        | -        | -        | -               |
| 5.00              | -                                  | -        | +        | -        | -               |

<sup>a</sup> All experiments were carried out with 1cmx1cm squares of aluminum foil which were spread with 5 $\mu$ l of a suspension of *Bacillus cereus* spores. These were then exposed to the 2.45GHz ion plasma under the conditions indicated. After incubation, foil squares were transferred in a sterile manner to tubes containing 2ml sterile L. broth which were then incubated overnight at 37°C. Tubes were then scored as "+" if they contained bacterial growth and "-" if they did not. Experiments were carried out in the Plasma-Preen II (Plasmatic Systems, Inc.). The air pressure in this instrument could not be monitored during the course of irradiation, but it was estimated to be 5 Torr - 10 Torr.

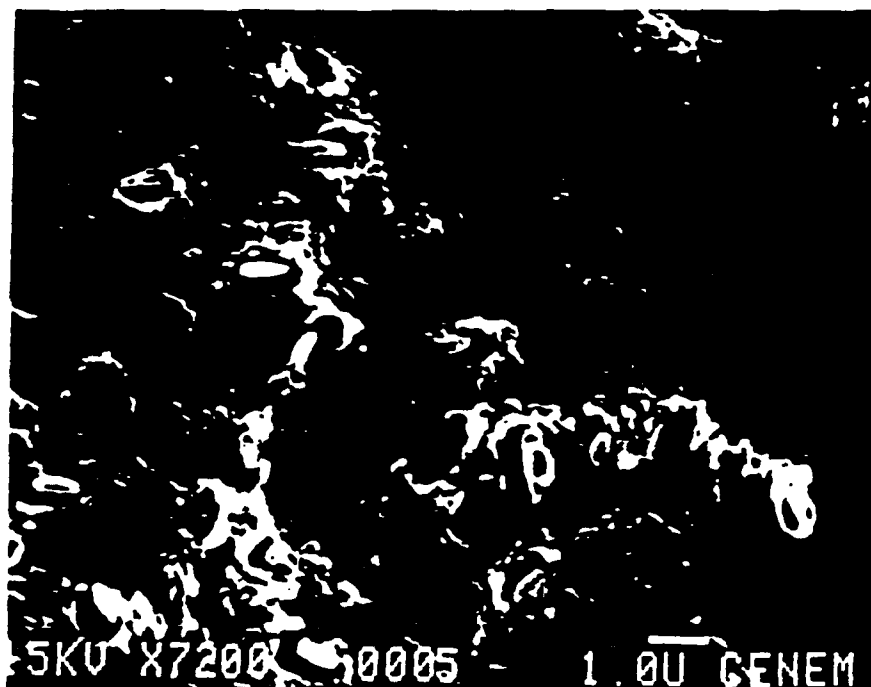
<sup>b</sup> ND= not determined.



(a)



(b)



**Figure 1:** Scanning electron microscope photographs of Bacillus cereus spores before (a) and after (b) exposure for 5 minutes to a high frequency (2.45GHz) glow discharge. A suspension of spores was spread on 1cm X 1cm squares of aluminum foil and air dried. After exposure to the ion plasma (550W in the Plasma-Preen II) specimens were coated with gold and examined in a JEOL 70T scanning electron microscope at a magnification of 7200X. Bar=1um.

## Section VI - Progress Decemer 15, 1988 to January 15, 1989

Studies during the current reporting period were devoted to analyzing the ability of ion plasmas to clean (as opposed to sterilize) stainless steel and other metallic surfaces. Most studies were performed with radio frequency (RF) ion plasmas generated in the Anatech PA-200 plasma etcher, although some experiments were done with direct current discharges.

### A. Removal of Virus from Aluminum Foil Targets

Initial experiments to measure the removal of biological material from metallic surfaces were carried out with radioactively labeled vesicular stomatitis virus (VSV). <sup>3</sup>H radioactive label was incorporated into the virus by growing it in the presence of <sup>3</sup>H-leucine. Purified, <sup>3</sup>H-labeled virus was first spread on 1cm x 1cm squares of aluminum foil and air dried. Foil squares were then exposed to an ion plasma under the experimental conditions to be tested; both RF and direct current glow discharges were examined. Radioactivity remaining on the foil square was then determined quantitatively by liquid scintillation counting as an overall measure of the ability of the ion plasma to remove biological material.

Figure 1 shows the results of a representative experiment carried out with an RF glow discharge (160 Watts; 1 Torr; Anatech PA-200 Plasma Etcher). It shows that <sup>3</sup>H label was lost as (roughly) a logarithmic function of etching time declining to less than 1% of its initial value after 20 minutes of irradiation. The rate loss was not greatly different in targets containig VSV in amounts corresponding to 6 (10ug), 12 (20ug) or 18 (30ug) monolayers of VSV. By visual inspection it could be seen that whereas virus-containing targets have a dull white film (presumably of virus) before irradiation, after 20 minutes of irradiation they had lost the film and reflected incident light. Targets were not found (by touch) to be heated after any etching time examined. The above results suggest that a large fraction (98% or more) of VSV can be removed from a metal surface after 20 minutes of irradiation and an RF glow discharge.

Figure 2 shows the results obtained with a direct current ion plasma. VSV - containing targets similar to those described above were etched in the Polaron E5100 sputter coater operated in argon at 0.1 Torr. The results showed that the rate of <sup>3</sup>H loss depended critically on the current. Greater than 90% of the <sup>3</sup>H label was removed in less than 2 minutes at 10mA or at 25mA; a much longer time was required at 1mA. Visual inspection of targets showed that the dull film of virus was removed after 1 minute of etching at either 10mA or 25mA. Targets were not significantly heated under any conditions examined. Removal of VSV from aluminum foil targets was faster in direct current plasmas produced at 10mA or 25mA than in comparable RF plasmas. For example, a period of 20 minutes of RF irradiation was required to achieve the same level of cleaning found (for the exposed surfaces tested here) after less than 2 minutes of irradiation in the 10mA direct current glow discharge.

## B. Removal of Blood from Stainless Steel and Aluminum Surfaces

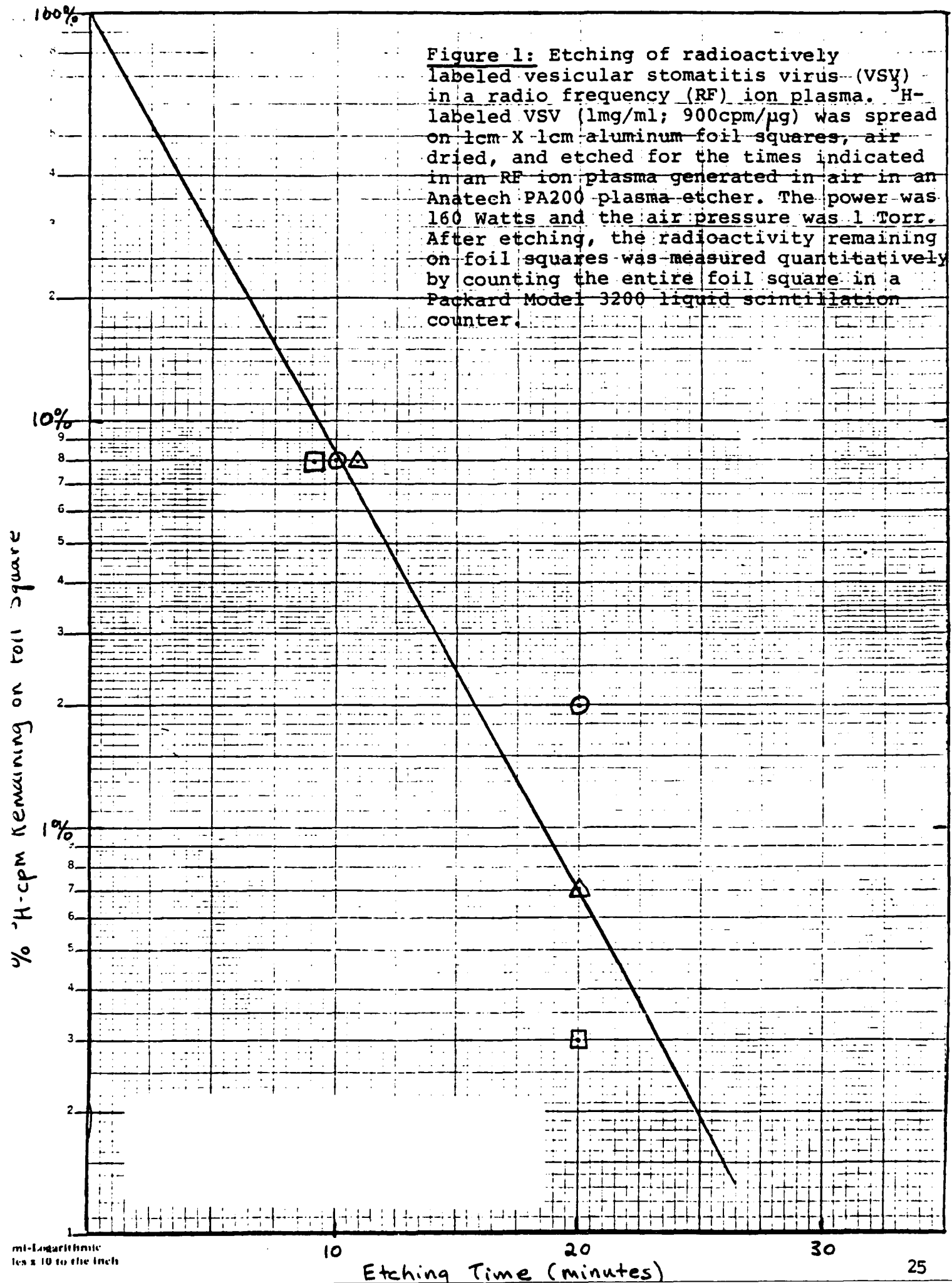
A great deal of our effort has been devoted to examining the removal of blood from metallic surfaces since blood is expected to be an important component of the biological material to be removed from contaminated surgical instruments. Experiments have involved spreading sheep blood on stainless tools (or other surfaces) and exposing them to various test ion plasmas. Surfaces were then examined visually for the presence of residual blood. The results of representative experiments with RF ion plasmas are shown in Figures 3 (stainless steel tweezers) and 4 (aluminum foil squares). In both cases we observed that significant cleaning took place during 10 minutes of exposure to the ion plasma (160 Watts; 1 Torr; Anatech PA-200 plasma etcher) and that both types of surfaces were clean after 30 minutes. Stainless steel tweezers were quite hot (too hot to touch) after 10 minutes or more of irradiation. No heating was observed in aluminum foil targets.

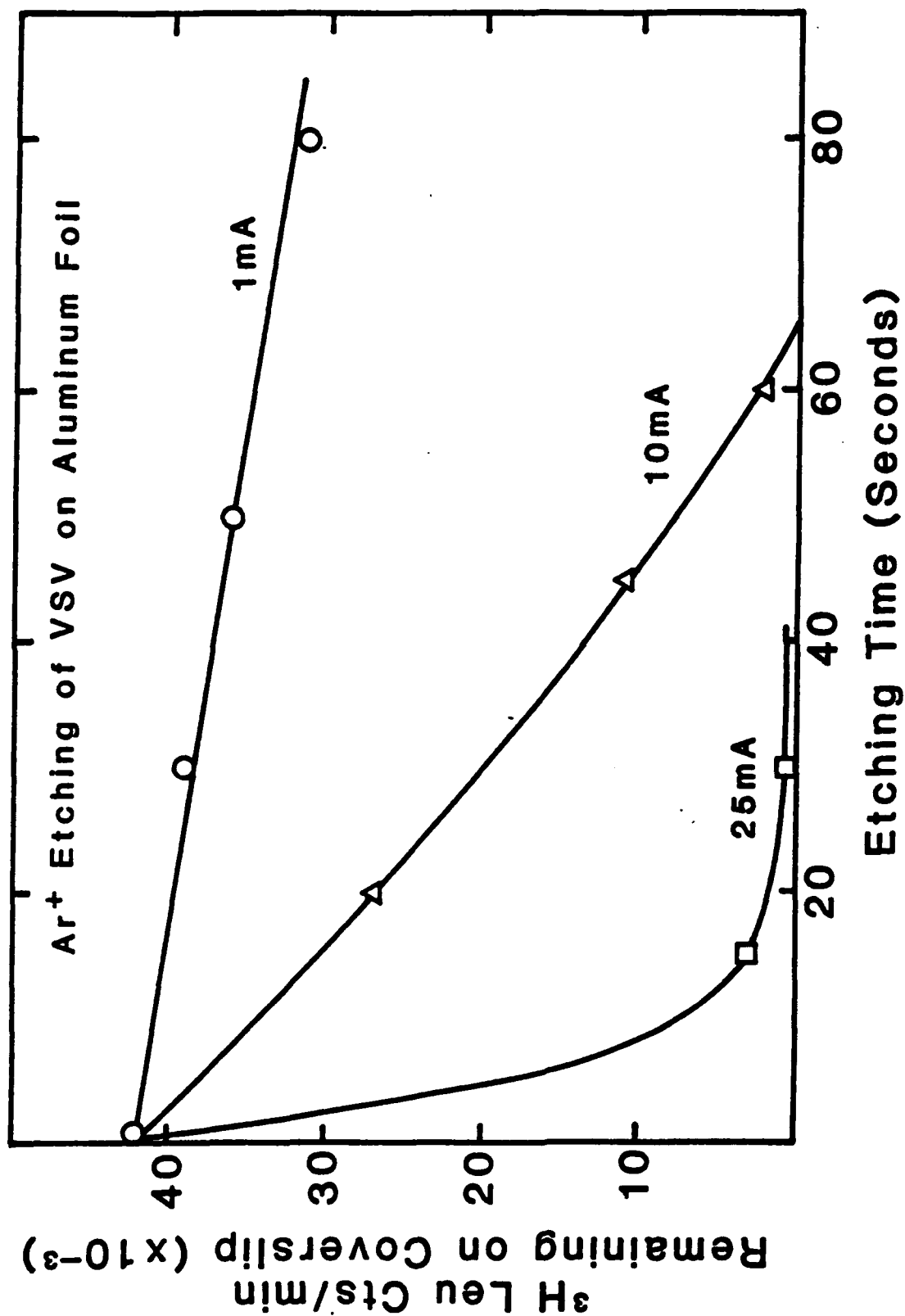
## C. Cleaning in Protected Areas of Nut and Bolt Contaminations

In contaminated surgical instruments the most difficult areas to clean are likely to be the threads of screws found in hinged instruments such as scissors and hemostats. To test the ability of ion plasmas to clean in such areas, we have carried out the following experiments. Small nuts and bolts have been dipped, separately, in black paint, dried and threaded together. In this state they were exposed to an RF ion plasma in the Anatech PA-200 operated at 160 Watts in air at 1 Torr. After various periods of irradiation, they were removed from the etcher, separated, examined visually and photographed.

The results, as shown in Figure 5, indicated that exposed surfaces were partially cleaned after 10 minutes and completely cleaned after 20 minutes of etching. Protected screw threads, however, were much more resistant to cleaning; substantial contamination was observed even after 60 minutes of irradiation. This result contrasts significantly with our observation (Section V, page 19, Table 1) that bacterial spores in the same areas are rendered non-viable after 10-30 minutes of exposure to an identical RF ion plasma. The results, therefore, indicate that the plasma sterilizes remote surfaces more readily than it cleans them. Stainless steel nut and bolt combinations were too hot to handle after 20 minutes or more of exposure to the RF ion plasma.

Figure 1: Etching of radioactively labeled vesicular stomatitis virus (VSV) in a radio frequency (RF) ion plasma.  $^3\text{H}$ -labeled VSV (1mg/ml; 900cpm/ $\mu\text{g}$ ) was spread on 1cm X 1cm aluminum foil squares, air dried, and etched for the times indicated in an RF ion plasma generated in air in an Anatech PA200 plasma etcher. The power was 160 Watts and the air pressure was 1 Torr. After etching, the radioactivity remaining on foil squares was measured quantitatively by counting the entire foil square in a Packard Model 3200 liquid scintillation counter.





**Figure 2:** Etching of radioactively labeled vesicular stomatitis virus (VSV) in a direct current ion plasma. <sup>3</sup>H-labeled VSV was spread on 1cm X 1cm aluminum foil squares and etched for the times indicated in an Ar plasma at 0.1Torr. Etching was carried out in the Polaron E5100 sputter coater operated at 1mA, 10mA or 25mA as indicated. After etching, radioactivity remaining on foil squares was determined as described in Figure 1.

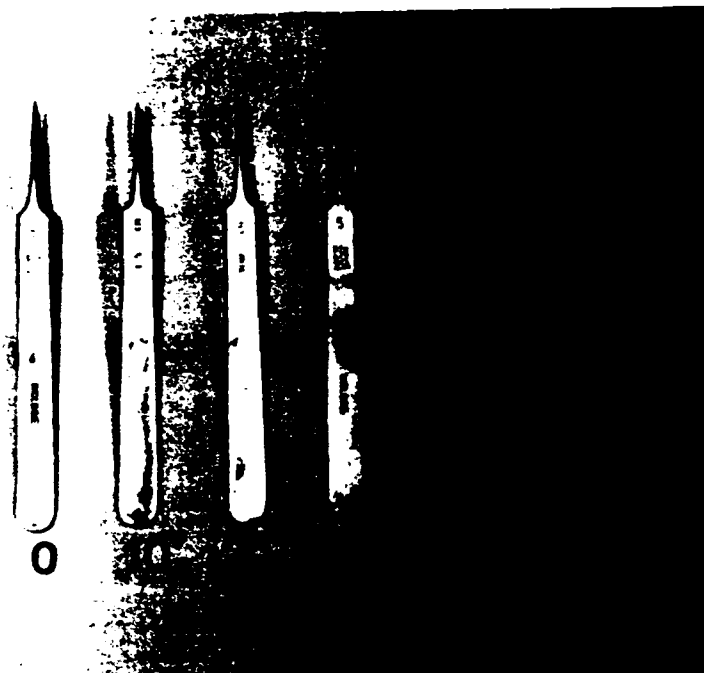


Figure 3: Removal of blood from stainless steel tweezers by etching in a radio frequency (RF) ion plasma. Identical stainless steel tweezers were coated on the handles with sheep blood, dried and irradiated for the times indicated (in minutes) in an RF ion plasma. The plasma was produced at 160 Watts and an air pressure of 1 Torr in an Anatech PA200 plasma etcher. After etching, tweezers were photographed in direct light.

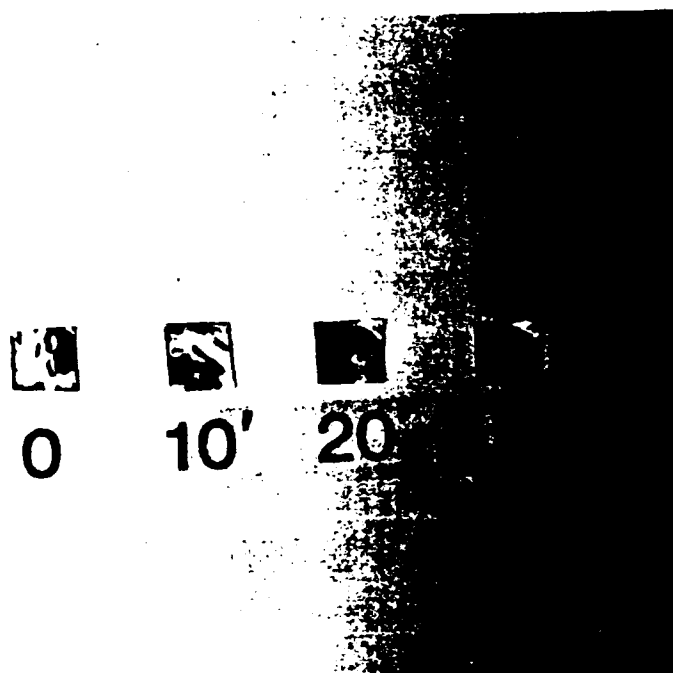


Figure 4: Removal of blood from aluminum foil squares by etching in a radio frequency ion plasma. Experiments were carried out as described in Figure 3 except that blood was spread on 1cm X 1cm squares of aluminum foil rather than on tweezers.

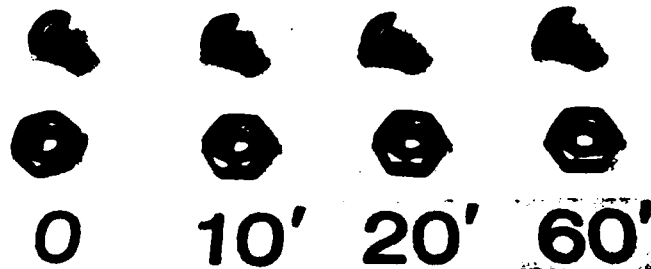


Figure 5: Removal of paint from stainless steel nut and bolt combinations by etching in a radio frequency (RF) ion plasma. Identical stainless steel nut and bolt combinations were dipped (separately) in black paint and dried. The nut was then threaded onto the bolt and the two were etched for the times indicated ( in minutes) in an RF ion plasma. The plasma was produced in the Anatech PA200 plasma etcher at 160 Watts and an air pressure of 1 Torr. After etching, nut and bolt were separated and photographed in direct light.

## Section VII - Summary August 15, 1988 to February 15, 1989

### A. Experimental Strategy

Our experimental strategy was to test different types of ion plasmas for their ability to clean and sterilize stainless steel surfaces. Tests were carried out with plasmas produced by direct current (DC), alternating current (AC), radio frequency (RF; 13.56 MHz) and high frequency (2.45 GHz) power sources. For each ion plasma type, we carried out experiments in which we varied the power density (i.e. Watts/cm<sup>3</sup> in chamber) and the air pressure. Each type of plasma was tested for its ability to clean by removing ink, blood and protein from stainless steel and aluminum foil surfaces. Tests of sterilization by ion plasmas involved use of targets (foil squares or instruments containing screw threads) that had been deliberately spread with bacterial endospores (from either *Bacillus subtilis* or *Bacillus cereus*). These materials were considered to have been sterilized by the ion plasma if no bacteria grew out when irradiated materials were incubated in bacterial growth medium (L.broth).

For our proposed application it was considered that a satisfactory ion plasma would be able to clean and sterilize stainless steel instruments in 20 minutes or less without damaging the surface of the instrument. The plasma would operate in air and ideally would not heat stainless steel instruments significantly during the cleaning and sterilization process.

Experimental tests were carried out with the commercial instruments listed below.

1. Polaron E5100 Sputter Coater (DC plasmas)
2. Anatech PA-200 Plasma Etcher (13.56 MHz; RF plasmas)
3. Anatech Hummer VI Sputter Coater (AC plasmas)
4. Anatech Hummer XP Plasma Etcher (AC plasmas)
5. Harrick PDC3XG plasma cleaner (RF; 30 Watts)
6. Plasma-Preen II (Plasmatic Systems, Inc., HF plasma; 2.45GHz)

### B. Results

**Direct Current Plasmas.** DC plasmas were found to be especially effective in cleaning and sterilizing exposed metallic surfaces. For example, ink or blood was removed from the exposed surfaces of aluminum foil squares in 5 minutes at 20 mA and an air pressure of 100 mTorr. Targets contaminated with spores were sterilized in 10 minutes or less under the same conditions. Vegetative *B. subtilis* cells were killed with a half-time of 15 sec at 10 mA and 100 mTorr.

DC plasmas, however, were found to be less effective in cleaning and sterilizing in the protected areas of screw threads such as those found on hemostats and scissors. Greater than 30 minutes were required for cleaning or sterilization in those areas as shown in Table 1.

The DC power density found to be required for cleaning and sterilizing exposed surfaces was calculated to be 0.01 Watts/cm<sup>3</sup> to 0.1 Watts/cm<sup>3</sup>.



The ability of the DC plasma to clean and sterilize was not greatly affected by the air pressure in the range of 100 to 500 mTorr. Neither stainless steel nor aluminum foil targets were appreciably heated after exposure to the DC plasma for 30 minutes at the maximum current (20mA) tested.

Radio Frequency (13.56MHz) Plasmas. Like DC plasmas, RF ion plasmas were found to be very effective in cleaning and sterilizing exposed metallic surfaces. For example, blood and ink were removed from exposed surfaces in 10 minutes at 100 Watts, while spores were killed in 5 minutes at 100 Watts. Unlike the DC plasma, however, RF discharges were able to sterilize in the protected areas of screw threads and in the lumens of hyperdemic syringe needles. Sterilization in these areas could be very rapid (10 minutes at 160 Watts; see Table 1), suggesting that glow discharges occur even in very confined spaces with an RF power source. Cleaning of blood or ink in screw threads was found to occur much more slowly than sterilization at the same sites. Whereas sterilization was complete in 10 minutes at 160 Watts, cleaning required more than 60 minutes (Table 1).

The effective power density for RF based cleaning and sterilizing was calculated to be 0.1-0.25 Watts/cm<sup>3</sup>.

Air pressure was determined to have a very significant effect on both cleaning and sterilization by the RF plasma. Pressures of 1.0-1.5 Torr were found to be optimal for both functions. These results indicate that the effect of air pressure will need to be examined carefully in the plasma cleaner finally proposed for use by the Army.

AC and HF Plasmas. Less work was done with AC and high frequency (HF) plasmas than with DC or RF. Our studies showed, however, that both AC and RF plasmas were quite effective in cleaning and sterilizing exposed metallic surfaces. The minimum times required were comparable to those observed for DC and RF plasmas (see Table 1). Heating of stainless steel instruments proved to be a significant problem with the HF plasma. Instruments exposed to the HF plasma for more than approximately 10 minutes at 550 Watts were too hot to handle at the end of the etching cycle. Similarly, in the case of the AC plasma, instruments were found to be heated before spores could be sterilized in the screw threads.

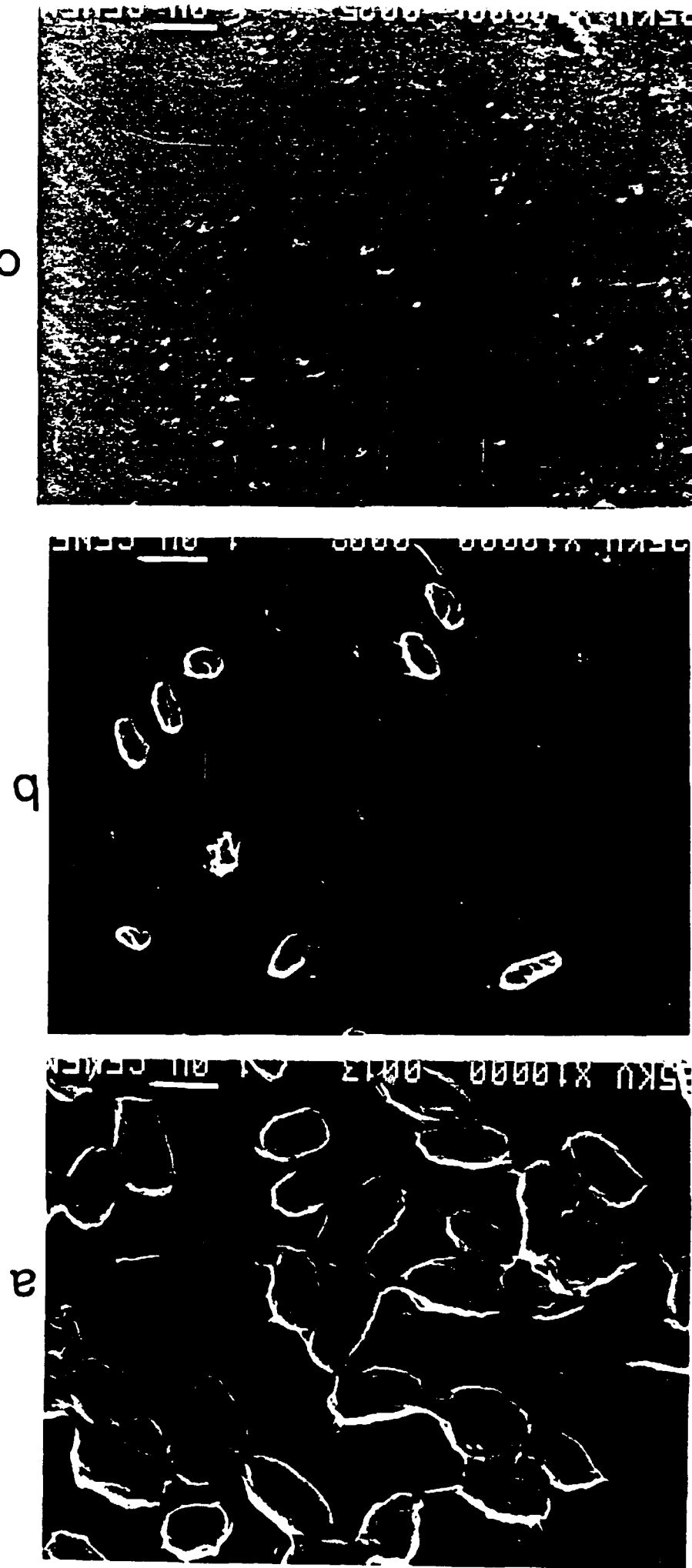
Electron Microscopy of Plasma Etched *Bacillus cereus* Endospores. Figures 1 to 4 show the effects of the four types of ion plasmas (DC, AC, RF and HF) on *Bacillus* endospores. They show that all four types of plasmas significantly erode spores as a part of the killing and sterilization process.

### C. Conclusions

As a result of our Phase I studies, we conclude that any of the four basic ion plasma modalities (DC, AC, RF and HF) tested could serve as the basis of operation for the plasma cleaner to be built for the Army. In all cases, cleaning and sterilization of exposed surfaces can be accomplished in less than 20 minutes, in air (at reduced

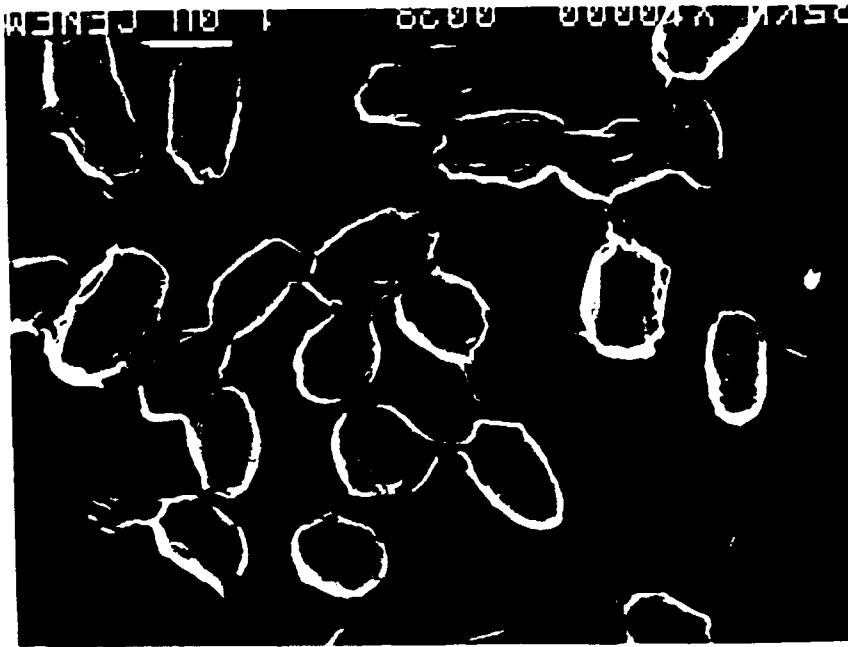
pressure), without significant heating and without damage to the surfaces of the stainless steel instruments to be cleaned. There is virtually no upper limit to the size of the chamber that can be employed in any of the four etching modalities examined. Comparisons among the four ion plasma types showed that DC plasmas are slightly better than the others in cleaning exposed surfaces. RF plasmas, however, were found to be preferable for sterilization in remote areas such as screw threads and the lumens of hypodermic needles. The power density required for operation of the plasma cleaner is expected to be in the range of 0.1-0.25 Watts/cm<sup>3</sup> regardless of which type of ion plasma is employed. In the case of RF plasmas, the air pressure needs to be carefully adjusted to the range of 1.0-1.5 Torr for optimal cleaning and sterilization.

Scanning electron micrographs of *Bacillus cereus* spores (a) and of spores after etching for 5 min (b) and 10 min (c) in a direct current ion plasma. Etching was carried out at 30 mA and an air pressure of 200 mTorr. Mag. x 10,000.

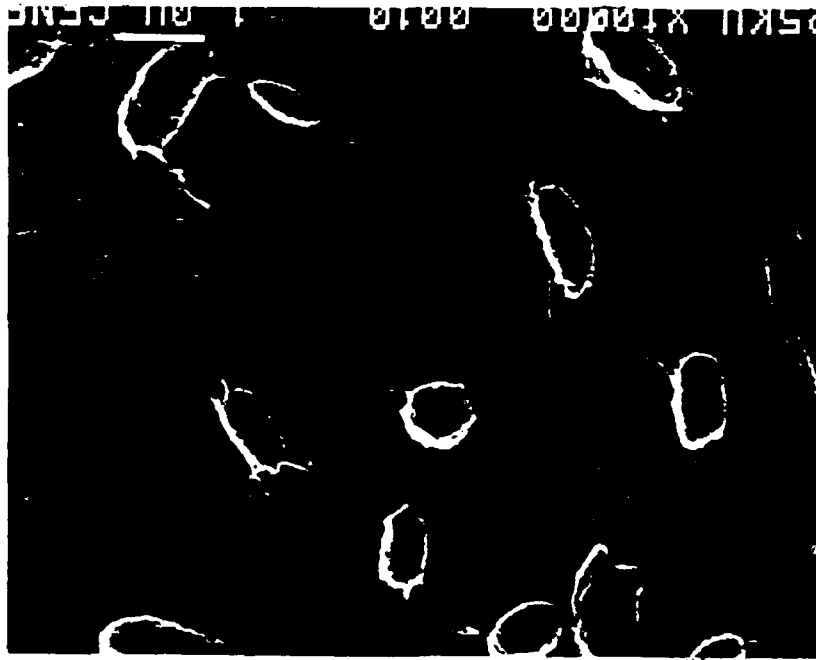


Scanning electron micrographs of *Bacillus cereus* spores (a) and of spores etched for 10 min (b) and 20 min (c) in an alternating current (60Hz) ion plasma. Etching was carried out at 30 mA in an Anatech Hummer VI sputter coater at an air pressure of 200 mTorr. Mag. x 10,000.

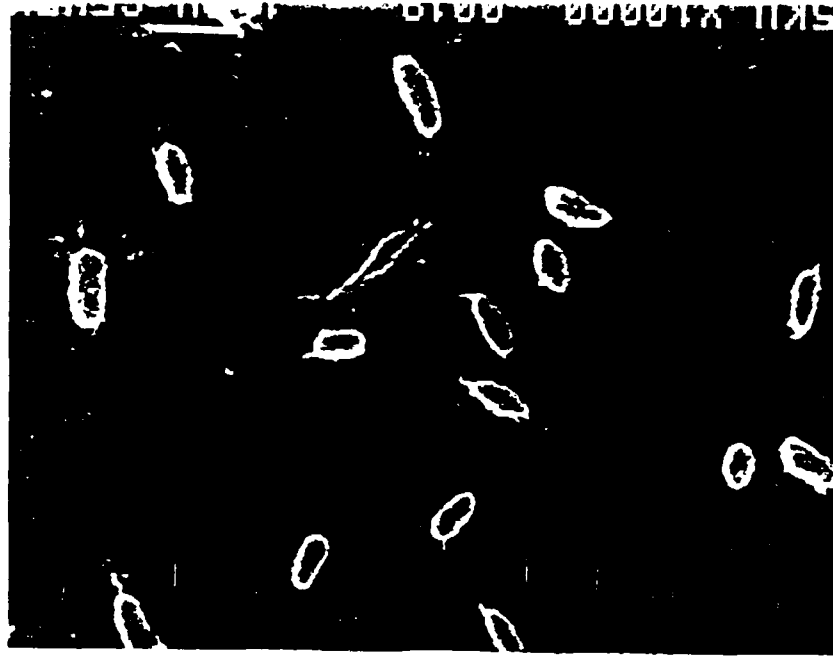
a



b

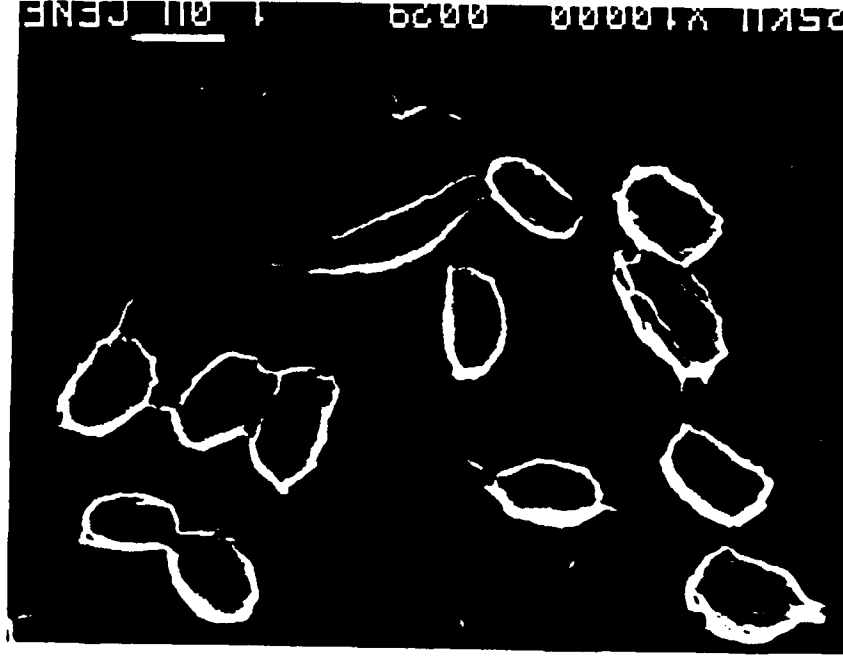


c



Scanning electron micrographs of *Bacillus cereus* spores (a) and of spores etched for 10 min. (b) and 20 min (c) in a radio frequency ion plasma. Etching was carried out at 160 Watts in an Anatech PA-200 plasma etcher. The air pressure was 1.5 Torr. Mag. x 10,000.

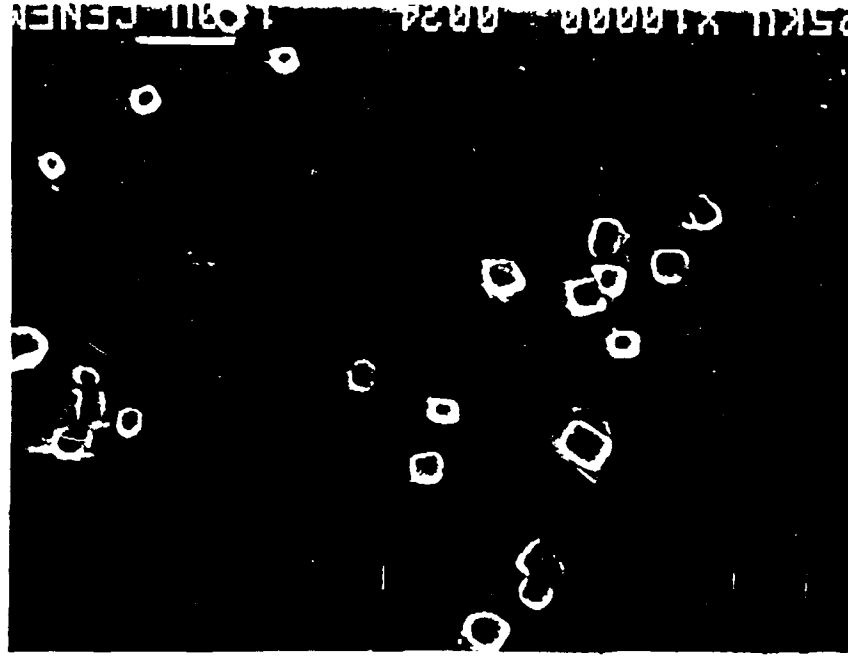
a



b

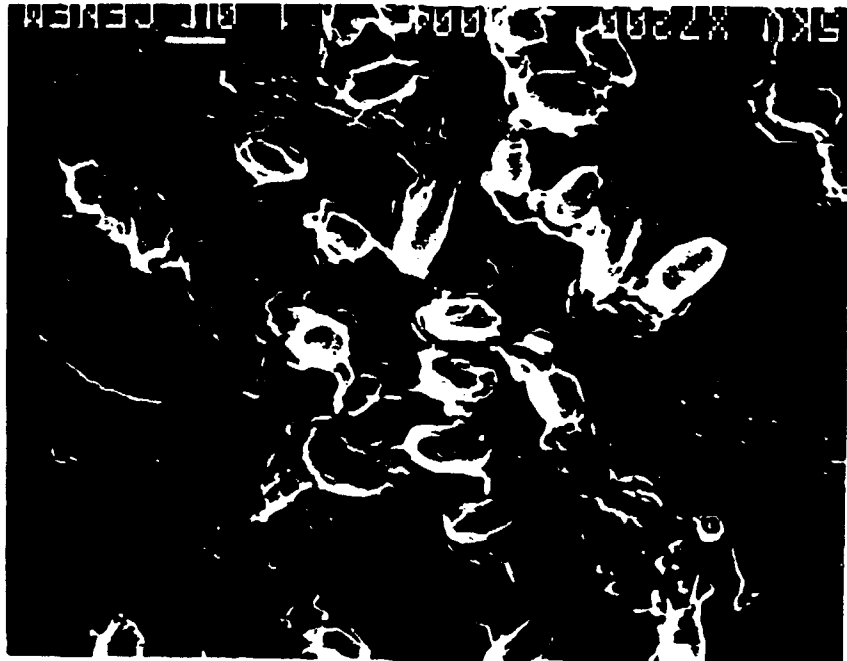


c

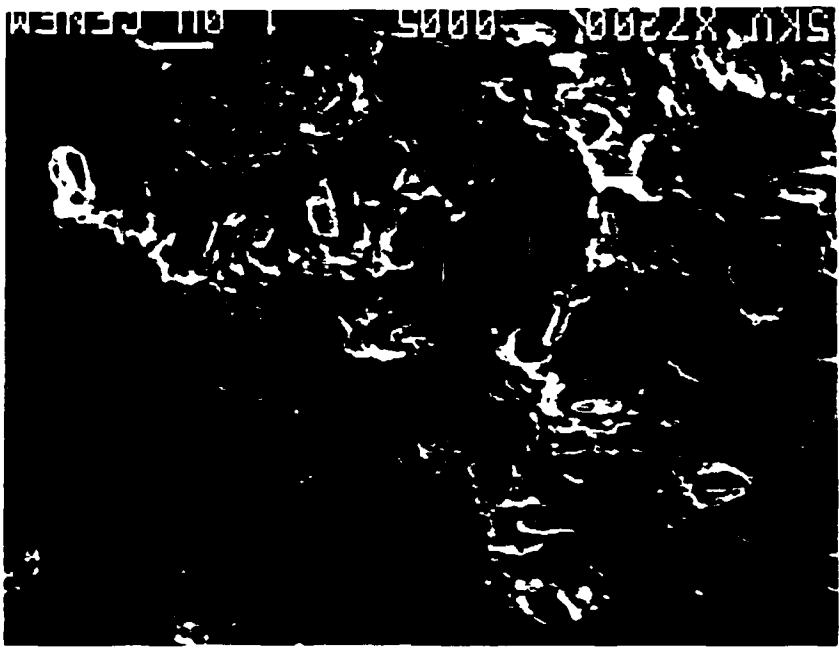


Scanning electron micrographs of *Bacillus cereus* spores (a) and of spores etched for 5 min (b) in a high frequency (2.45GHz) ion plasma. Etching was carried out at 550 Watts in a Plasma-Preen II plasma cleaner at an air pressure of approximately 1.5 Torr. Mag. x 10,000.

a



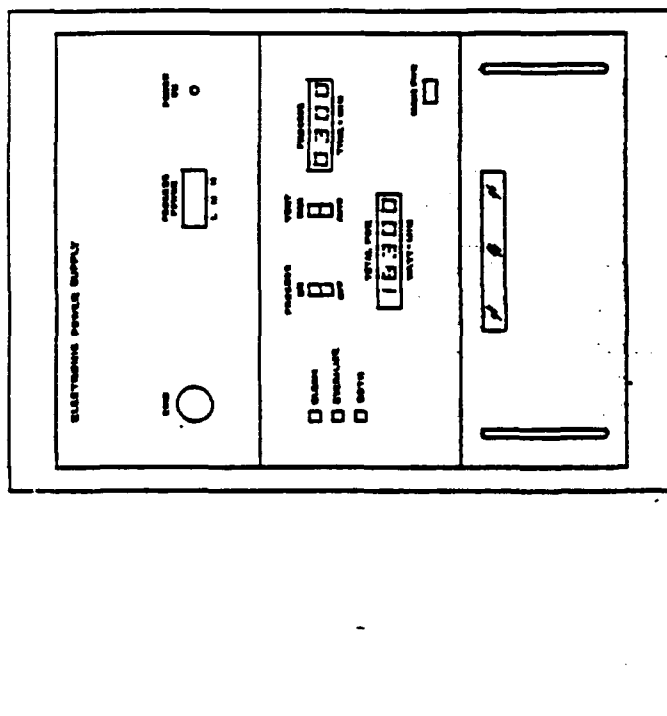
b



## Section VIII - Design Parameters

A Plasma Etcher to Clean and Sterilize Surgical Instruments. The system can be scaled up or down by tray size. For larger trays, larger power supplies will be required. For smaller trays, smaller power supplies can be used. The size of the equipment can also be affected by the decision to limit process to clean or, alternatively, to sterilize in a separate unit. Power requirements are also a function of time to clean or sterilize. Proposed parameters are:

|                        |                                                                        |
|------------------------|------------------------------------------------------------------------|
| Cabinet, Process       | 22"W x 36"H x 26"D, approximate                                        |
| Cabinet, Powed Supply  | 22"W x 24"H x 26"D, approximate                                        |
| Instrument Tray        | 20"W x 12"H x 20"D                                                     |
| Tray Loading/Unloading | insert, lock, process, unlock, remove                                  |
| Door                   | observation window                                                     |
| Weight                 | estimate 300 pounds each cabinet                                       |
| Main Power             | on/off                                                                 |
| Process Select         | total time or watt minutes                                             |
| Mode                   | clean, sterilize, or both, DC, AC or RF<br>are potential power sources |
| Power                  | low, medium, high                                                      |
| Vacuum System          | rotary vane pump with automated pressure s                             |
| Watt Density           | .1 w/cm <sup>3</sup> to .25 w/cm <sup>3</sup>                          |



## BENCH HEIGHT

**FRONT VIEW**

**CONCEPTUAL DRAWING FOR PLASMA ETCHER  
TO CLEAN/STERILIZE SURGICAL INSTRUMENTS**

ANATECH CONTRACT # 12242

ARMY # DAMD17-00-C-0190

[illegible]





**PROJECT TITLE:** DEVELOPMENT OF DESIGN PARAMETERS AND CONCEPTUAL  
DRAWING FOR A PLASMA ETCHER TO CLEAN  
AND STERILIZE SURGICAL INSTRUMENTS

**PERFORMING ORGANIZATION**  
ANATECH Ltd

**PRINCIPAL INVESTIGATOR**  
ROBERT W. BARR

**CONTRACT NO.** DAMD17-88-C-8190

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