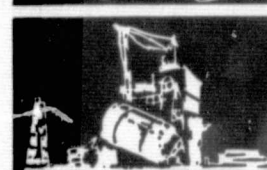
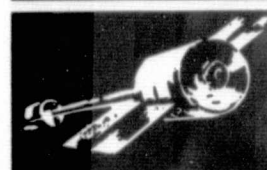
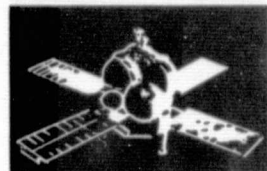
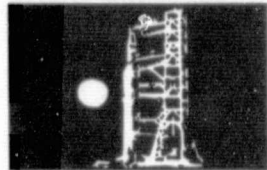
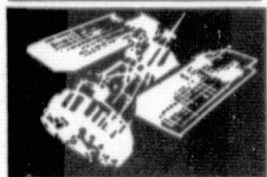


## **General Disclaimer**

### **One or more of the Following Statements may affect this Document**

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.
- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.
- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.
- This document is paginated as submitted by the original source.
- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

**SPACE  
DIVISION**



GE REPORT NO. 75SDS4231  
JUNE 1975

NASA CR-

144401

**URINE SAMPLING AND COLLECTION SYSTEM**

**OPTIMIZATION AND TESTING**

**FINAL REPORT**

(NASA-CR-144401) URINE SAMPLING AND  
COLLECTION SYSTEM OPTIMIZATION AND TESTING  
Final Report (General Electric Co.) 111 p  
HC \$5.25

N75-30795

CSSL 06B

Unclas  
G3/54 33996

CONTRACT NAS 9-13049

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

LYNDON B. JOHNSON SPACE CENTER

HOUSTON, TEXAS 77058



**GENERAL  ELECTRIC**

## FOREWORD

This report documents modifications and further test of the Urine Sampling and Collection System hardware developed under NASA Contract NAS 9-10741, Phase B4. NASA direction was provided by Mr. R. L. Sauer, Contract Technical Monitor.

## Table Of Contents

	<u>Page</u>
1.0 SUMMARY . . . . .	1
2.0 BACKGROUND . . . . .	3
3.0 OPTIMIZATION AND TESTING . . . . .	4
3.1 USCS Modifications . . . . .	4
3.1.1 Residual Volume Minimization . . . . .	4
3.1.2 Thermoelectric Cooling . . . . .	6
3.1.3 System Repackaging . . . . .	7
3.2 ABSS Testing . . . . .	9
3.2.1 Introduction . . . . .	9
3.2.2 Microbiological Samplers . . . . .	13
3.2.2.1 Methods . . . . .	13
3.2.2.1.1 Average Fluid Volume . . . . .	13
3.2.2.1.2 Sterilization . . . . .	16
3.2.2.1.3 Sensitivity To Test Organisms . . . . .	16
3.2.2.1.4 Quantitative Recoveries Of Test Organisms From MS . . . . .	16
3.2.2.2 Results Of The Assessment Of The Microbiological Sampler (MS) . . . . .	17
3.2.2.2.1 Average Fluid Volume Retained By The MS . . . . .	17
3.2.2.2.2 Sterilization Of The MS . . . . .	17
3.2.2.2.3 Sensitivity Of MS To Test Organisms . . . . .	17
3.2.2.2.4 Results Of Quantitative Recoveries Of Test Organisms From The MS . . . . .	20
3.2.2.3 Conclusions . . . . .	21
3.2.2.4 Recommendations . . . . .	21
3.2.3 Chemical Cross-Contamination Studies . . . . .	21
3.2.3.1 Methods . . . . .	21
3.2.3.1.1 Baseline Conditions . . . . .	21
3.2.3.1.2 Residual Volume - Direct Measurement . . . . .	23
3.2.3.1.3 Residual Volume - Indirect Measurement . . . . .	23

## Table Of Contents

	<u>Page</u>
3.2.3.1.4 Phosphate (Inorganic Phosphorus) . . . . .	25
3.2.3.1.5 Dip Stick Analysis . . . . .	25
3.2.3.2 Biochemical Testing Results . . . . .	25
3.2.3.2.1 Residual Volume - Direct Measurement . . . . .	25
3.2.3.2.2 Residual Volume - Indirect Measurement . . . . .	26
3.2.3.2.3 Residual Volume - Effect On Urine Phosphorous . . . . .	26
3.2.3.2.4 Residual Volume - Effect On Dip Stick Analysis . . . . .	28
3.2.3.3 Conclusions - Biochemical Testing . . . . .	30
3.2.3.4 Recommendations - Biochemical Testing . . . . .	31
3.2.4 Microbiological Response: Betadine/Disinfect Studies . . . . .	31
3.2.4.1 Objectives/Discussion . . . . .	31
3.2.4.2 Experimental Outline And Base . . . . .	33
3.2.4.3 Methods And Procedures . . . . .	34
3.2.4.4 Experimental Results . . . . .	36
3.2.4.4.1 Effect Of Sequential Urine Inputs On Microbial Population Of The ABSS . . . . .	36
3.2.4.4.2 ABSS/Betadine Disinfect Studies . . . . .	40
3.2.4.4.2.1 Introduction . . . . .	40
3.2.4.4.2.2 Effect Of Betadine On Microbial Contaminants Within Fluid Pathways Of The ABSS . . . . .	41
3.2.4.4.2.3 Effect Of Betadine On Urine Waste Tank . . . . .	42
3.2.4.4.3 <u>In Vitro</u> Assay Of Betadine In Water And Urine Versus E. Coli . . . . .	45
3.2.4.4.4 Simulation Of WCS Operation With Betadine Disinfectant . . . . .	47
3.2.5 Miscellaneous Information - ABSS Testing . . . . .	50
3.2.4.5 Conclusions And Recommendations . . . . .	51
3.2.4.5.1 Conclusions . . . . .	51
3.2.4.5.2 Recommendations . . . . .	52

## Table Of Contents

	<u>Page</u>
4.0 APPENDIX . . . . .	54
4.1 Test Plans And Protocols . . . . .	55
4.1.1 ABSS Micro/Biochemical Assay . . . . .	55
4.1.2 Betadine Rinse - WCS Study . . . . .	63
4.1.3 ABSS/Betadine Rinse Study - Part I . . . . .	66
4.1.4 Betadine/ABSS Rinse Study - Part II . . . . .	70
4.1.5 Hemaglobin Titration . . . . .	73
4.1.6 ABSS - Microbiological Assessment And Extended Biochemical Cross-Contamination Study . . . . .	75
4.1.7 Microbiological Samper - Preliminary Studies . . . . .	78
4.1.8 7-Day ABSS Operational Trial . . . . .	82
4.1.9 Quantitative Recovery Of Bacteria From Microbial Samplers . . . . .	89
4.1.10 Protocol For Determining Efficacy Of Betadine For Use In ABSS Urine System . . . . .	92
4.2 Thermoelectric Cooling Design Details . . . . .	100

## URINE SAMPLING AND COLLECTION SYSTEM OPTIMIZATION AND TESTING

### 1.0 SUMMARY

A Urine Sampling and Collection System (USCS) engineering model was developed for NASA-JSC by GE under NASA Contract NAS 9-10741, Phase B4. The USCS provided for the automatic collection, volume sensing and sampling of urine from each micturition: The purpose of the engineering model was to demonstrate verification of the system concept.

The objective of the USCS Optimization and Testing program was to update the engineering model, to provide additional performance features and to conduct system testing to determine operational problems. Optimization tasks were defined as modifications to minimize system fluid residual and addition of thermoelectric cooling.

The USCS optimization tasks were completed as planned. However, prior to the start of system testing, and at NASA direction, the modified USCS engineering model was diverted for urgent use under NASA Contract NAS 9-13519, ASTP Fluid Transfer Measurement Experiment. Subsequently, NASA directed that in lieu of tests on the USCS engineering model, similar tests be conducted using the Automated Biowaste Sampling System (ABSS) Urine Subsystem as the test hardware (developed under NASA Contract NAS1-11443).

The ABSS was tested in the laboratory to determine biochemical and microbiological responses, including Betadine disinfectant studies, to the presence of urine. Testing was divided into 4 discrete experiments totaling 29 days

of operation which included 28.3 liters of urine (143 inputs). Results are summarized below:

### Biochemical Responses

- ABSS embodies a constant 18 ml fluid residual within the fluid pathways.
- The 18 ml residual is composed of an unknown proportion of rinse water and the previous urine.
- Successive urine inputs are diluted by the residual.
- Urine inputs containing high concentrations of biochemical constituents will cross-contaminate succeeding urine inputs.

### Microbiological Responses

- Fluid pathways within the ABSS contain a number of reservoirs (dead-ends, cracks, etc.) which quickly capture and hold available microbiological organisms within the ABSS.
- These pockets, or microbial reservoirs, re-infect (cross contaminate) all incoming fluids.
- Frequent rinsing with normal urine inputs did not reduce the high titers ( $\sim 10^7$ ) maintained within these pockets.
- The waste urine tank at ambient temperatures provides excellent media for rapid multiplication of microbial organisms with concomitant production of:
  - High titers of microorganisms ( $\sim 10^8$ )
  - Gas Pressure - Positive or Negative
- Peracetic acid (2%) flush was unable to eliminate the pockets of contamination within the ABSS fluid pathways.

### Betadine Disinfection Studies

- Betadine at concentrations of up to 578 ppm of free  $I_2$  was ineffective in either reducing or controlling populations of the E. coli marker, or the naturally occurring urine contaminants within the fluid pathways of the ABSS.
- Betadine at concentrations averaging 727 ppm of free  $I_2$  appeared to inhibit the formation of gas pressure (positive or negative) in the waste urine reservoir of the WCS simulation.



The ABSS Microbiological Samplers (MS) are devices designed to provide an unadulterated (with previous fluids) urine sample for microbiological assay. Testing was performed to determine the sensitivity, and quantitative recovery of bacteria from the MS. Data indicated the MS provides both a sensitive and quantitative means of obtaining a real-time urine sample for microbiological testing. However, operational experience indicated that manipulation of the samplers was awkward, entailed a definite contamination risk, and required excessive operator involvement.

## 2.0 BACKGROUND

A Urine Sampling and Collection System (USCS) engineering model was developed for NASA-JSC by GE under NASA Contract NAS 9-10741, Phase B4. The USCS provided for the automatic collection, volume sensing and sampling of urine from each micturition. The purpose of the engineering model was to demonstrate verification of the system concept. This was accomplished; the engineering model was delivered to NASA-JSC during December 1971 and successfully demonstrated. However, because of program limitations, system level testing of the USCS engineering model was limited essentially to checkout activity.

The objective of the USCS Optimization and Testing program was to update the engineering model, as developed under Contract NAS 9-10741, to provide additional performance features and to conduct system testing to determine operational problems. Optimization tasks were defined as modifications to minimize system fluid residual and addition of thermoelectric cooling.

The USCS optimization tasks were completed as planned. However, prior to the start of system testing, and at NASA direction, the modified USCS engineering model was diverted for urgent use under NASA Contract NAS 9-13519, ASTP Fluid

Transfer Measurement Experiment. Subsequently, NASA directed that in lieu of tests on the USCS engineering model, similar tests be conducted using the ABSS Urine Subsystem as the test hardware (developed under NASA Contract NAS1-11443).

Thus the program results reported in the following section describe the USCS modifications followed by ABSS Urine Subsystem test results.

### 3.0 OPTIMIZATION AND TESTING

#### 3.1 USCS Modifications

The USCS, as developed under Contract NAS 9-10741, is described in detail in GE Report No. 70SD5414.

##### 3.1.1 Residual Volume Minimization

Residual volume directly effects the degree of cross-contamination - from micturition to micturition - and consequently the amount of flush water necessary to reduce the cross-contamination to an acceptable value. Table 3-1 shows the results of tests with the USCS (unmodified) oriented with the axis of the phase separator vertical (in line with the gravity vector) and with the phase separator axis horizontal but with the system rotated so that the phase separator outlet port was located in line with, at right angle to or opposite to the gravity vector. Table 3-2 shows the average value of phase separator residual for the four orientations evaluated. Note that the influence of gravity is significant and that a relatively small residual can be anticipated under zero g conditions.

TABLE 3-1

RESIDUAL VOLUME AS FUNCTION OF  
USCS PHASE SEPARATOR ORIENTATION

<u>Test No.</u>	<u>Liquid Input</u>	<u>System</u>	<u>Residual Phase Separator Only</u>	<u>Orientation (1)</u>
1	250 gms.	30.0 gms.	15.0 gms.	1
2	250	28.5	14.5	1
3	250	31.0	14.7	1
4	250	42.0	7.0	2
5	250	43.0	11.0	2
6	250	44.5	12.0	2
7	250	46.0	3.0	3
8	250	7.5	3.0	3
9	250	15.5	11.0	1
10	250	15.5	11.0	1
11	250	11.5	1.5	4
12	250	19.5	14.5	1
13	250	21.0	16.0	1
14	250	18.5	13.5	1
15	250	17.5	12.5	1
16	250	24.5	7.5	2
17	250	25.0	7.0	2
18	250	23.5	6.0	2
19	250	15.0	2.5	3
20	250	13.0	2.0	3
21	250	9.0	1.5	4
22	250	8.5	1.5	4
23	250	16.0	12.0	1
24	250	18.0	14.0	1
25	250	16.0	12.0	1
26	250	24.5	2.5	3
27	250	24.5	3.0	3
28	250	16.5	2.5	4
29	250	16.5	2.5	4
30	250	25.0	3.0	3
31	250 gms.	16.5 gms.	2.5 gms.	4

(1) Orientation 1 - Phase separator rotational axis vertical.  
Orientations 2, 3 and 4 - phase separator rotational axis horizontal; pump outlet at 12 o'clock, 9 o'clock and 6 o'clock respectively (phase separator rotation CW at 400 rpm) viewed from impellor end.

TABLE 3-2

AVERAGE RESIDUAL VOLUME IN PHASE SEPARATOR

<u>Orientation<sup>(1)</sup></u>	<u>Phase Separator Avg. Residual Volume</u>
1	13.4 gms.
2	8.4 gms.
3	2.7 gms.
4	2.0 gms.

(1) See Table 3-1 for definition.

---

Thus for best simulation of zero g operation, orientation of the phase separator with the rotational axis horizontal and with the pump outlet oriented between a 6 and 9 o'clock position (for CW rotation) is recommended. A reduction in tubing ID and length is recommended for reducing the residual in the remainder of the system. Note also that the accumulator has a preferred orientation for minimum residual when operated under one g conditions.

### 3.1.2 Thermoelectric Cooling

The USCS, as originally designed, provided for cooling of the sample container via a coolant loop from an external source. The addition of thermoelectric units was to make the USCS a self contained system with minimal interface problems for flexibility of installation. Also to alleviate possible microorganism growth problems in the system, cooling was extended to include, in addition to the sample container, the phase

separator and accumulator and associated pump, valves and plumbing. To minimize the cooling load, the USCS components to be cooled (to 5 to 10°C) were relocated in an insulated compartment. Cooling was provided by 6 Cambion 801-1015-01 thermoelectric modules via forced air circulation. Thermal energy was dissipated from the hot side junction using forced air circulation. An estimated 72 watts of cooling is required. Design details are covered in Appendix 4.2. Subsequent laboratory testing of the repackaged system (see Section 3.1.3) verified the effectiveness of this design.

### 3.1.3 System Repackaging

System repackaging was required to accommodate the addition of thermoelectric cooling and reorientation of the phase separator and reduction in tubing length needed to minimize the system residual. This was accomplished by increasing the width of the unit from 8 to 15.5 inches, with all other external dimensions remaining unchanged (see Figure 3-1). Internally, the modified USCS is divided into 3 sub-compartments. One compartment contains all the components involved in fluid handling, i.e., phase separator (less motor), accumulator, pump and solenoid valves, urinal and sample container. This compartment is thermally insulated and cooled by forced air over the thermoelectric element cold junction heat exchanger. A second compartment contains the thermoelectric elements, hot junction heat exchanger and blower for heat rejection to ambient. The third section contains the electronic logic, signal

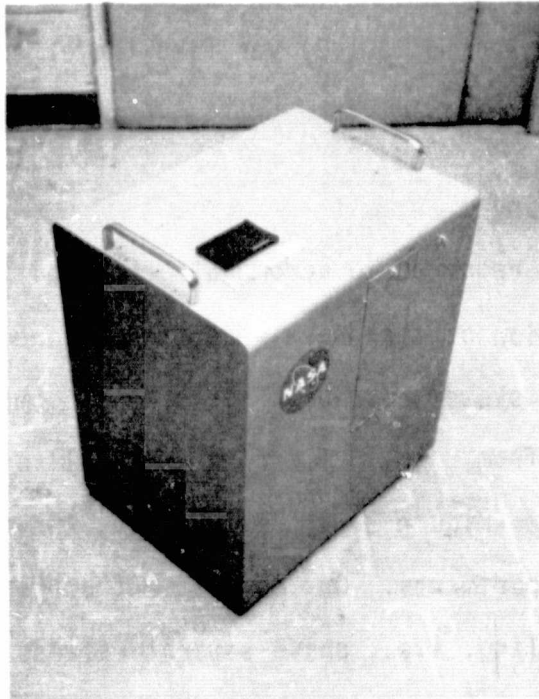


Figure 3-1. Repackaged USCS (Control Switches And  
Volume Readout Meter On Top Surface;  
Urinal And Sample Container Access  
Doors On Side)

conditioning, power supplies, phase separator motor and other support hardware. Overall system operation and performance capability did not change from that described in GE Report No. 70SD5414 (except for reduced residual and additional cooling).

### 3.2 ABSS Testing

The ABSS Urine Subsystem, described in detail in GE Report No. 74SD4208, Part I, was designed as an advanced version of the USCS. As such, many of the design features from the USCS were incorporated into the ABSS Urine subsystem. Figure 3-2 shows the system block diagram; Figure 3-3 and Figure 3-4 show the sampling and disinfect operating sequences. A major difference from the USCS (as modified) is that cooling of collected samples only is provided in the ABSS Urine Subsystem. Also the ABSS Urine Subsystem was designed to accommodate a six man crew (as compared to one for the USCS).

#### 3.2.1 Introduction

The Automated Biowaste Sampling System represents, in part, a highly sophisticated approach to urine collection, volume measurement, and sampling. If representative urine samples are to be obtained which will exhibit values relevant to their real biochemical and microbiological content, the question of sample integrity must be addressed.

Thus, sample integrity was examined in a series of laboratory experiments designed to highlight the following areas of biochemical/microbial interest:

- Evaluation of the Microbiological Sampler as a device/technique for obtaining real-time, unadulterated urine samples for microbial analysis;
- Amount and effect of the constant residual fluid volume in the ABSS, i.e. loss of low-level constituents by dilution, or carry-over (cross-contamination) from one sample to the next;

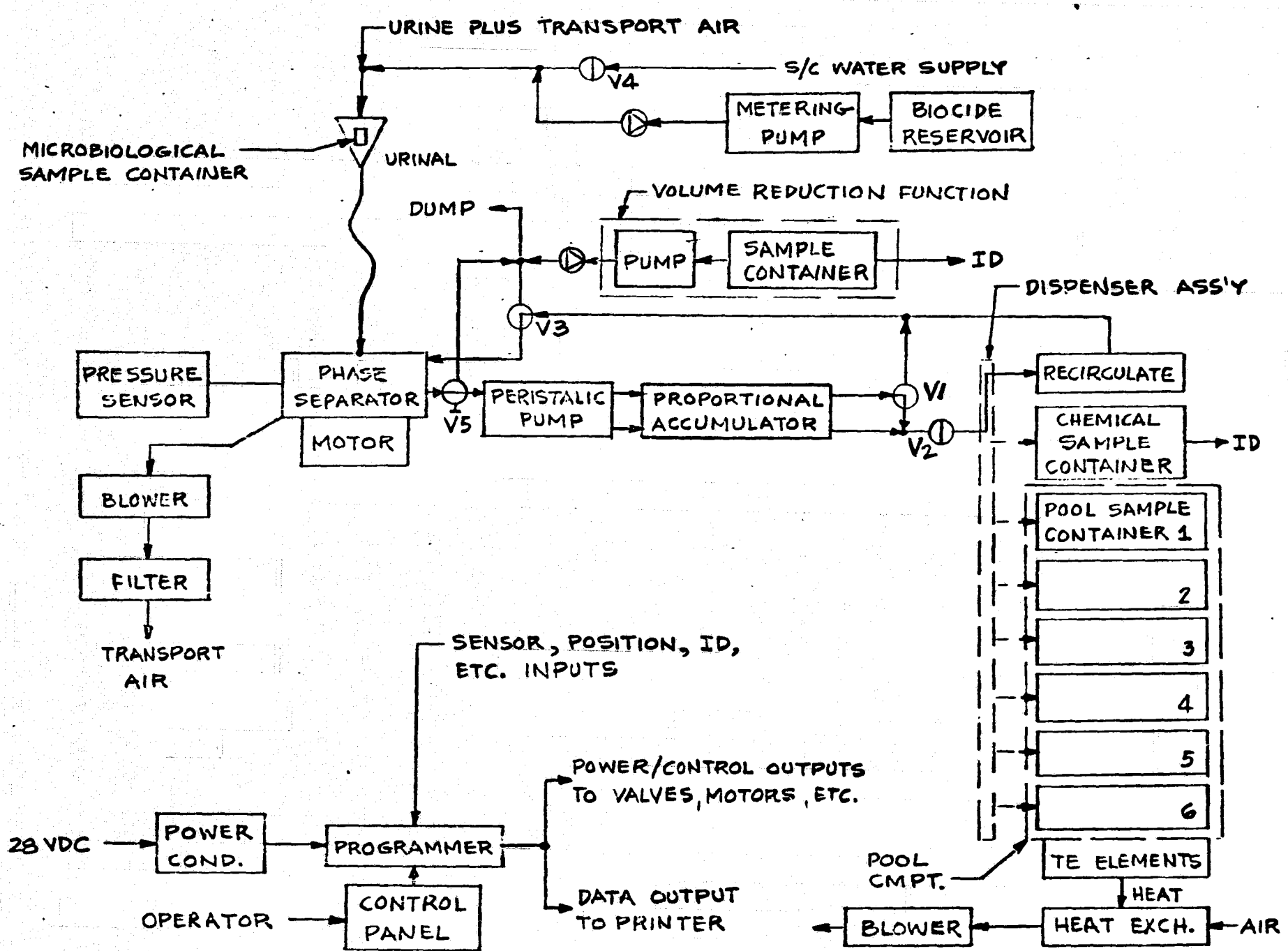


Figure 3-2. ABSS Urine Subsystem Block Diagram



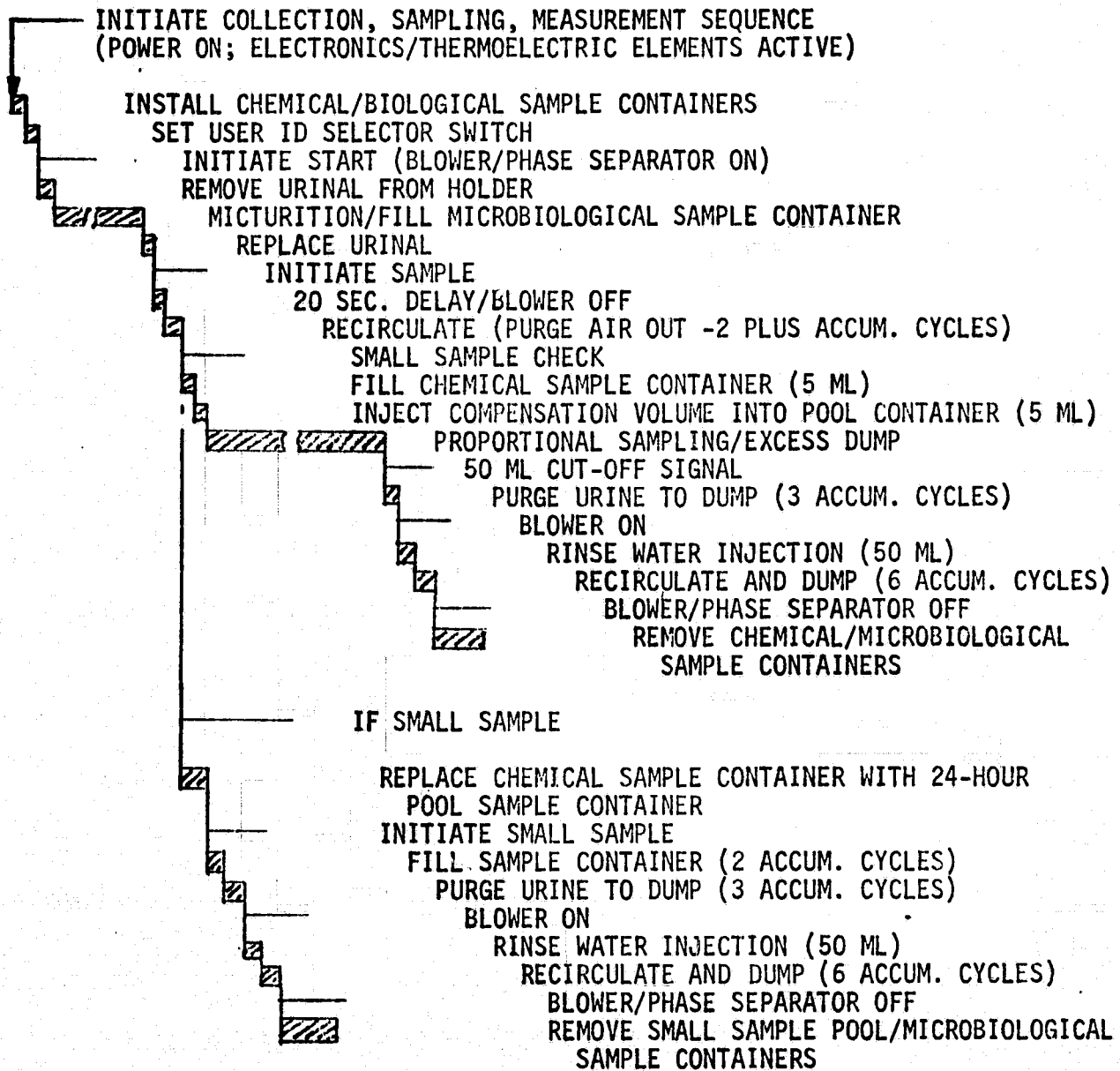
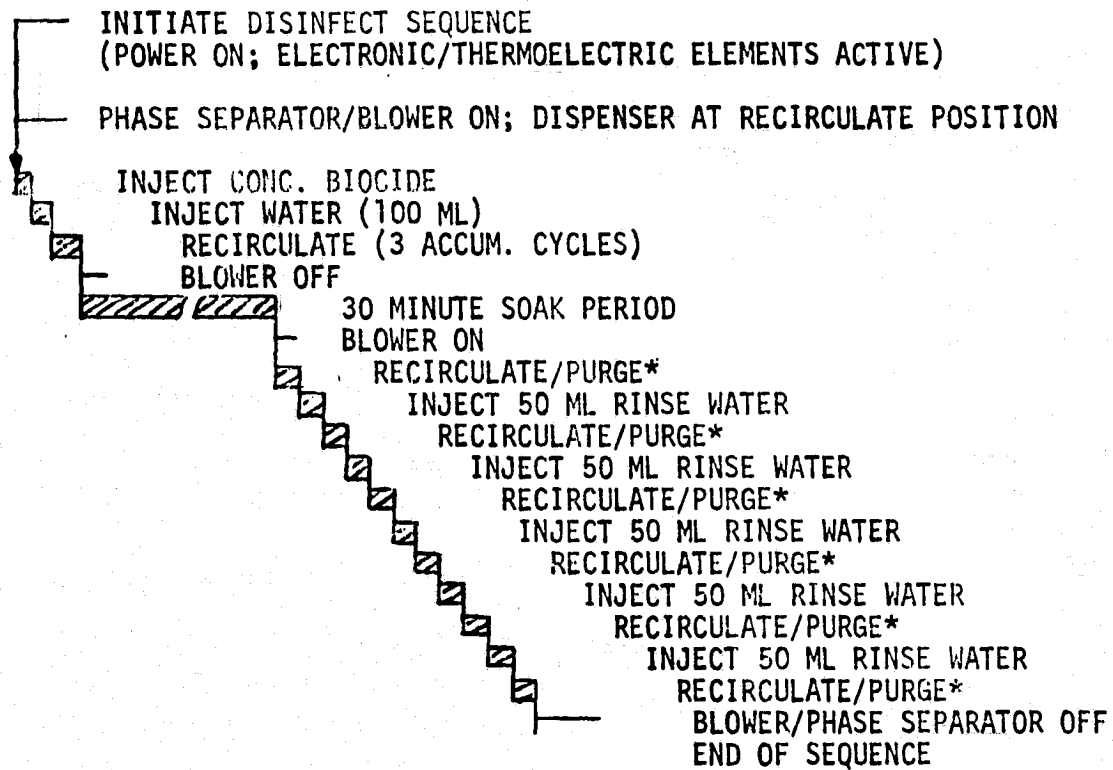


Figure 3-3. Sampling Sequence (Not To Scale)



\* 3 RECIRCULATE FOLLOWED BY 3 DUMP CYCLES OF THE ACCUMULATOR

Figure 3-4. Disinfect Sequence (Not To Scale)

- Response of the ABSS following bacterial contamination either from infected urine or as the result of adventitious agents;
- Examination of the effect of the "disinfect-rinse" cycles on contaminating bacteria within the instrument.

### 3.2.2 Microbiological Samplers (See Figure 3.2.2-1, 2, 3)

The Microbiological Samplers (MS) were designed to allow real-time sampling of the urine stream prior to contact with any of the mechanical interfaces of the ABSS, and, therefore, before potential contamination. Testing was structured to investigate the following basic areas:

- Average amount of fluid retained in each MS;
- Sterilization of the MS;
- Sensitivity of the MS to test microorganisms; and
- Quantitative recoveries of test organisms from the MS.

#### 3.2.2.1 Methods

##### 3.2.2.1.1 Average Fluid Volume

The Microbiological Samplers were tested to determine the average amount of fluid retained in each of the stainless steel "cages" which held the wick. Experience suggested that gauze squares were easier to standardize and insert into the "cages" than the cotton.

Gauze pads measuring 7 x 8 cm were rolled up and substituted for the cotton as a wick in the stainless steel "cages" within the MS. This was done because of the difficulty in inserting and removing cotton, wet or dry, and in standardizing the amount to be used.

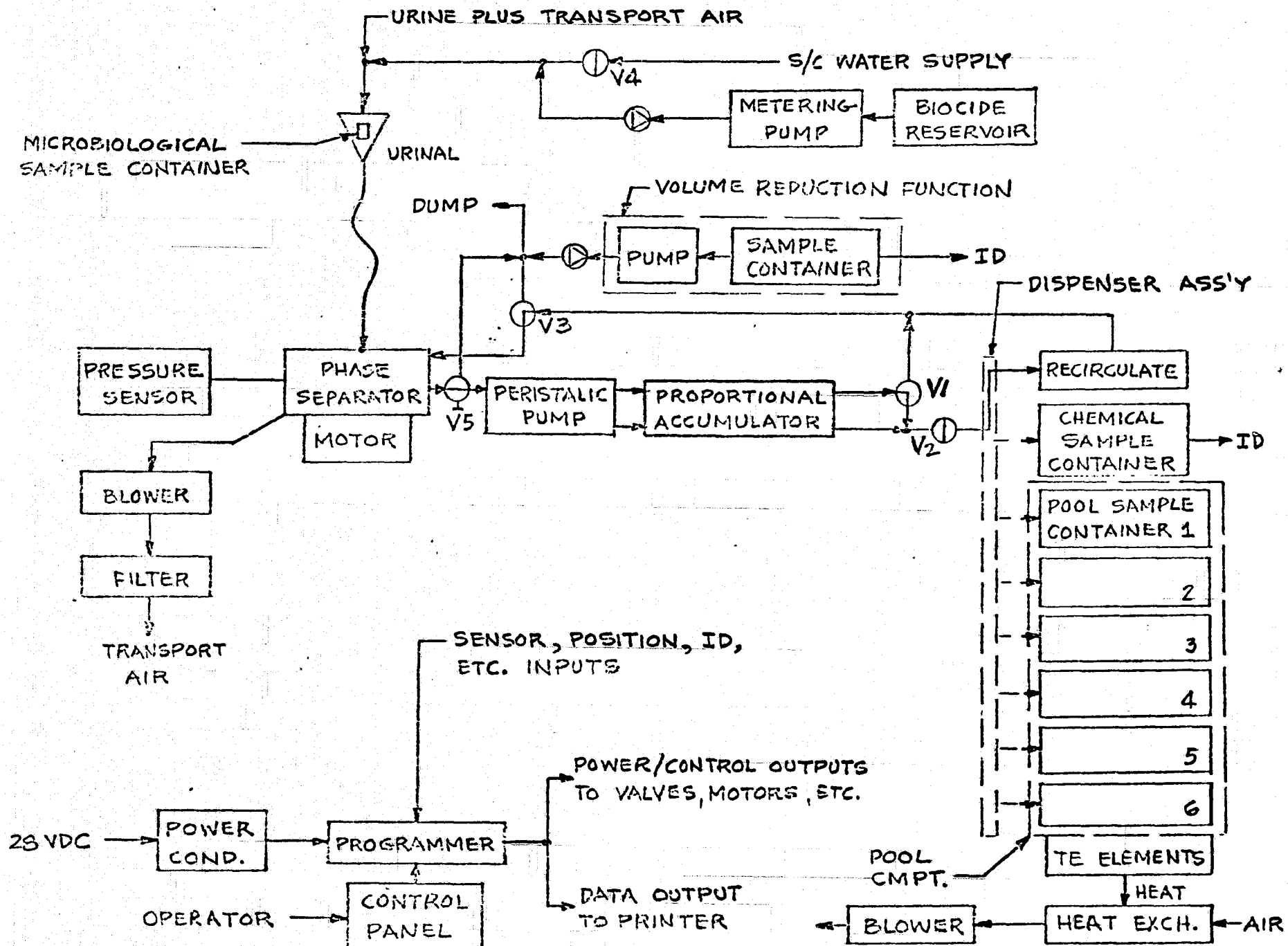


Figure 3.2-2. ABSS Urine Subsystem Operating Model Block Diagram

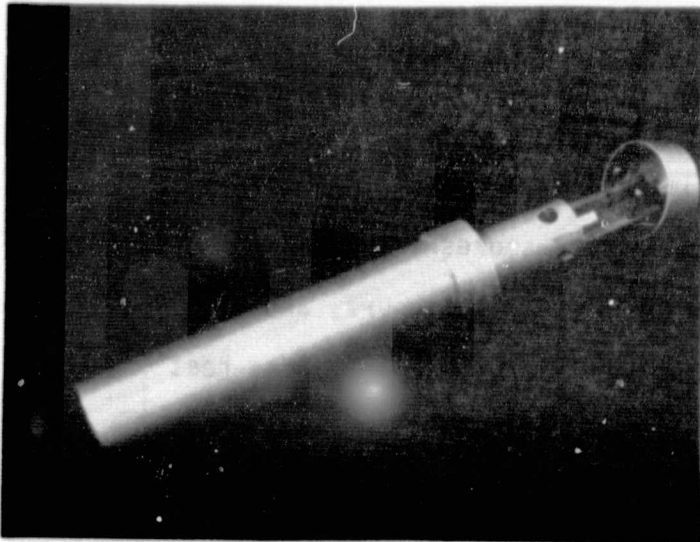


Figure 3.2.2-1. Microbiological Sampler-  
Partially Extended

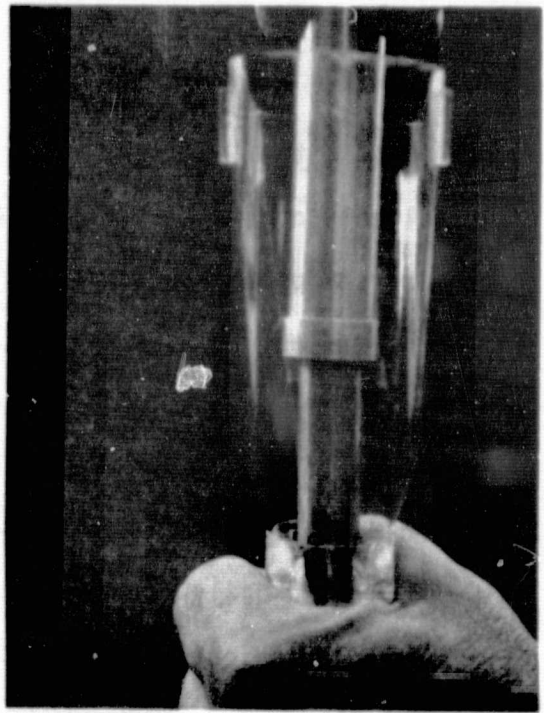


Figure 3.2.2-3. Microbiological Sampler-  
Insertion Into Urinal

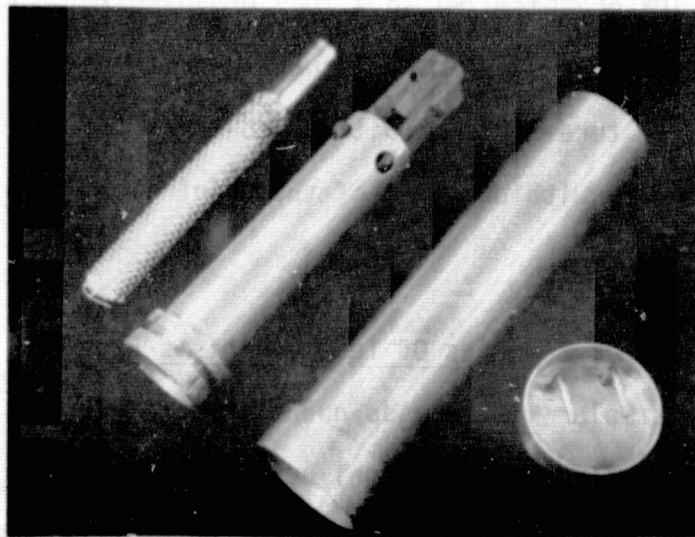


Figure 3.2.2-2. Microbiological Sampler-  
Component Parts

### 3.2.2.1.2 Sterilization

Sterilization of the complete Micro Sampler assembly was studied by inserting a spore strip containing B. subtilis var. niger\* spores into each wick and placing the entire assembly at 121°C for 16 hours, 20.5 hours, and 24 hours. At each time period, duplicate assemblies were removed from the dry-heat sterilizer, the stainless steel cage containing the wick and spore strip placed into a tube of trypticase-soy broth, and incubated at 35°C. Tubes were observed daily for one week. Those showing no growth received a fresh spore strip indicator and the tube was re-incubated at 35°C and observed for growth.

### 3.2.2.1.3 Sensitivity To Test Organisms

Sensitivity of the Micro Sampler to bacteria, i.e. ability to detect bacterial contaminants in the urine, was studied by pouring 100 ml quantities of various aqueous dilutions of E. coli (ATCC #15144), representing contaminated urine, into a urine receiver-cup containing a sterile sampler. Programmed bacterial levels ranged from  $10^6$  organisms/ml to  $< 10^{-2}$  organisms/ml. After exposure, all samplers (cage and wick only) were incubated in trypticase-soy broth at 35°C for one week. Cultures not exhibiting growth at the end of the week were inoculated directly with one drop of the original E. coli culture and re-incubated.

### 3.2.2.1.4 Quantitative Recoveries Of Test Organisms From MS

The capacity of the samplers to provide quantitative recoveries of test organisms was studied. Fifty ml aliquots of normal urine containing E. coli (ATCC #15144) at  $\sim 10^6$  organisms/ml were poured over each of the sterile samplers inserted

\* Obtained From The Wilmot-Castle, Rochester, N. Y. 14602, Lot #B-24

withing the urine cup of the ABSS. After each sampler had been exposed, the stainless steel "cage" containing the gauze/bacteria was deposited into a flask containing 48 ml of sterile trypticase-soy broth and then placed on an Eberbach shaker set to operate at ~ 50 excursions/minute for 15 minutes. In one case, duplicate flask-samplers were shaken for 30 minutes. This solution of broth and bacteria was considered a 1:25 dilution. Another 1:4 dilution was made in distilled water to give a  $10^{-2}$  dilution. Additional 10-fold dilutions were made to allow a titration of 3 replicate plates/dilution in trypticase-soy agar.

### 3.2.2.2 Results Of The Assessment Of The Microbiological Sampler (MS)

#### 3.2.2.2.1 Average Fluid Volume Retained By The MS

Average retention of fluid in these wicks was determined to be 2.2 ml/wick after pouring 14, 100 ml increments of water through replicate MS's prepared with gauze wicks.

#### 3.2.2.2.2 Sterilization Of The MS

Results of the long dwell-time, lower dry heat sterilization of the Samplers are shown in Table 3.2.2.2.2. As indicated in the Table, 16 hours at 121°C was sufficient to sterilize the stainless steel cages (supports) and wicks.

#### 3.2.2.2.3 Sensitivity Of MS To Test Organisms

E. coli (ATCC #15144) was used to produce varying amounts of viable organisms in 100 ml quantities to simulate contaminated urine. Each of these dilutions

TABLE 3.2.2.2.2

**RESULTS OF VARIABLE DWELL TIMES AT 121°C  
USING SPORE STRIPS OF B. SUBTILIS, VAR.  
NIGER<sup>1</sup> AS STERILITY CONTROL INDICATORS  
FOR STERILIZATION OF THE MICROBIAL SAMPLERS**

<u>Samples</u>	<u>Sterilizing Time</u>	<u>Results Of Incubation<sup>2</sup> At 35°C</u>		
		<u>24 Hours</u>	<u>1 Week</u>	<u>Spore Strip Add - Back</u>
1	16 Hours	0	0	+
2	16 Hours	0	0	+
3	20.5 Hours	0	0	+
4	20.5 Hours	0	0	+
5	24 Hours	0	0	+
6	24 Hours	0	0	+
Spore Strip Control	N/A	+	+	+

<sup>1</sup> Obtained From Wilmot Castle, Rochester, N. Y., 14602, Lot No. B-24

<sup>2</sup> Incubation Medium Was Trypticase-Soy Broth

0 = No Discernable Growth

+ = Growth

were poured over the sterile MS wicks which had been inserted in the urinal cup. After exposure to the test dilutions, each wick was placed into a flask containing sterile trypticase-soy broth and incubated at 35°C for one week. Results are displayed in Table 3.2.2.2.3.



TABLE 3.2.2.2.3

GROWTH OF BACTERIA CAPTURED BY MICROBIAL SAMPLERS  
 FOLLOWING EXPOSURE TO 100 ML DILUTIONS OF E. COLI<sup>1</sup>  
POURED THROUGH THE WICK

<u>Dilutions Tested</u>	<u>Approx. No. Of E.coli/ml In Dilution</u>	<u>Theor. Total No. Organisms Exposed To Wick</u>	<u>Growth After<sup>2</sup> 1 Week At 35°C (Duplicate Samples)</u>
10 <sup>-2</sup>	3 x 10 <sup>6</sup>	3 x 10 <sup>8</sup>	+
			+
10 <sup>-4</sup>	3 x 10 <sup>4</sup>	3 x 10 <sup>6</sup>	+
			+
10 <sup>-6</sup>	3 x 10 <sup>2</sup>	3 x 10 <sup>4</sup>	+
			+
10 <sup>-7</sup>	3 x 10 <sup>1</sup>	3 x 10 <sup>3</sup>	+
			+
10 <sup>-8</sup>	3	3 x 10 <sup>2</sup>	+
			+
10 <sup>-9</sup>	0.3	3 x 10 <sup>1</sup>	+
			0 <sup>3</sup>
10 <sup>-10</sup>	0.03	3 x 10 <sup>0</sup>	0
			0

<sup>1</sup> E.coli Culture ATCC #15144

<sup>2</sup> Incubation Medium, Trypticase-Soy Broth

<sup>3</sup> After 48 Hours Incubation, Broth Turned Green In Color, No Evidence Of Growth

As shown in the Table, the sensitivity of the Samplers to capture of, and compatibility with, microorganisms was demonstrated with urine samples containing total numbers of E.coli as low as 30 (0.3 organisms/ml). These results were obtained following immediate application of the wick to an appropriate growth medium and temperature; no attempt was made to hold the exposed wicks at refrigerator or freezer temperatures before assaying for growth.

### 3.2.2.2.4 Results Of Quantitative Recoveries Of Test Organisms From The MS

Results of the quantitative recovery studies are tabulated in Table 3.2.2.2.4.

TABLE 3.2.2.2.4

QUANTITATIVE RECOVERY OF E. COLI FROM MICROBIAL SAMPLERS  
FOLLOWING 15 AND 30 MINUTE AGITATION\* - SAMPLERS EXPOSED  
TO 50 ML ALIQUOTS OF URINE CONTAINING  $10^6$  ORGANISMS/ML

<u>Trial No.</u>	<u>Average Titer Of Duplicate Samples</u>	
	<u>15 Minute</u>	<u>30 Minute</u>
1	$3 \times 10^5$	$2 \times 10^6$
2	$2 \times 10^6$	$3 \times 10^6$
3	$2 \times 10^5$	
4	$2 \times 10^5$	
Control	$5 \times 10^6$	

\*Samplers Shaken In Eberbach Shaker At 50 Excursions/Minute

As shown, after 15 minutes of shaking the amount of E. coli recovered was essentially 1 log lower than the control; with 30 minutes shaking, recoveries approached the levels of the control. Additional testing would be required to establish the optimum degree of agitation, medium, time, and procedure, but these preliminary data indicate that the use of the Microbial Sample for the quantitative recovery of microorganism from urine is feasible.

### 3.2.2.3 Conclusions

The studies reported here demonstrate the Microbial Samplers as presently configured provide both a sensitive and quantitative means of obtaining a real-time urine sample for microbiological testing.

On the other hand, operational experience with the samplers has produced the following comments:

- Manipulation of the samplers - implacement, recovery, removal of the wick, etc. - was judged to be awkward; really needed "3 hands" to operate adequately.
- The potential risk of contamination with urine (and therefore bacteria) to the operator and/or the spacecraft environment was considered high.
- Use of the sampler required excessive time and involvement on the part of the operator.

### 3.2.2.4 Recommendations

In view of these conclusions it is recommended that the following actions be considered:

- Development of a modified form of the MS which significantly reduces the potential contamination risk. Adaptation of various commercially available urine sampling devices, e.g. Smith, Kline & French Labs Clinicult, could be accomplished with minimum cost to NASA.
- Incorporation of a quantitative capability within the design of the MS so that all information can be obtained more quickly, safely and conveniently. Again, adaptability of commercially available products could be accomplished.

## 3.2.3 Chemical Cross-Contamination Studies (See Figure 3.2.3-1, 2, 3)

### 3.2.3.1 Methods

#### 3.2.3.1.1 Baseline Conditions

The following were considered baseline conditions for these experiments.

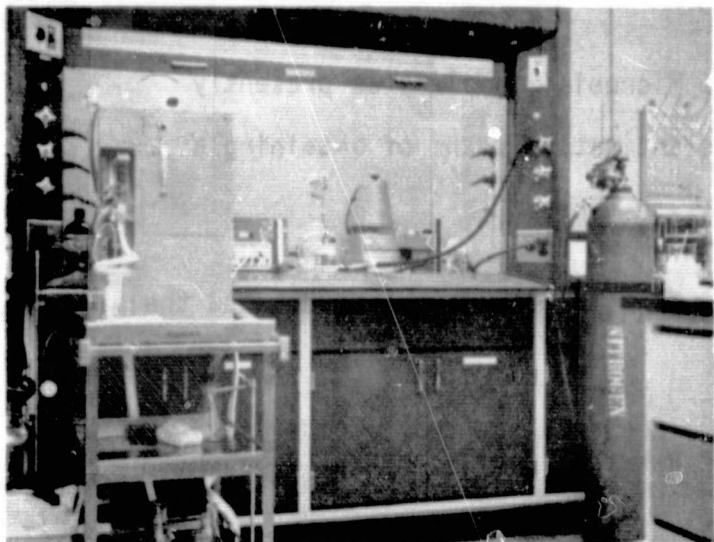


Figure 3.2.3-1. ABSS-General Test Configuration

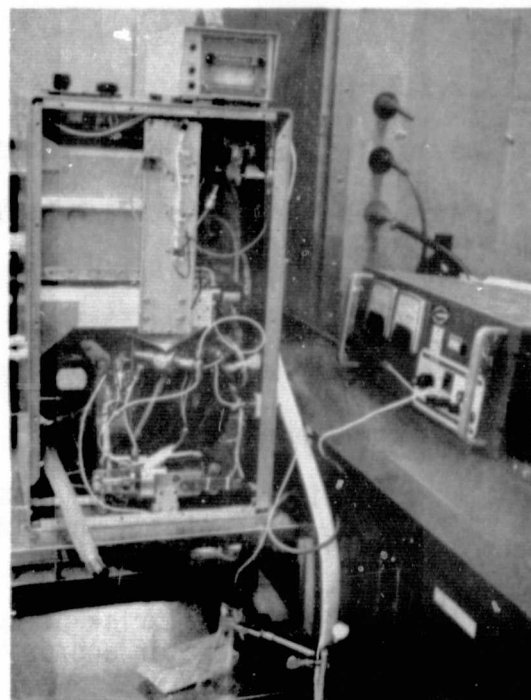


Figure 3.2.3-2. ABSS-Proportional Sampling Syringe (Accumulator) And Power Supply

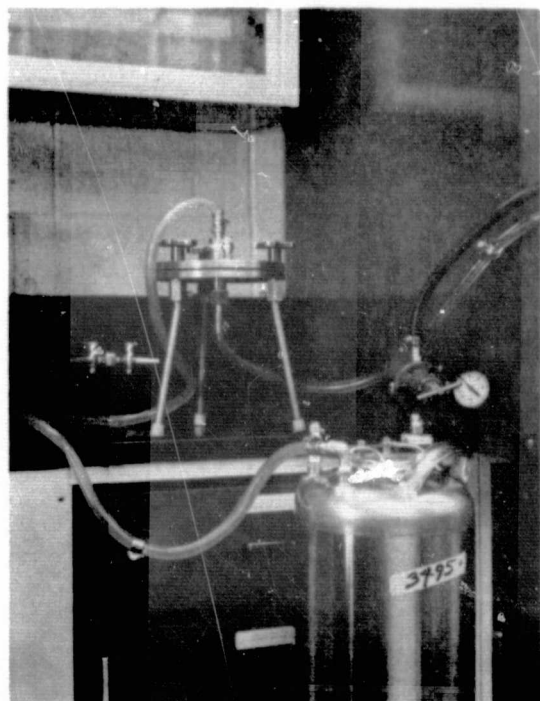


Figure 3.2.3-3. ABSS-Rinse Water Reservoir And Cold Sterilization (Filtration) Arrangement

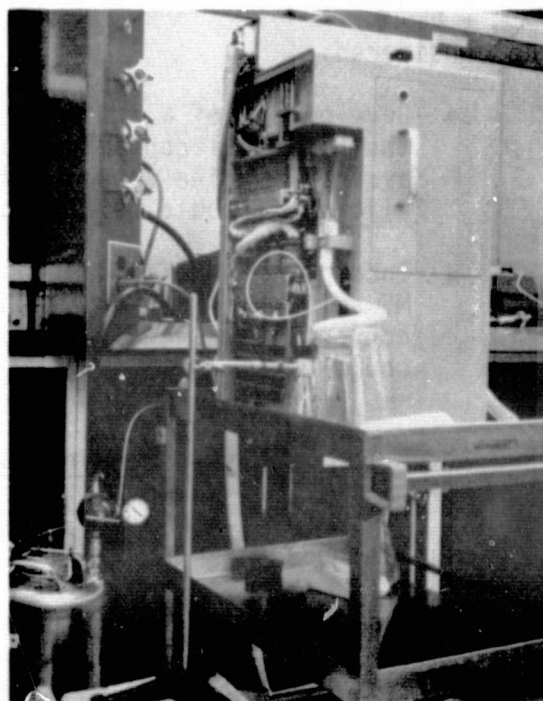


Figure 3.2.3-4. ABSS-Male/Female Urine Receiving Cup And Electronics Packages

This was imposed in order to reduce the effects of some variables, e.g. male versus female urine on the biochemical studies:

- All urine employed for these tests was male, freshly collected (within two hours) from a single micturation;
- Minimum volume employed was 120 ml;
- Samples of each urine were tested for presence and level of potential microbial organisms;
- Volume of urine, both input and output, was determined by weight;
- No attempt was made to disinfect or otherwise decontaminate the fluid pathways within the ABSS before start of the tests.

#### 3.2.3.1.2 Residual Volume - Direct Measurement

The constant fluid volume which remained within the fluid pathway of the ABSS was measured by: 1) draining and air-drying where possible, all fluid lines, phase separator, and valves. 2) adding a known weight of water (1 gm = 1 ml); and 3) direct measurement by weight of the amount recovered. The difference between the two weights was considered to be the residual volume (See Figure 3.2.3.1.3).

#### 3.2.3.1.3 Residual Volume - Indirect Measurement

Quantities of an aqueous solution of Rhodamine B\*, a highly water-soluble dye with a known absorbance value at 550 nm, was introduced to the ABSS which had been thoroughly flushed with water, i.e. zero optical density. The resulting dilution of the effluent dye samples as measured through the change in optical density (OD) and, subsequently, the volume of water necessary to produce that change could be calculated (See Figure 3.2.3.1.3).

---

\*Stock dye solution was prepared by diluting a saturated aqueous solution of the dye to a final dilution of  $4 \times 10^{-3}$  (1/4000).

$$1. \quad RV = in V_t - out V_t$$

RV = Residual Volume (ml)

$$2. \quad DF_s = \frac{OD_s}{OD_c}$$

V = Volume (ml) of fluid involved

$$3. \quad DF_d = \frac{OD_d}{OD_c}$$

DF = Dilution Factor

$$4. \quad RV_s = \frac{V_s - (V_s \times DF_s)}{DF_s}$$

OD = Optical Density of appropriate sample

%R = Percent Recovery of fluid derived from either s or d

$$5. \quad RV_d = \frac{V_d - (V_d \times DF_d)}{DF_d}$$

d = Dump Port fluid

s = Sample Port Fluid

$$6. \quad \%R_s = \frac{V_s}{V_t} \times 100$$

c = Control fluid

t = Total fluid involved

$$7. \quad \%R_d = \frac{V_d}{V_t} \times 100$$

CDF = Combination Dilution Factor (weighted for proportional vol. of fluid involved).

$$8. \quad CDF = (DF_s \times \%R_s) + (DF_d \times \%R_d)$$

Figure 3.2.3.1.3. Formulae Used In Calculation Of Residual Volume - Direct And Indirect

#### 3.2.3.1.4 Phosphate (Inorganic Phosphorus)

The phosphorus content of each urine (pre-use aliquot and effluent sample) was determined by a spectrophotometric method derived from Taussky.\* The complete method is presented in Appendix 4.1.

#### 3.2.3.1.5 Dip Stick Analysis

The Ames Multistix dip-and-read test strips were used to provide a range of seven, semi-quantitative urine parameters. These were: pH, protein, glucose, ketones, urine bilirubin, blood, and urine urobilinogen. Color comparator charts were furnished with each bottle of dip sticks along with reading - time restrictions for each test.

In order to study the sample to sample effect of a high concentration of any single constituent, a concentrated solution of hemoglobin was prepared by mixing equal quantities of freshly drawn venous blood and distilled water. The resulting lysis of the RBC's produced a hemaglobin solution which was able to elicit a positive response on the Multistix blood-reagent pad up to a dilution of  $10^5$ . This solution was then used to add a fresh urine input ( 1/100 diln.) in order to study potential chemical carryover (cross-contamination) within the ABSS.

#### 3.2.3.2 Biochemical Testing Results

##### 3.2.3.2.1 Residual Volume - Direct Measurement

Four separate measurements with a "dry" instrument, i.e. air drying and disassembly as required, indicated that the average residual volume of fluid which would consistently remain within the fluid pathways of the ABSS was  $18.1 \text{ ml} \pm 0.3$ . This amount was used in calculating theoretical dilutions of subsequent fluid inputs to the ABSS.

---

\* Taussky, H.H. and Shorr E. J. Biol. Chem. 202: 675-85; 1953

#### 3.2.3.2.2 Residual Volume - Indirect Measurement

Table 3.2.3.2.2 summarizes the results of the experiments to determine the residual volume by indirect measurement, i.e. dye dilution technique. The percent dilution was calculated from the ratio of the optical density (OD) of the effluent to the original OD. The volume of fluid required to produce that percentage dilution can then be calculated (See Figure 3.2.3.1.3; #2, 3). Examination of the data suggests that the accuracy of the technique increases as the input volume increases.

The data also highlights the regular differences which occur between the calculated dilutions for the Sample Port effluent and those for the Dump Port. This suggests that the mixing of the residual fluid with each input fluid may not be homogenous.

This is demonstrated by comparing the calculated percent dilution derived from the Sample Port data; as the volume of input fluid increases, the calculated value becomes closer to the theoretical value.

#### 3.2.3.2.3 Residual Volume - Effect On Urine Phosphorous

As a part of a one-week experiment to study biochemical responses of the ABSS under actual use conditions inorganic phosphorus was assayed in an attempt to demonstrate the effect of the residual fluid on the chemical constituents in urine samples obtained from the Sample Port. Since routine operation of the ABSS included a water rinse of 80-85 ml following collection and distribution of the urine, it was assumed that a large portion of the residual fluid would be composed of the rinse water. Therefore, assays of phosphorus within urine aliquots obtained from the Sample Port should demonstrate reduction from the control (input sample) as a result of this dilution.



TABLE 3.2.3.2.2

RESIDUAL VOLUME - INDIRECT MEASUREMENT

PERCENT CHANGE IN OPTICAL DENSITY OF  
RHODAMINE B AT 550 NM AS AN INDICATOR  
OF THE DILUTION EFFECT OF THE RESIDUAL  
VOLUME ON VARIOUS INPUT VOLUMES

AVG. 4 VOL. 4	IN-PUT FLUID		OUT-PUT FLUID			% DILN OF COMBINATION 3	
	THEORETICAL 1 % DILN		SAMPLE PORT % DILN	% INPUT 2	DUMP PORT % DILN		% INPUT 2
90 ML	83.3		80.2	11.2	79.2	88.8	79.4
150 ML	89.3		88.5	11.3	86.1	88.7	86.3
300 ML	94.3		95.6	11.5	95.8	88.5	95.8

- 1 - Based on the avg. vol. added and the 18 ml residual fluid volume.
- 2 - Calculated from measured volume of original in-put delivered to each port.
- 3 - Calculated from Sample and Dump Port data and weighted for proportional volume.
- 4 - Avg. of a minimum of three replicate determinations/vol. level.

Ten consecutive urine samples from the ABSS and their corresponding controls were assayed for inorganic phosphorus content according to the method described by Taussky (See Appendix 4.1). Table 3.2.3.2.3 which summarizes the effect of residual fluid on P content, indicates a consistent reduction in the P found in the urine samples from the ABSS vs. the P in the control urine.

A Student's "t" test for non-paired experiments was performed on the data. The analysis demonstrated that the mean of the differences between the theoretically determined percent dilutions and those calculated from the experimental data was not significantly different from zero. Therefore, it was concluded that urine chemical constituents were diluted by the residual fluid. The magnitude of the dilution which occurs would appear to be dependent upon:

- The proportion of the residual fluid composed of rinse water versus the previous urine input.
- The level of the chemical constituent from the previous urine to be monitored.
- The sensitivity of the assay techniques.

#### 3.2.3.2.4 Residual Volume - Effect On Dip Stick Analysis

Of the seven routine urine constituents incorporated within the Multistix dip-and-read test strips, no naturally occurring abnormal levels were demonstrated by any of the urine used in the experiment. To study the effect of abnormally high chemical constituents, a hemaglobin reagent, prepared from fresh venous blood was added to a single urine input sample (run #53) at approximately a 1:100 dilution, and inserted into the instrument. The sample aliquot normally delivered by the ABSS demonstrated complete sensitivity (3+) to the presence of the high concentration of hemaglobin. Two more urine inputs of 330 ml and 141 ml (runs #54 and 55) were introduced to the ABSS after 24 and 48 hours, respectively. The sample obtained from Run #55 was still capable of demonstrating a minimum sensitivity (+) to the hemaglobin test pad on the

TABLE 3.2.3.2.3

SUMMARY OF URINE PHOSPHORUS DILUTION  
VALUES FROM CONSECUTIVE URINE SAMPLES  
FROM THE ABSS SAMPLE PORT

<u>RUN NO.</u>	<u>INPUT VOL.-ML</u>	<u>THEORET. % DILN<sup>1</sup></u>	<u>% DILN. OF P. DETN'D<sup>2</sup></u>
54	329	95	92
55	141	89	95
56	208	92	103
57	ND	ND	ND
58	193	91	93
59	390	96	89
60	133	88	89
61	122	87	90
62	314	95	75
63	333	95	86

ND = Not done

<sup>1</sup> = Theoretical dilution is based on the 18 ml residual volume assumed to be rinse water.

<sup>2</sup> = As calculated by the "t" test for paired experiments, the mean of the differences between theoretical and determined % dilutions is not significantly different from 0 ( $p > 0.1$ , but  $< 0.2$ )  $t = 1.64$

dipstick. Considering the water rinses of approximately 85 ml which were routinely associated with each input sequence, plus the two subsequent urine inputs, the total dilution effect on the hemaglobin reagent was calculated to be approximately 3000 fold. The original hemaglobin reagent was assayed at a titer of  $10^5$ ; the dilution from the urine input (run #53) of approximately 1:100 reduced this titer to  $10^3$ , making the total calculated dilution effect within the instrument to be  $3 \times 10^3$  or 1:3000. Thus, the carryover from the original abnormal hemaglobin was detectable in the sample after two additional urine inputs through the ABSS confirming, at least by the conditions of this test, the potential for cross-contamination from sample to sample.

The extent of this cross-contamination is dependent upon two variables: 1) the level or concentration of the biochemical constituent, and 2) the sensitivity of the assay technique for the constituent. Therefore, it would appear that the ABSS user-sequence should be consulted when abnormal amounts of biochemical constituents are detected in sample pools obtained from instruments.

### 3.2.3.3 Conclusions - Biochemical Testing

Results of the preliminary biochemical testing phase have demonstrated the following:

1. A consistent residual volume of approximately 18 ml remains in the ABSS during normal operation;
2. The residual volume causes dilution within the urine aliquot derived from the sample port; and
3. Carry-over of chemical constituents (cross-contamination) from sample to sample does occur.

Therefore, the following conclusions can be drawn:

- Potential carry-over of biochemical constituents from one urine input to the next, an uncontrollable variable, negates the use of proportional urine samples from the ABSS for research quality, bio-medical investigations.
- Arbitrarily, if the residual volume was reduced by one half, thereby reducing the dilution effect, the ABSS could provide adequate samples for routine clinical level testing, i.e. crew-health monitoring.

Note - Although not addressed in this Section, the presence of reservoirs of microbial contaminants would also negate the use of the ABSS for either use. Data presented in Section 3.2.4 confirm the presence of this contamination.

#### 3.2.3.4 Recommendations - Biochemical Testing

Future designs for urine sampling hardware should incorporate the following:

1. Shorter fluid pathways (reduces residual fluid);
2. Elimination of dead-ends, crevices, etc. which can impinge and hold fluid;
3. Reduction of complexity, i.e. elimination of valves where possible, and capability for "plug" flow to avoid mixing one urine with another. This would allow elimination of the water rinse incorporated within each cycle, thus reducing another interface, including reservoir, valves, lines, etc.

It is recognized that incorporation of these suggestions may reduce the degree of automaticity and/or increase operator involvement to a slightly higher level.

However, it is expected that the overall improvement in sample integrity will offset the disadvantages.

#### 3.2.4 Microbiological Response: Betadine\*/Disinfect Studies

##### 3.2.4.1 Objectives/Discussion

Experience with the ABSS during the biochemical, cross-contamination study, i.e. carryover of hemoglobin, suggested that microbiological contaminants would behave similarly. Therefore, a specific objective of the microbiological assessment was to study the behavior of adventitious urine microorganisms, as well as added microbial markers (contaminants), both from the urine sample aliquot (sampling mode), and from the waste urine tank. It was of interest to determine for example, if successive, urine inputs would significantly

---

\* Trademark for Povidone-Iodine, distributed by the Purdue-Frederick Company, Norwalk, Conn. 06856.

reduce or at least lower the viable bacterial population level within the ABSS. Some of the factors affecting this question are summarized below:

- Type of contaminant in each urine input (bacteria or fungus, hardy or labile, potential variety of microbial mix);
- Population size of the contaminant(s);
- Internal structure of the fluid pathways which would promote pockets or reservoirs of contamination, e.g. crevices, dead-ends, etc.;
- Volume and frequency of the urine inputs.

Because thorough investigation of all factors was beyond the intended scope of these studies, the total effect was measured primarily by assay of the viable microbial titer of successive urine samples delivered by the ABSS, and of selected samples obtained from the waste urine tank.

Another objective of these studies was the investigation of the efficacy of Betadine as an effective agent for both disinfection of the ABSS, and stabilization of microbial growth within the urine waste holding tank. It is necessary to establish the concentration of Betadine, in terms of available iodine required to provide effective destruction, or at least inhibition (stabilization), of microbiological contaminants found in the urine inputs.

Still another objective was the investigation of the efficacy of Betadine as a disinfectant applied on a use-by-use basis in a simulation of the Waste Collection System (WCS) as proposed for operation on the Space Shuttle Program. Because there is no requirement to measure urine volume, provide, a proportional sample, or a water rinse cycle, the WCS imposes simpler, less sophisticated operational demands than the ABSS. Disinfection in the WCS will be effected by insertion of uniform amounts of Betadine with each urine

input. Therefore, for these tests the ABSS was modified mechanically (described below) so as to simulate, as closely as possible, the operational characteristics of the WCS and the subsequent effects of the Betadine on the collected urine.

#### 3.2.4.2 Experimental Outline And Base

Experimental data for those objectives described above was derived from a series of 3 laboratory tests as follows:

1. Effect of frequent urine inputs on the microbial population within the ABSS without operation of the "Disinfectant" cycle -
  - Total Elapsed Time = 12 days
  - Total Urine Inputs = 87
  - Total Urine Volume = 17.7 liters
2. Effect of a single Betadine rinse on the microbial levels within the ABSS (as determined from effluent samples) -
  - Total Elapsed Time = 3 days
  - Total Urine Inputs = 20
  - Total Urine Volume = 3.1 liters
3. Effect of use-by-use addition of Betadine to a urine collection system which simulates the WCS, i.e. no sample mode, no water flush -
  - Total Elapsed Time = 4 days
  - Total Urine Inputs = 24
  - Total Urine Volume = 4.8 liters

Experimental conditions which were considered as baseline are listed below:

- All urine was collected from a single micturation and used within a maximum of 2 hours;
- Male and female urine was employed;
- All urine inputs were assayed for level of pre-existing microbial population;
- Rinse water used by ABSS was sterilized through use of in-line membrane filters (0.22 $\mu$ );
- Volume of rinse water employed was adjusted to ~ 70 ml/rinse following routine urine inputs, and 100 ml with the "Disinfect" cycle;

- ABSS was decontaminated before each experimental series with 2% peracetic acid;
- Volume of every input urine was determined by weight; and
- Once each operational day,  $10^6$  organisms/ml of E.coli was added, via urine input, for use as a marker organism in assaying experimental effects.

### 3.2.4.3 Methods And Procedures

Detailed procedures and materials used for each list sequence are reproduced in Appendix 4.1. The following represent general methods applied to each test.

#### Urine Pre-existing Microbe Assay

Replicate 1 ml quantities of each urine were incubated in trypticase-soy agar plates at 35°C. In cases where colonies were too numerous to count (TNTC), the count was arbitrarily recorded as  $10^3$ . In cases where more than one colony type was present, no attempt was made to separate the two types in the count.

#### Sterile Rinse Water

A stainless steel pressure can was used as a reservoir to hold the distilled rinse water. Sterility of the water was achieved by passage through a 142 mm membrane filter of 0.22 micron average pore diameter held in a Millipore stainless steel holder. A pressure of 17 psi (879 mm Hg) on the reservoir tank was sufficient to insure a final flow rate into the ABSS of 70 ml per rinse (normal rinse cycle), and during the "Disinfect" cycle, 100 ml of water.

#### Decontamination of ABSS

A mixture of 2% Peracetic Acid and Naccanol surfactant was used as the agent to decontaminate the ABSS fluid pathways before each experiment (See Protocol for exact formula and preparation). Five hundred ml quantities of the 2% acid were circulated within the ABSS and allowed to stand for 30 minutes. Two of these treatments were followed by 6-7, 500 ml portions of sterile, distilled water rinses - or until the pH of the effluent returned to 6.5 or higher. Sterility tests on neutral effluents from the sterile water rinses indicated that microbial contamination was not eliminated by this procedure. Levels of growth in trypticase-soy agar were about  $10^1$  colonies/ml. No further effort was made to eliminate this residual contamination.



### Volume of Urine Inputs

All micturations were collected in clean, 450 ml plastic containers which exhibited a uniform tare weight. Each urine was weighed before addition to the ABSS. The final volume was regarded as the total less the container tare weight. (Note - for these purposes, 1 gram of urine was considered to be equal to 1 ml.)

### Addition of E.coli as a Marker Organism

E.coli (ATCC #15144) was incubated for 18 hours at 35°C in a 500 ml flask containing 50 ml of trypticase-soy broth. Inoculum was 1 drop of a previous 18-hour culture held at 4°C. A fresh culture was prepared for each operational day. The titer of each 18-hour culture was assumed to be approximately  $5 \times 10^8$ . Therefore a 1:100 dilution was arbitrarily made in each urine to produce a final titer of E.coli of approximately  $5 \times 10^6$ . Actual counts were made on each "loaded" urine when the effluent sample was assayed.

### Titration of Effluent Samples and Urine Pool Samples

In order to judge the effect, if any, of the experimental conditions, effluent samples and urine-pool samples were assayed in Eosin-Methylene-Blue (EMB) Agar which exhibits dark red centers with the E.coli (Marker). Because other microorganisms could be inhibited by the EMB agar, parallel titrations were also performed in trypticase-soy agar to obtain a total microbial count.

### Modification of ABSS for WCS Simulation

The ABSS was modified mechanically to simulate the operational characteristics of the WCS. Fluid lines directly off of the proportional accumulator were altered to feed directly to the urine pool, thus by-passing the normal recirculate, sample, flush, and solenoid valve sequences. Rinse water was also turned off. To simulate addition of the disinfectant,

15 ml of full-strength (10%) Betadine was added to each urine input simultaneously with the insertion into the instrument. The total amount of Betadine added was calculated on a projected daily frequency and volume so as to provide a minimum of 600 ppm of free  $I_2$  in the urine pool at the end of each operational day. The net result of this modification was significantly faster processing of the rinse through the instrument. Also, with the substantially reduced fluid pathway, the peracetic acid rinse was significantly more successful in reducing contaminants: After two acid rinses and 5 sterile water rinses the viable count was < 1 organism/ml.

## Betadine Concentrations

Betadine is the tradename for a mixture of 10% iodine complexed with polyvinyl pyrrolidone, which provides 1% available or free I<sub>2</sub>. Therefore, at full-strength there is 10,000 ppm (10 gram/l. or 10,000 mg/l) available I<sub>2</sub>. All calculations as to initial and final iodine concentrations in the Betadine reservoir or in the urine pool were based upon those figures. It was assumed that the providone -I<sub>2</sub> complex behaves like a weakly dissociated salt, i.e. additional iodine is released as the free iodine is consumed.

### 3.2.4.4 Experimental Results

#### 3.2.4.4.1 Effect Of Sequential Urine Inputs On Microbial Population Of The ABSS

E.coli was added to an early urine input (1st or 2nd) once each operational day at a level of  $\sim 10^6$  organisms/ml. This was considered representative of a pathological level in a possible urinary infection, and therefore served as a marker organism to study the passage of a potential bacterial contaminant through the ABSS or its impingement within the fluid pathways. Since normal urines generally contain variable quantities of microorganisms ( $< 10^0$  to  $10^3$ ), it was necessary to determine if subsequent normal urine inputs, with low levels of bacteria, would flush out the marker, or become impinged within the fluid pathways and compete for nutritional dominance (See Section 3.2.4.2-1).

Decontamination of the ABSS with 2% peracetic acid was attempted before the start of the test; low levels of microorganisms were still recoverable in the effluent, even after 2 courses of treatment with the acid ( $\sim 10^2$  organisms/ml). For this study, selected samples of the urine effluents were assayed in both EMB and trypticase-soy agar to determine the levels of the marker (E. coli) and any other unidentified microorganisms which were recoverable. The test covered 7 operational days but encompassed a 12-day span, i.e. 3-day weekend between 3rd and 4th operational days, with a total of 87 urine inputs (average 12 per day) averaging 203.6 ml per input (17.7 liter total).

Table 3.2.4.4.1 presents the experimental results. It is apparent that the frequent urine inputs were not able to flush out the marker organisms, but were also contributing additional microorganisms to the system with subsequent multiplication and maintenance of titers at  $10^7$  to  $10^8$ . This strongly suggests that the fluid pathways (including phase separator and solenoid valves) contain abundant crevices and dead-end spaces which can serve as excellent reservoirs for bacteria. In addition, the inability of 2% peracetic acid to reach and lyse the contaminants lodged within the ABSS supports this conclusion.

Additional examination of the data suggests that the  $10^7$  per ml level probably represents a maximum titer, at least for the organisms in this study which were successful. Factors such as volume, nutrient supply, and mix of organisms are interacting limitations.

Additional information was also gathered from this experiment: The effects of accumulating quantities of urine, as in a normal use-condition, on the multiplication of the microbial population and corresponding production of gas in the urine waste tank was determined. At the end of each operational day, all lines from the ABSS were closed off with pinch clamps, and the air space above the urine was connected to a water manometer and allowed to stand overnight at room temperature ( $\sim 16$  hours). Each morning the pressure was recorded and the clamps removed, before sampling of the urine tank, and before the first urine input of the operational day.

Results are reported on Table 3.2.4.4.1-2. As shown, titers of both the marker (E. coli) and other contaminants quickly reached a level of  $10^7$  organism/ml (by day 2), and remained there. At the same time, the urine

TABLE 3.2.4.4.1-1

EFFECT OF SEQUENTIAL URINE INPUTS  
ON MARKER ORGANISM (E.COLI) AND  
ADVENTITIOUS ORGANISMS FOUND WITHIN  
FLUID PATHWAYS OF ABSS (SAMPLE EFFLUENT)

DAY	URINE INPUT DATA			URINE SAMPLE PORT ALIQUOT <sup>3</sup> COUNT/ML					
	TOTAL NO./DAY	TOTAL DAILY VOL. (ml)	AVE. DAILY VOL./INPUT (ml)	FIRST SAMPLE		SECOND SAMPLE		FINAL SAMPLE	
				<u>E.COLI</u> <sup>1</sup>	OTHER <sup>2</sup>	<u>E.COLI</u>	OTHER	<u>E.COLI</u>	OTHER
1	15	3312	214	4 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	4 x 10 <sup>5</sup>	< 10 <sup>3</sup>	4 x 10 <sup>3</sup>	< 10 <sup>1</sup>
2	14	2287	163	2 x 10 <sup>6</sup>	1 x 10 <sup>3</sup>	5 x 10 <sup>6</sup>	< 10 <sup>4</sup>	7 x 10 <sup>4</sup>	4 x 10 <sup>4</sup>
3	10	2428	243	> 10 <sup>4</sup>	> 10 <sup>4</sup>	8 x 10 <sup>7</sup>	3 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	9 x 10 <sup>5</sup>
4	12	2431	203	4 x 10 <sup>6</sup>	9 x 10 <sup>6</sup>	8 x 10 <sup>5</sup>	3 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	2 x 10 <sup>5</sup>
5	14	2872	205	NP	> 10 <sup>6</sup>	3 x 10 <sup>6</sup>	1 x 10 <sup>7</sup>	NP	5 x 10 <sup>5</sup>
6	12	2471	206	3 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>	1 x 10 <sup>7</sup>	4 x 10 <sup>7</sup>	3 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>
7	10	1912	191	NP	2 x 10 <sup>8</sup>	1 x 10 <sup>7</sup>	4 x 10 <sup>7</sup>	1 x 10 <sup>6</sup>	9 x 10 <sup>6</sup>

1 - E.coli (ATCC 15144) at ~ 10<sup>6</sup> organisms/ml added in second urine input each day (except 1st and 4th day).

2 - EMB Agar used to differentiate marker organism (E.coli) from naturally occurring contaminants.

3 - ABSS fluid pathways decontaminated with Peracetic Acid before start of test period.

NP = Count not possible, probably due to competitive inhibition with other contaminants.

TABLE 3.2.4.4.1-2

EFFECT OF SEQUENTIAL URINE  
INPUTS ON GAS PRODUCTION AND  
MICROBIAL TITERS WITHIN THE  
URINE WASTE POOL

DAY	URINE INPUT		URINE POOL			
	TOTAL DAILY VOL.	CUMUL. VOL.	INPUT NO.	COUNT/ML <sup>1</sup>		PRESSURE CHANGE (CM H <sub>2</sub> O <sup>2</sup> )
				<u>E. COLI</u>	OTHER	
1	2430	2430	12	0	3 x 10 <sup>8</sup>	- 2.3
2	2872	5302	14	1 x 10 <sup>7</sup>	> 10 <sup>8</sup>	-19.7
3	2471	7773	12	1 x 10 <sup>8</sup>	5 x 10 <sup>8</sup>	-54.5
4	1912	9685	10	2 x 10 <sup>8</sup>	2 x 10 <sup>8</sup>	ND

- 1 Samples for counts were drawn 24 hours after initial input for days 1, 2 and 3, and 72 hours after initial input for day 4.
- 2 Pressure was recorded from last input of day to first input of the next day (16 hours).

waste tank exhibited an ever increasing negative pressure from the endogenous respiration of the accumulated quantities of microbial contaminants. The urine reservoir consisted of a glass jug of ~ 16 liter capacity; by the end of day 3, the jug was approximately 0.6 full. This serves to explain, in part, the negative pressure of ~ 21 inches of water exhibited by the urine pool; i.e. reduced airspace and increased volume of urine/bacteria would tend to pyramid the effects of the withdrawal of gas from the available supply. The data underlines the potential risks in untreated urine waste pools, since the factors which tend to control the potential results, e.g. quantity, mix of organisms, etc. are essentially uncontrolled variables.

#### 3.2.4.4.2 ABSS/Betadine Disinfect Studies

##### 3.2.4.4.2.1 Introduction

The speed with which the adventitious microbial contaminants became established within the ABSS and the multiplication level they attained, as demonstrated by the previous experiment (see Table 3.2.4.4.1) suggests the need for a daily purge with an appropriate disinfectant. Betadine has been recommended as a potential candidate.

In structuring the Betadine-disinfection experiments, the underlying theory was that to be successful, the disinfectant was not primarily required to kill/inactivate all the microbial population, but merely to maintain the contaminants at an acceptable level, i.e. stabilize. This was especially true with respect to the urine tank which is a potential reservoir for a wide range of organisms. To the extent that the disinfectant-rinse can reduce the pockets of contaminants within the ABSS, the long-term growth potential in the waste reservoir could be reduced.

### 3.2.4.4.2.2 Effect Of Betadine On Microbial Contaminants Within Fluid Pathways Of The ABSS

An attempt was made to study the effect of the disinfectant on the level of contaminants within the instrument through the action of Betadine. The water flush of 70 ml following each urine input will produce a dilution of the 18 ml residual urine within the fluid pathways, which then results in a theoretical urine concentration of ~ 20%:

$$\frac{18 \text{ ml urine (residual)}}{18 \text{ ml} + 70 \text{ ml H}_2\text{O rinse}} \times 100 = 20\% \text{ (approximately)}$$

With this reduction in the quantity of protein (organic material). The Betadine was expected to be capable of antimicrobial activity against the indwelling contaminants, thus reducing the potential cross-contamination effect on the effluent urine samples. The concentration of Betadine to be delivered to the fluid pathways (phase separator, lines, etc.) was set at 600 ppm. This concentration was determined by a trade-off with the volume of the disinfectant reservoir needed versus the actual concentration of the Betadine applied within the fluid pathways. After mixing with the 18 ml of the 20% urine/water residual, the final concentration of Betadine was then calculated to be ~ 536 ppm:

$$\frac{150 \text{ ml (Betadine Flush @ 600 ppm)}}{150 + 18 \text{ ml residual}} \times 600 = \sim 536 \text{ ppm}$$

Note - 50 ml Betadine from disinfectant reservoir (at concentration of 1800 ppm) was mixed with 100 ml water, recirculated through instrument, and allowed to stand for 30 minutes.

Two disinfectant trials in the ABSS were carried out with these concentrations of Betadine. The experiments were conducted on two separate days following daily addition of the marker (E. coli), and at least 7 additional normal urine inputs. The effluent urine sample immediately before and after the "Disinfect" cycle (which requires ~ 1 hour, including the 1/2 hour dwell time) were titrated in both trypticase-soy and EMB agars. Results of this data are displayed in Table 3.2.4.4.2.2.

Betadine at the concentration indicated was ineffective in significantly reducing the viable titers of the indwelling contaminants. The small reductions observed, approximately 1 log, are probably the result of a dilution effect.

#### 3.2.4.4.2.3 Effect Of Betadine On Urine Waste Tank

In an attempt to control high levels of microbial contaminants within the urine waste tank, Betadine was added to the reservoir each operational day before insertion of the first urine input (See Section 3.2.4.2-1). Samples of the final urine/Betadine mix were assayed for the microbial count at the end of each operational day. Results are exhibited in Table 3.2.4.4.2.3.

As shown, microbial counts increased by 2 log increments each day from  $10^4$  to  $10^8$  organisms/ml. The amount of Betadine added each morning was based on projected volume of urine inputs. By day 3 concentration of the disinfectant was judged low, thus an additional 200 ml was added on day 3 in an effort to increase the average concentration. However, as shown by the data, the higher level of Betadine was unable to affect the steady increase in bacterial multiplication.

It was obvious, therefore, that higher concentrations of Betadine were required to achieve a stable population of ~  $10^6$  organisms/ml.



TABLE 3.2.4.4.2.2

EFFECT OF BETADINE DISINFECTANT  
ON MARKER ORGANISMS (E. COLI) AND  
RANDOM CONTAMINANTS WITHIN FLUID  
PATHWAYS OF ABSS (SAMPLE ALIQUOT)

TRIAL NO.	CONDITION	URINE INPUT		URINE SAMPLE COUNTS			
		VOL	PRE-COUNT	TSA	EMB AGAR		TOTAL
					E. COLI	OTHER	
1	Pre-Disinfection	212	$2 \times 10^1$	$2 \times 10^4$	$8 \times 10^4$	$5 \times 10^5$	$5 \times 10^5$
	Betadine Disinfection* @ 536 ppm	← Approximately 1 Hour →					
	Post-Disinfection	112	$1 \times 10^3$	$2 \times 10^4$	$1 \times 10^3$	$1 \times 10^4$	$1 \times 10^4$
2	Pre-Disinfection	208	$9 \times 10^1$	$6 \times 10^5$	$8 \times 10^4$	$5 \times 10^5$	$5 \times 10^5$
	Betadine Disinfection* @ 536 ppm	← Approximately 1 Hour →					
	Post-Disinfection	201	$<10^0$	$2 \times 10^6$	$6 \times 10^4$	$5 \times 10^6$	$5 \times 10^6$

\* Disinfection = Betadine at 600 ppm (1800 ppm in Reservoir) with 1/2 Hour Dwell Time

TSA = Trypticase Soy-Agar

EMB = Eosin-Methylene Blue Agar

**TABLE 3.2.4.4.2.3**

**EFFECT OF ADDITION OF BETADINE  
TO URINE WASTE TANK OF ABSS  
AT START OF EACH OPERATIONAL DAY**

Day	Urine Input		Betadine <sup>1</sup>		Urine Pool Count <sup>2</sup>	
	Total Daily Vol. (ml)	No. Of Inputs	Amount Added (ml)	Calc. I <sub>2</sub> Conc. In ppm (Final) <sup>3</sup>	<u>E. Coli</u>	Other
1	3312	15	100	233	1 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>
2	2287	14	100	345	7 x 10 <sup>4</sup>	2 x 10 <sup>6</sup>
3	2429	10	300	586	2 x 10 <sup>8</sup>	2 x 10 <sup>8</sup>

1. Betadine used was full strength 10% Povidone Iodine, as supplied by the manufacturer.
2. Samples for counts were drawn at end of operational day (24 hours).
3. Final concentration of I<sub>2</sub> in ppm represents cumulative totals at end of each day's urine inputs.

#### 3.2.4.4.3 In Vitro Assay Of Betadine In Water And Urine Versus E. Coli

An effort was made to better understand the potential efficacy of Betadine as a possible agent for decontamination of the ABSS/WCS as well as for control of microbial populations within the urine waste pool. A preliminary in vitro assessment of bactericidal activity of Betadine in both water and fresh urine was organized as follows: Duplicate dilutions of Betadine undiluted, 1:2, 1:10, 1:30, 1:100, and 1:1000 were made in water and urine so that the total volume of each dilution was 100 ml. The concentrations of Betadine were 10,000, 5,000, 1,000, 300, 100 and 10 ppm respectively; while the corresponding concentrations of water/urine were 0, 50%, 90% 96.6%, 99%, and 99.9% respectively. E. coli was added to each combination in an amount calculated to produce  $\sim 10^6$  organisms/ml. After 30 minutes incubation at room temperature, an aliquot was removed from each flask and placed in a thiosulfate buffer (to neutralize any residual Betadine) at a 1:10 dilution. Portions of this dilution were added to trypticase-soy broth, incubated at 35°C, and observed daily for evidence of microbial growth.

The data is displayed in Table 3.2.4.4.3. As shown, all concentrations of Betadine tested were effective against the standard challenge of E. coli when the diluent was water. With urine, none of the concentrations were effective. It may be speculated that the sharp differences demonstrated by this preliminary test were due to the presence of protein in the urine which was capable of complexing the freely disassociated iodine, and then driving the povidone-iodine mixture to the extinction of all iodine. This situation has been demonstrated with quaternary ammonium disinfectants and has been cited as the reason for their relative ineffectiveness in the presence of organic material.

**TABLE 3.2.4.4.3**

**RESULTS OF IN VITRO ASSAY OF DILUTIONS  
OF BETADINE<sup>1</sup> IN WATER AND URINE VERSUS  
E. COLI AT A CONCENTRATION OF 10<sup>6</sup> ORGANISMS/ML  
FOLLOWING 30 MINUTE INCUBATION AT ROOM TEMPERATURE**

Betadine Conc. - ppm		% Water- Urine					
		10,000	5,000	1,000	300	100	10
0	Water	+					
	Urine	N/A					
50	Water		+				
	Urine		0				
90	Water			+			
	Urine			0			
96	Water				+		
	Urine				0		
99	Water					+	
	Urine					0	
99.9	Water						+
	Urine						0

+ = Inactivation of E. coli, i.e. No Growth After Incubation

0 = No Inactivation of E. coli.

#### 3.2.4.4.4 Simulation Of WCS Operation With Betadine Disinfectant

Previous data has shown that normally occurring microbial contaminants in urine will, if unattended, quickly multiply in the waste urine tank with concomitant odor, gas pressure, and infection dangers to the crew. In addition, any crack, crevice, or dead-end space within the fluid pathways of the instrument is a potential reservoir of microbial contaminants which will continue to inoculate microorganisms into the waste tank. The Waste

Collection System (WCS) is a significantly less sophisticated instrument with correspondingly shorter fluid pathways, and a simpler operational sequence.

For these reasons, it was of value to modify the ABSS so that in operation it simulated the WCS, and then assess the potential efficacy of Betadine in this system as a disinfectant in order to achieve stability in the urine waste pool. The following changes were made in the ABSS:

1. Lines from the accumulator were reconnected directly to the urine waste tank. This automatically provided the following:
  - Elimination of the entire programmed sequencing of the ABSS, e.g. no recirculation, no air punge, etc.;
  - Prevention of the operation of the accumulator - urine flowed directly through as though a part of the fluid lines;
  - Significant reduction in the time required to process any urine input.
2. Rinse water cycle was eliminated.
3. Fifteen ml of Betadine (10% Povidone-Iodine) was added to each urine input simultaneously with the addition to the instrument. The 15 ml amount was based on an expected average input volume of 250 ml and an arbitrarily selected frequency of 8 inputs/operational day. This would provide an average free iodine concentration of  $\geq$  600 ppm by the end of each day's intake.

In an experiment designed to assess this modified WCS, aliquots were drawn from the urine waste pool at the start of each day, after the median urine (4th or 5th); and after the final one. Each aliquot was titrated immediately in trypticase-soy agar. The urine waste reservoir was re-connected each evening to a water manometer and allowed to stand at room temperature overnight (See Section 3.2.4.2-3). Pre-test decontamination with 2% peracetic acid reduced measurable bacterial counts within the modified ABSS to < 1 organism/ml. Table 3.2.4.4.4 records the results of this study.

As shown, total counts of the urine waste tank were low ( $10^2$ ) through the first day, and increased to  $10^6$  by day 3 with no measurable pressure being recorded. Visual observation of the tank showed that the urine/Betadine mixture remained translucent throughout, although there was an increasing accumulation of debris at the bottom of the reservoir. By day 4 - after standing overnight, but with no new addition of urine - the count had risen to  $10^7$ , still with no measurable pressure, positive or negative. The average Betadine concentration within the urine tank over the 4 day test period was equivalent to 727 ppm free iodine.

These results suggest the following interpretations:

1. Levels of Betadine at average concentrations of 700 ppm free iodine are able to show bacterial activity to the extent that gas production/consumption is significantly reduced over the time period encompassed by the test.
2. Lack of production of any pressure and the maintenance of translucence with the urine pool reservoir suggests that some level of "stability" has been approached.
3. However, continued increase in the total count indicates that multiplication of the microbial population is not being held in check by the concentrations of Betadine present.
4. Therefore, higher concentrations of Betadine will be required to establish and maintain a "stabilized" urine tank. In all probability, the increase in weight and volume of Betadine which is suggested by these experiments would be unacceptable.

TABLE 3.2.4.4.4

WCS SIMULATION<sup>1</sup>: ADDITION OF 15 ML BETADINE<sup>2</sup>/URINE INPUT;  
NO SAMPLE MODE; NO WATER RINSE - EFFECT ON URINE TANK

Day	Total Daily Urine Vol/ Input No.	Urine Tank Sample Sequence	Cumulative Fluid Totals			Urine Tank	
			Urine Betadine Vol.-ml	Urine Conc.-%	Free I <sub>2</sub> Conc.-ppm	Count/ml <sup>3</sup>	Overnight Gas Production
1	1499/8	First	181	91.7	827	2 x 10 <sup>2</sup>	ND
		Middle	1160	93.5	647	4 x 10 <sup>2</sup>	
		Last	1619	92.6	741	3 x 10 <sup>2</sup>	
2	1484/8	First	1618	92.6	741	5 x 10 <sup>2</sup>	< 1 mm
		Middle	2731	92.8	714	3 x 10 <sup>5</sup>	
		Last	3223	92.5	745	4 x 10 <sup>5</sup>	
3	1770/8	First	3223	92.5	745	4 x 10 <sup>6</sup>	< 1 mm
		Middle	4507	93.0	699	4 x 10 <sup>6</sup>	
		Last	5113	92.9	704	4 x 10 <sup>6</sup>	
4	—	First	5113	92.9	704	5 x 10 <sup>7</sup>	< 1 mm

1. Following changes were made on ABSS to provide the WCS Simulation: 1) Discharge lines from accumulator connected directly to urine waste tank; 2) Water rinse disconnected.
2. Betadine full strength (10,000 ppm) was added to each urine before circulation through WCS; total daily amount of Betadine added = 120 ml (8 x 15).
3. Bacteria plate count for first sample on Day 2, 3 and 4 represents overnight incubation at ambient temperature.

### 3.2.5 Miscellaneous Information - ABSS Testing

During operation of the ABSS and throughout the various tests/studies, relevant information, facts, operational detail, etc. have been accumulated which do not bear directly on any of the specific problems being addressed. Listed below is a compilation of this information which can be useful in the total evaluation of the ABSS and for use in future design/experiments.

● Total Fluid Input	102 liters
No. of Inputs	341
● Total Urine Input	28.3 liters
No. of Inputs	143
Ave.	198 ml
Male Urine	
No.	92
Ave.	209 ml
Geom. Mean	202 ml
Total	19.2 liters
Female Urine	
No.	51
Ave.	178 ml
Geom. Mean	174 ml
Total	9.1 liters
● Operational Information	
Recirculate Cycle	45 Sec.
Sample Cycle	0.47 Sec./ml
Air and Water Purge	190 Sec.
Total Operation Time	34.2 hours
● Naturally Occurring Microbial Titters	
Male	$6 \times 10^2$ org./ml
Female	$2 \times 10^3$ org./ml

**Note:** Pre-use counts were accomplished by incubating replicate plates containing 1 ml of urine in trypticase-soy agar. Urine counts on Female sample were often overgrown and the titters were arbitrarily set at  $10^3$ . Therefore, average count of female urines is probably slightly higher.



### 3.2.4.5 Conclusions And Recommendations

#### 3.2.4.5.1 Conclusions

Results of the studies presented in this Section which involve the response of the ABSS to urine microbial contaminants and Betadine disinfection have demonstrated the following:

#### Microbiological Responses

- Fluid pathways within the ABSS contain a number of reservoirs (dead-ends, cracks, etc.) which quickly capture and hold available microbiological organisms within the ABSS.
- These pockets, or microbial reservoirs, re-infect (cross-contaminate) all incoming fluids.
- Frequent rinsing with normal urine inputs did not reduce the high titers ( $\sim 10^7$ ) maintained within these pockets.
- The waste urine tank at ambient temperatures, provides excellent media for rapid multiplication of microbial organisms with concomitant production of:
  - High titers of microorganisms ( $\sim 10^8$ )
  - Gas pressure - positive or negative
- Peracetic acid (2%) was not able to flush pockets of contamination within the fluid pathways of the ABSS.

#### Betadine Disinfection Studies

- Betadine at concentrations of up to 578 ppm of free  $I_2$  was ineffective in either reducing or controlling populations of the E. coli marker, or the naturally occurring urine contaminants within the fluid pathways of the ABSS.
- Betadine at concentrations averaging 727 ppm of free  $I_2$  appeared to inhibit the formation of gas pressure (positive or negative) in the waste urine tank of the WCS simulation.

Therefore, the following conclusions are suggested:

1. The ABSS, in its current design, is inappropriate as an instrument to provide representative urine samples for research-level testing,

due to the inherent susceptibility of the fluid pathways to form pockets of contamination and then re-infect (cross-contaminate) succeeding urine inputs/samples.

2. Betadine is generally unacceptable as a disinfectant and decontaminant for the ABSS, due probably to presence of quantities of organic material (primarily protein) which inactivates the available iodine as fast as it is released.
3. Stabilization, or maintenance of the microbial population below pre-set limits, e.g.  $\sim 10^6$  organisms/ml, is probably an unrealistic goal, especially with Betadine. Objective should be for total inactivation of all microbial activity as a solution for the control of biological events within the fluid pathways and waste urine reservoir.
4. Modification of ABSS to simulate the WCS activity provided, from the microbiological response viewpoint, a very positive improvement: Significantly fewer pockets of contamination, faster processing, and inhibition of gas pressure.

#### 3.2.4.5.2 Recommendations

Experience with the ABSS and Betadine as the disinfectant underlined the factors, mentioned earlier, which influence the microbial activity within a urine collection system, i.e. type and mix of microbial organisms, population size, antimicrobial spectrum of the disinfectant, and volume/frequency of urine inputs. The permutations among these variables and others would involve an unacceptably high volume of testing. An effective solution to this dilemma is to provide an overkill with the disinfectant employed, i.e. efficacious, broad spectrum of antimicrobial activity. Therefore, the following recommendations suggest themselves:

- Compile and investigate new candidates for use as decontaminants in a Waste Collection System (urine, feces, condensate, wash fluids, etc.) with a combination of the following:
  - Both chemical and antibiotic agents, e.g. streptomycin, neomycin, etc. as potential disinfectants;

- Use of two or more agents in combination;
- Possibility of incorporation of the agent(s) into dry (freeze-dried ?) or pelletized form which will:
  - a. Reduce or eliminate potential toxicity
  - b. Decrease volume requirements
  - c. Reduce weight demands.
- Redesign of fluid pathways so as to accomplish:
  - Elimination of cracks, crevices, dead-ends within internal structure of fluid pathways.
  - Severe reduction in length of fluid pathway.

## 4.0 APPENDIX

### 4.1 Test Plans And Protocols

- 4.1.1 ABSS Micro/Biochemical Assay
- 4.1.2 Betadine Rinse - WCS Study
- 4.1.3 ABSS/Betadine Rinse Study - Part I
- 4.1.4 Betadine/ABSS Rinse Study - Part II
- 4.1.5 Hemaglobin Titration
- 4.1.6 ABSS - Microbiological Assessment And Extended Biochemical Cross-Contamination Study
- 4.1.7 Microbiological Sampler - Preliminary Studies
- 4.1.8 7-Day ABSS Operational Trial
- 4.1.9 Quantitative Recovery Of Bacteria From Microbial Samplers
- 4.1.10 Protocol For Determining Efficacy Of Betadine For Use In ABSS Urine System

### 4.2 Thermoelectric Cooling Design Details

~~PRECEDING PAGE BLANK NOT FILMED~~

## 4.1 Test Plans And Protocols

### 4.1.1 ABSS Micro/Biochemical Assay

Purpose To provide a "first-cut study" on the microbiological and biochemical response of the ABSS within the following parameters:

- Short-term and long-term effect of known bacterial contaminants within the instrument;
- Efficacy of Betadine as an antibacterial flush;
- Ability of the Microbiological Sampler to provide efficacious, real-time samples for microbiological sampling; and
- Continuing assessment of the potential effects of chemical cross-contamination on the ABSS.

Discussion The complex chemical and microbiological inter-relationships within a sophisticated instrument such as the ABSS cannot be completely explored or defined with one experiment. It is planned that this experiment, therefore, will involve the basic elements of those parameters, and will serve as a foundation for additional experiments to provide more explicit definition.

Method A. Microbiological Elements

1. Short-term/long-term effect of known contaminants
  - Test organism - E.coli, ATCC #15144.
  - Carrier - Urine, obtained as fresh, random volumes from males only.

- Level of infection - approximately  $10^6$  organisms/ml
- Baseline Conditions
  - ABSS will be dried-out as per chem. cross-contam. studies, and fresh male urine added to instrument to "load" the ABSS with urine, as under normal user conditions.
  - Pre-test aliquots will be taken from every urine input for assay-Biochem./Micro.
  - Effluent from Sample and Dump ports will be collected separately from each urine input. Collection will be made in pre-weighed, sterile containers.
  - All urine inputs will be weighed before addition to ABSS.
  - ABSS will be assayed before start of experiment for background microbiological load.
- Schedule of urine sample inputs - These inputs will be using the fresh, male urine collected on random basis.
  - "Seeded" sample - 0 time
  - 1st sample - 5-10 minutes post
  - 2nd sample - 1 hour post
  - 3rd sample - 24 hour post
  - 4th sample - 48 hour post
  - 5th sample - 72 hour post
- Assays - All pre and post samples will be titered in Trypticase-Soy Broth and Agar according to a dilution schedule in a following section.

2) Efficacy of Betadine as an anti-bacterial flush.

- Organism - E.coli ATCC #15144 (As Above).
- Carrier - Urine freshly collected, male, random volume (As Collected).
- Betadine concentration - 10% in water solution furnished in commercial containers.
- Amount - As prescribed by automatic "Disinfect" cycle together with rinse water.
- Sample schedule
  - Seeded Urine -  $10^6$  organisms/ml
  - Betadine Flush - 5-10 minutes post
  - 1st Sample - 1 hour post
  - 2nd Sample - 24 hour post

3) Efficacy of Microbiological Sampler - See separate protocol for Micro Sampler. Assay is carried on away from ABSS, since the instrument itself is not critical to the ability to provide real-time sample for microbiological assay.

B. Biochemical Elements

1. Biochemical Parameters

- Phosphates - As inorganic phosphorus - See separate protocol for details of assay. (Colorimetric Assay).
- Specific Ion Analysis - Electrochemical
  - Chloride ( $\text{Cl}^-$ )
  - Sodium ( $\text{Na}^+$ )
  - Potassium ( $\text{K}^+$ )
  - Calcium ( $\text{Ca}^{++}$ )

- Dip Stick Analysis (Ames) - Color Comparison

- pH
- Glucose
- Protein
- Acetone
- Occult Blood
- Bilirubin
- Urobilinogen

Occult blood test, from list above, is extremely sensitive to hemaglobin (Hgb). Therefore, a stock solution of Hgb will be obtained by mixing fresh, whole blood with distilled H<sub>2</sub>O. This stock will be titered (See Separate Protocol) for limit of sensitivity to the "occult blood pad" on the dip stick by dilution in water.

2. Sample Schedule - Biochemical tests as listed above will be performed on each urine sample as defined under the Microbiological Elements section.

- Pre-add'n aliquot - 20 ml.
- Post-add'n - from Sample/Dump lines - as collected.
- All urine will be weighed (After Removal of Pre-add'n aliquot).
- All samples (Sample/Dump) will be weighed and recorded for calculation of urine recovered versus urine added.



### 3. Assay

- Phosphate - As soon as possible after obtaining each sample, a 1:100 dilution in H<sub>2</sub>O will be made and stored at ~ 4°C until assay can be performed. Assay will be done so as to provide complete data (1 pre and 2 post) on each sample simultaneously.

#### Materials

1. TS Agar - 5 liters, in 500 ml lots.
2. Petri Plates - ~ 180 = 9 packages.
3. Sterile flasks for primary collection of urine  
12 500 ml flasks.
4. Sterile flasks for collection of samples.
  - Sample Port - 15 250 ml flasks
  - Dump Port - 15 500 ml flasks
5. Sterile 2-hole stopper assemblies - All stoppers will contain opening for tubing from Sample port and cotton stuffed air-relief tube wrapped individually in brown paper and sterilized in the autoclave.
  - Sample Port - 15 2-hole #6-1/2 stoppers
  - Dump Port - 15 2-hole #8 stoppers
6. Sterile in-line air filter for nitrogen line.
7. Sterile, individually wrapped rubber tubing with glass delivery tube for insertion into Sample port at each urine addition.

15 units

8. Sterile pipettes, 10, 5, 2 ml - as needed.
9. Sterile H<sub>2</sub>O - in 900 ml lots - 6 - 8.
10. Sterile 99 ml blanks - 60.
11. Sterile 20 ml serum vials for holding pre-test samples - 15.
12. Ames Multi-stix for urine tests.
13. Reagents for the Phosphate assay - see phosphate protocol for details.
  - TCA
  - Molybdate Reagent

## Timeline/Procedure

### Monday - 24 March

1. Wash several lots of sterile water through ABSS. Capture final 50 - 100 ml portion with sterile assembly and assay for microbial background.
2. Drain fluid and dry-out fluid pathways of instrument.
3. Refill instrument with pre-weighed urine. Pre-sample urine before putting into instrument. Sample and titrate aliquots from Sample and Dump ports of instrument. (3 titrations/sample to values of 0.1 ml, 1 ml, 10 ml)
4. Introduce 2nd urine sample into instrument after having added hemoglobin reagent. Pre-sample urine before putting into instrument. Sample and titrate (3 per sample to values of 0.1 ml, 1 ml, 10 ml).
5. Inoculate two (2) 500 ml flasks containing 50 ml TSB with E. coli.

### Tuesday - 25 March

1. Take pre-sample of first urine. Inoculate remaining urine with E. coli to approximately  $10^6$  organisms/ml.  
Titer E. coli inoculum,  $10^6$ ,  $10^7$ ,  $10^8$   
Titer seeded urine,  $10^5$ ,  $10^6$ ,  $10^7$
2. Add seeded urine to ABSS. Take sample aliquots from Sample and Dump ports and titer  $10^5$ ,  $10^6$ ,  $10^7$ .
3. Pre-sample urine (unseeded); titer 0.1, 10, 1 ml. Take sample aliquots from Sample and Dump ports and titer  $10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$ .
4. Add unseeded urine sample (urine sample #5). Pre-sample urine before addition to ABSS and titrate to 0.1, 1, 10 ml. Take aliquot samples from Sample and Dump ports and titrate to  $10^1$ ,  $10^2$ ,  $10^3$ . (Two plates each)

### Wednesday - 26 March

1. Add 6th urine sample, unseeded, to ABSS after first taking pre-sample aliquots at values of 0.1, 1, 10 ml. Take aliquot samples from Sample and Dump ports at values of 0.1, 1, 10 ml. (Two plates each)

### Thursday - 27 March

1. Add 7th urine sample, unseeded, to ABSS. Pre-sample urine before addition to ABSS and titrate to 0.1, 1, 10 ml. Take aliquot samples from Sample and Dump ports at values of 0.1, 1, 10 ml (Two plates each).
2. Inoculate two (2) 50 ml flasks with E. coli and incubate at 37°C.

### Friday - 28 March

1. Add 8th urine sample (unseeded) to ABSS. Pre-sample before addition taking 0.1, 1, 10 ml samples. Collect aliquots from Sample and Dump ports. Titrate at values of 0.1, 1, 10 ml (Two plates each).
2. Pre-sample 9th urine sample taking 0.1, 1, 10 ml samples.

Into the remaining urine inoculate E. coli to obtain a concentration of  $10^6$  organisms per ml using fresh culture from 27 March. Sample and titrate to values of  $10^5$ ,  $10^6$ ,  $10^7$ .

Add seeded urine to machine, process, and collect aliquots from Sample and Dump ports. Titrate to values of  $10^3$ ,  $10^4$ ,  $10^5$  (Two plates each).

3. Activate "disinfect" cycle on ABSS.
4. Add 10th urine sample after taking pre-sample aliquots of 0.1, 1, 10 ml. Sample Dump and Sample ports and titrate to 0.1, 1, 10 ml (Two plates each).  
  
Perform add-backs to confirm ability of media to support growth using spore strips.
5. Pre-sample 11th urine at aliquots of 0.1, 1, 10 ml. Add sample to ABSS and Sample Dump and Sample ports and titrate to 0.1, 1, 10 ml (Two plates each).

### Monday - 31 March

1. Pre-sample 12th urine at aliquots of 0.1, 1, 10 ml. Add sample to ABSS and Sample Dump and Sample ports and titrate to 0.1, 1, 10 ml (Two plates each).

#### 4.1.2 Betadine Rinse - WCS Study

##### Purpose

To investigate the effect of use-by-use application of Betadine to a urine collection system which operates in the absence of a water flush and without proportional sampling requirements.

##### Discussion

The WCS, as currently designed, will receive urine, remove the gases, and deposit the liquid in a closed, variable volume storage container. At some point within the WCS a defined volume of Betadine will be injected into the system during each use. The total daily amount of Betadine will be enough to provide a minimum effective concentration of  $I_2$  for the maximum expected volume. It is necessary to determine whether Betadine equivalent to 600 ppm free  $I_2$  in the waste container is effective in preventing the growth of microorganisms.

One of the problems associated with such growth is the possibility of increased or decreased pressure within the waste container due to production or assimilation of gases.

##### Procedure

In order to simulate conditions in the WCS, the lines from the accumulator in the ABSS will be disconnected and made long enough to reach a waste container. The ABSS will be treated twice, for 15 minutes each time, with 500 ml 2% peracetic acid containing 0.05% Nacconol 60TL. Sterile water rinses of about 500 ml will be put through the unit until the pH of the effluent is approximately equal to that of sterile water. A titration of the organisms present will be made on the final rinse effluent.

For three days, eight urines/day will be circulated through the altered unit into a sterile six liter Florence flask fitted for attachment to a manometer. One urine/day will contain E.coli at  $10^6$  organisms/ml. Before introduction, 15 ml of commercial (10%) Betadine will be injected into the sample. This amount is sufficient to give 600 ppm free  $I_2$  in the waste container if the average input is 250 ml. No water flush will be used and ABSS will be turned off between samples.

An aliquot of the waste fluid will be treated with sodium thiosulfate to neutralize the  $I_2$  and titrated on trypticase soy agar and on EMB agar after the first, fifth and last samples on the first day and initially, and after the fifth and eighth samples on the second and third day.

A manometer will be attached to the waste container at the end of each day to measure changes in pressure overnight.

Operational Procedure:

1. Weigh urine, add E coli 1/100 if called for, and introduce.
2. Titrate E.coli at  $10^{-7}$ ,  $10^{-8}$
3. Take aliquots of waste after first (or before first on days 2 and 3) sample, and fifth and eighth samples.
4. Dilute aliquots 1/10 in neutralizer-phosphate buffer prepared by taking 11 ml of sodium thiosulfate, 4%, in 99 ml phosphate buffer.
5. Dilute  $\frac{1}{100}$   $\frac{1}{10}$   $\frac{1}{100}$  in phosphate.
6. Plate 0.1 ml on EMB x2 and on trypticase soy x 2.
7. Attach manometer at the end of the day and record pressure the next morning.

### 4.1.3 ABSS/Betadine Rinse Study - Part I

Purpose To determine the level of available iodine ( $I_2$ , in PPM) necessary to neutralize or decontaminate the expected water/urine/bacteria mixture present within the ABSS following a normal usage day.

Discussion The normal water rinse of  $\sim 70$  ml, following the input of urine to the ABSS, will dilute the residual urine (from the input) to about 20%, i.e. 18 ml residual urine + 70 ml rinse =  $\sim 20\%$  urine. When the Betadine/water disinfect rinse is employed, the 20% urine residual is the fluid within the instrument which will be encountered. Therefore, it is necessary, as a first step, to determine in vitro what levels of  $I_2$  will be effective against various mixtures of contaminated urine/water.

Procedure Theory The study will employ a matrix of 4 x 4, i.e. 4 levels of Betadine encompassing the concentrations which are expected to be employed versus 4 mixtures of urine/water/bacteria also encompassing the range of dilutions expected. Graphically, this is represented by the block below:

Avail. $I_2$ in PPM	100	300	600	1000
% Urine				
0				
10				
20				
30				



The "Disinfect" cycle is normally employed as follows: 50 ml is pumped from the disinfectant reservoir followed by 100 ml of sterile rinse water. This 150 ml mixture becomes a 1:3 dilution of the Betadine from the reservoir. When the 150 ml is circulated throughout the fluid pathways, it then mixes with 18 ml residual mixture of water/urine. This ratio of  $\frac{150}{168}$  is equivalent to  $\frac{10}{11.2}$ . Therefore, 1.2 ml of each water/urine/bacteria (contaminant) mixture will be placed in each row of 4 tubes. The 4 dilutions of Betadine containing 100, 300, 600, and 1000 PPM, respectively, will be added at the level of 10 ml to each rank of 4 tubes so that each dilution of Betadine will be exposed to each concentration of water/urine/bacteria in the approximate dilution ratio expected within the ABSS.

The dwell-time of these mixtures will be 30 minutes (dwell-time of disinfectant rinse in the ABSS) followed by 1:10 dilutions in sterile sodium thiosulfate, to neutralize any residual I<sub>2</sub>, and two additional 1:10 dilutions in sterile phosphate buffer. Each of these dilutions will be plated using EMB Agar, incubated at 35°C for 24 hours, and enumerated to determine the titer of each experimental mixture.

Procedure  
Opera-  
tional

- 1) Set up the sterile 16-tube matrix with appropriate labels.
- 2) Prepare the urine/water mixtures in separate tubes as follows:
  - 0% urine - 19.8 ml sterile H<sub>2</sub>O
  - 10% urine - 2 ml urine + 17.8 ml sterile H<sub>2</sub>O
  - 20% urine - 4 ml urine + 15.8 ml sterile H<sub>2</sub>O
  - 30% urine - 6 ml urine + 13.8 ml sterile H<sub>2</sub>O.

- 3) To produce the bacterial contaminant for each mixture, add 0.2 ml of an 18 hour culture of E.coli (ATCC 15144) to each tube for a 1:100 dilution of the bacteria. This should provide a final concentration of  $\sim 10^6$  organisms/ml for each water/urine mixture.
- 4) Betadine dilutions - Since the Betadine solution will be further diluted in the ABSS by the introduction of the rinse water (100 ml) for an additional 1:3, the initial dilutions will be as follows:

<u>Final Betadine Conc. Desired-PPM</u>	<u>Final Dilution Required</u>	<u>Stock Betadine</u>	<u>Sterile Water</u>
100	1:100	0.5 ml	+ 49.5
300	1:33	1.5	+ 48.5
600	1:15	3.3	+ 46.7
1000	1:10	5.0	+ 45.0

- 5) Make 20, 9 ml blanks of Na Thiosulfate/Phosphate buffer (See Materials For Formula).
- 6) Make 35, 9 ml blanks of phosphate buffer (See Materials).
- 7) Add 1.2 ml of the appropriate water/urine/bacteria mixture to each row of the 16 tube matrix.
- 8) Add 10 ml of each Betadine dilution to the appropriate rank of water/urine/bacteria mixture at 10 second intervals so that the dwell-time for each of the 16 experimental mixtures can be controlled to exactly 30 minutes.

- 9) Remove 1 ml from each tube in the same order it was added at 10 second intervals and place in the 9 ml blank of neutralizer/buffer - mix thoroughly.
- 10) Make 2 more successive 1:10 dilutions from each tube and plate 0.1 ml on 2 replicate EMB plates/dilution for a final titration of  $10^2$ ,  $10^3$ ,  $10^4$ . Incubate at 35°C for 24 hours.

Materials

- 1) 20 mm screw-cap tubes for matrix - sterile, 18 needed.
- 2) 9 ml neutralizer/buffer blanks - sterile, 18 needed.
- 3) 9 ml buffer blanks - sterile, 35 needed.
- 4) Neutralizer - See previous protocol.
- 5) Buffer - See previous protocol.
- 6) E.coli - Inoculate 18 hours pre with 1 drop from previous culture. Incubate at 35°C.
- 7) EMB plates - 100 needed.
- 8) Spreaders - Approximately 35 needed, sterile.
- 9) Betadine - Stock.

#### 4.1.4 Betadine/ABSS Rinse Study - Part II

##### Purpose

To examine the effect of daily Betadine rinses in the ABSS using levels of Betadine that have been determined in vitro to be effective against the expected water/urine/bacteria concentrations.

##### Discussion

Programmed operation of the ABSS following input of urine calls for the conclusion of ~70 ml of rinse water after passage of the urine. The expected 18 ml residual of the urine within the system when mixed to this 70 ml water results in approximately a 20% urine mixture as the new 18 ml residual. Previous in vitro testing has established that 600 ppm of Betadine will effectively inactivate (neutralize) the contaminant in this 20% urine/water mixture. Therefore, the thrust of this experiment will be to use this Betadine concentration within the "Disinfect" cycle of the ABSS and determine if any significant effect of the Betadine, so added, can be discerned under actual "use" conditions.

##### Baseline Conditions

1. Test will be conducted for two use-days using the programmed single "Disinfect" cycle per day.
2. ABSS will be decontaminated/cleaned in 2% peracetic acid before start of test.
3. Minimum number of urine inputs will be 10/day.
4. One "loaded" urine sample, i.e. E. Coli at  $10^6$ /ml, will be added to the instrument at the start of each test day.
5. Selected samples of the effluent "Samples" port will be taken each day and titrated for total count of bacteria.
6. Titrations will be conducted on aliquot samples of the urine pool.
7. All samples except the pre-urine sample, will be titrated in both EMB and TSB.

## Timeline/procedure

### Monday - 30 June

1. Place urine pool reservoir (19L jug) in position for use - all connections.
2. Run final sterile water rinse through, collect sample, and titrate in TSB for possible level of contaminants in system at start - use spore strip in tube as control for residual peracetic acid.  
  
Dilns - 10<sup>2,3,4</sup>(2 replicates/diln.)
3. Inoculate flask of TSB broth w̄ drop from previous culture - 4 p.m. - to give 18 hour growth at 35°C (E. coli).
4. Prepare Betadine solution and add to reservoir bag.

### Tuesday, 1 July

0 min	1 ml Pre-sample - Urine 1	1-1
	Add <u>E. coli</u> (1/100 diln) and titrate	1-2
	4,5,6 Sample - Urine 1	1-3
30 min.	1 ml Pre-sample Urine 2	1-4
	3,4,5 Sample Urine 2	1-5
1-4 hrs.	1 ml Pre-sample, Urine 3,4,5	1-6
		1-7
		1-8
4 hrs.	1 ml Pre-sample - Urine 6	1-9
	2,3,4 Sample Urine 6	1-10
5-7 hrs.	1 ml Pre-sample Urine 7, 8	1-11
		1-12
7 hrs.	1 ml Pre-sample Urine 9	1-13
	2,3,4 sample Urine 9	1-14
7:15	Disinfect cycle using Betadine at final diln. of <u>600</u> ppm.	
8 hrs.	1 ml Pre-sample Urine 10	1-15
	2,3,4 Sample Urine	1-16
8:15	Aliquot of Urine Pool 3,4,5,6	1-17
-	Inoc. <u>E. Coli</u> flask	

Wednesday, 2 July

0 min.	4,5,6 Sample Urine Pool	2-18
0 - 15 min.	1 ml Pre-sample - Urine 11	2-19
	2,3,4 Sample Urine 11	2-20
	1 ml Pre-sample - Urine 12	2-21
	4,5,6 add E. Coli, enter, Sample	2-22
1 hour	Pre-sample - Urine 13	2-23
	3,4,5 Sample - Urine 13	2-24
1-4 hours	Pre-sample Urine 14	2-25
	15	2-26
	16	2-27
	17	2-28
5-6 hours	Pre-sample Urine 18	2-29
6:15	Pre-sample Urine 19	2-30
	2,3,4 Sample - Urine 19	2-31
6:30	Disinfect Cycle	
7:30	Pre-sample Urine 20	2-32
	2,3,4 Sample Urine 20	2-33
7:45	3,4,5,6 Sample Urine Pool	2-34

Thursday - 3 July

0 min.	4,5,6 Sample Urine Pool	3-35
--------	-------------------------	------

Disinfect with Peracetic Acid Soln. with usual sterile H<sub>2</sub>O rinses and pH checks.

Materials

1. Sample Flasks - 250 ml with stoppers/air valve-sterile - need 17.
2. EMB Plates - Approx. 50 dilns x 2 plates/diln = 100 plates x 20 ml/plate; 5 flasks or 2.5 liters EMB Agar.
3. TSB Agar - Approx. 70 dilns. x 2 plates/diln - 140 plates x 20 ml/plate = 7 flasks or 3.5 liters TSB Agar.
4. 9 ml water diln. Blank - Sterile - 90 needed.
5. 99 ml water diln. Blanks - sterile - 20 needed.
6. Betadine diln,- TBD.

#### 4.1.5 Hemaglobin Titration

Purpose To provide a stock solution of free hemaglobin of a known titer with respect to the chemically sensitive pad for occult blood on the Ames Multistix tabs for urine assessment.

Discussion Investigation of the potential chemical cross-contamination of individual urine inputs to the ABSS requires in part, an easily detectable biochemical constituent in the urine which will act as a tag or tracer. High sensitivity of the Ames Multistix to Hgb. make this an ideal system for that purpose.

Method Freshly drawn, non-heparanized blood will be mixed immediately with sterile, distilled H<sub>2</sub>O to produce hemolysis. This solution of hemolysin/hemaglobin will be considered the stock solution. Dilutions will be made, first in water, then in urine, and tested for a positive reaction to the occult blood pad on the Ames Multistix. Results of this titration will be used as the basis for addition to fresh urine samples during the test period of the ABSS.

Materials Sterile 5 ml syringe.  
Sterile dist. H<sub>2</sub>O - 1 liter.  
Supply of Ames Multistix.  
Freshly collected male urine.

Procedure

1. Draw 3-4 ml. venous blood and add immediately to equivalent amount of sterile H<sub>2</sub>O.
2. Centrifuge, if necessary, to remove any debris or cell wall remnants from RBC. Store supernatant at 5°C when not in use.
3. Make following dilutions of H<sub>g</sub>b. stock in H<sub>2</sub>O:  
10<sup>-1</sup> to 10<sup>-9</sup> in 10 fold dilutions.
4. Test each dilution by adding 1 drop of H<sub>g</sub>b solution to appropriate test pad on Multistix to determine titer.
5. Repeat test at dilutions of smaller increments on either side of the titer from 4 above.
6. Repeat on appropriate dilutions of fresh urine.



#### 4.1.6 ABSS - Microbiological Assessment And Extended Biochemical Cross-Contamination Study

Purpose To make a preliminary investigation of the microbiological responses for the ABSS in its present configuration, and to extend the scope of the chemical/biochemical cross-contamination studies.

Objectives The specific investigative objectives may be subdivided as follows:

A. Biochemical Parameters to be studied

1. Phosphates as inorganic phosphorus - colorimetric analysis.
2. Specific ion analysis - electro chemical
  - Chloride ( $Cl^{-}$ )
  - Sodium ( $Na^{+}$ )
  - Potassium ( $K^{+}$ )
  - Calcium ( $Ca^{++}$ )
3. Dip Stick Analysis - Chemical comparisons
  - pH
  - Glucose
  - Protein
  - Acetone
  - Occult blood (independent variable)
  - Bilirubin
  - Urobilinogen

**B. Microbiological Parameters to be studied**

1. Efficacy of the microbiological sampler.
2. Effect of microbiologically contaminated sample
  - Persistence/contamination potential following urine inputs.
  - Potential of fluid pathway within ABSS for impinging/incubating bacteria from contaminated sample.
3. Efficacy of Betadine decontamination system.

**Discussion** All investigative areas must be studied as a scheduled unit following establishment of all analysis techniques before start of primary experiment.

**A. Baseline Conditions**

1. All measurements conducted using freshly collected male urine acquired on a random basis with respect to volume and time.
2. ABSS will be loaded (full) with urine before experiment begins.
3. Analyses will be done on pre/post samples for all investigative parameters.
4. Sample and dump port effluents will be collected separately for each urine output.
5. All urine inputs will be weighed pre and post (sample-post collection and dump post collection).
6. Free hemaglobin will be added to selected urine samples to check detection sensitivity of Ames Dip-Stix.

B. Pre-Test Analysis Experiments to be Developed.

1. Standardization and check-out of Orion Specific Ion Analyzer.
2. Titration of hemaglobin in fresh urine vs. dip-stix.
3. Check-out of Micro-Sampler
  - Establish constant amount of absorbant cotton/gauze to be added to each Sampler wick.
  - Determine average amount of fluid absorbed by standardized wick.
  - Study sterilization of Samplers with dry heat (about 125°C for 16-24 hours).
  - Titrate limit of sensitivity of wick with known dilution of test organism.
4. Titration of test organism
  - Growth on nutrient agar
  - Titer on pour plates and spread plates.
5. Titrate glucose additive in urine for potential use as another independent variable.

#### 4.1.7 Microbiological Sampler - Preliminary Studies

Purpose To acquire baseline information on the Micro Sampler such as (1) standard amount of cotton/gauze needed in wick, (2) average amount of fluid captured by wick, (3) sterilization procedures for sampler system, and (4) establish "in vitro" efficacy of sampler with dilutions of test organism.

Discussion Information acquired in these preliminary tests will be required for determination of schedule and assay requirements for in-use test of ABSS.

Materials

1. Absorbant cotton.
2. Gauze.
3. Sterile 250 ml flasks containing Nutrient Broth.
4. Stock colution of E.coli containing about  $10^8$  org/ml.
5. Sterile tubes containing 9 ml H<sub>2</sub>O for dilution.
6. " bottles containing 99 ml H<sub>2</sub>O for dilution.
7. Sterile 500 ml flasks containing 250 ml of Nutrient Broth.
8. Sterile petri dishes and 1 ml pipettes.
9. Commercial spore strips for sterilization control.
10. Betadine disinfectant.
11. Sterile 1-litre flasks containing about 800 ml. distilled H<sub>2</sub>O.
12. Sterile tongs.

Procedure

A. Standard amount of cotton/gauze for wick

1. Pack several SS wicks with cotton and others with gauze to a nominal amount.
2. Weigh each wick so packed before and after to determine amount of cotton used.
3. Do same for wicks stuffed with gauze.

B. Average amount of fluid captured by wick

1. Make several wicks with both cotton and gauze and pour 100 ml quantities distilled H<sub>2</sub>O over wick while in assembly inserted into urine cap.
2. Weigh wick, pre and post, to determine amount of water absorbed by wick.
3. Choose either cotton or gauze for use in wick on basis of performance with these tests.

C. Sterilization Procedures

1. Pack all 6 wicks with material chosen in tests above together with a spore strip.
2. Thoroughly clean aluminum containers for wicks and insert all samplers and test spore strips within each container.
3. Place 4 containers within dry heat oven and set for 125°C. Allow to remain at that temp. for 24 hours.
4. Remove from oven, cool, and aseptically transfer SS wick from each container to a sterile flask containing Nutrient Broth.

5. Incubate at 35°C and check for growth daily and record for 2 weeks.
6. To all flasks which exhibited negative growth, add back fresh spore strip and reincubate at 35°C for 2 additional weeks - monitor and record.

D. Efficacy of Sampler "In Vitro"

1. All Sampler wicks are packed with standard amounts of absorbant material, assembled, and sterilized with dry heat as above.
2. Make aqueous dilutions in 100 ml quantities of a stock E. coli culture containing about  $10^8$  org./ml as follows:  
     $10^3$  org./ml;  $10^2$  org./ml;  $10^1$  org./ml;  
    and two (2) 100 ml. bottles of  $10^0$  org./ml.
3. Prepare large tube (sufficient size to easily hold urine receptacle from ABSS) and partially fill with 20% Betadine solution.
4. Immerse urine collector in Betadine solution and allow to remain for 5 minutes.
5. Retrieve collector with sterile tongs and rinse thoroughly with one bottle (800 ml) of sterile water. Collect final 20-30 ml. of rinse in sterile flasks and test entire amount for presence of variable contaminants in NB. Check for 1 week and add back 1 drop of  $10^8$  org./ml E. coli for those flasks which do not demonstrate obvious growth of microorganisms.

6. Insert sterile Micro Sampler in urine collector as prescribed by design and pour each 100 ml dilution of E. coli into the cup and allow fluid to drain over the sampler in simulation of urine.
7. Remove Micro Sampler from cup and aseptically drop the wick into the N.B. Immerse collector in Betadine solution and treat as #5 above.
8. Continue through each dilution using duplicate testing at the 10° dilution. Micro Sampler #6 is used as control-at conclusion of E. coli dilution wash collector and pour 100 ml sterile H<sub>2</sub>O over control and treat as experimental samples.
9. Incubate all samples at 35°C - check daily for 1 week - add back E. coli to all negative samples.
10. Record all results.

#### 4.1.8 7-Day ABSS Operational Trial

##### GENERAL OBJECTIVE:

The overall objective shall be to complete a restricted but overall seven-day test of the ABSS with frequent urine inputs, under a nominal "Operation" schedule. In performing this 7 day user-test certain specific objectives shall be attained.

##### SPECIFIC OBJECTIVES:

The specific objectives of the test shall be as follows:

1. Effect of high frequency urine inputs on the bacterial contaminants within the ABSS.
2. Effect of Betadine on
  - a) Typical urinary bacterial infection (E. coli)
    - In vivo (within the ABSS)
    - In Vitro (standard disinfectant titration)
  - b) Preliminary assessment of long term effects of Betadine on the urine within the waste "holding tank".
3. Examination of the capability of the Microbial Sampler (MS) to quantitatively sample the urine stream.

##### BASELINE CONDITIONS:

The following conditions are considered as baseline for the 7-day trial with the ABSS:

- Test days will be a total of 7 but not necessarily continuous, e.g. no operation over weekend is planned.
- Day = 12 hours.
- Use of male and female urine.



- Minimum urine inputs/day = 10; up to 12-15.
- Entire system will be sterilized before start of 7 day trial including fluid pathways within ABSS and rinse water reservoir.
- Assay of Betadine activity will be performed according to a reference technique (separate protocol).
- Disinfect cycle will be operated once each operational day.
- "Loaded" (i.e., addition of bacteria) urine samples will be inserted into the ABSS each day.
- Each urine input will be sampled for control on initial level of bacterial organisms.
- Selected urine effluents will be collected and sampled at the Sample Port only - approximately 4/day.
- All urine effluents to "Waste" will be collected in common holding tank and the pool will be sampled once each operational day and titrated for microbial activity.
- Microbial Sampler tests will be performed in duplicate (freezing versus non-freezing as per protocol developed during earlier phase of program).

MATERIALS NEEDED:

1. Sterile urine collection containers - approximately 120 required (purchase from Fisher-Falcon Plastics).
2. Sample port collection containers - 250 ml flasks without stoppers and air vent - need approximately 25.
3. Betadine disinfectant - needed ~ 420 ml undiluted as supplied by manufacturer.
4. Large glass carboy for effluent waste pool - sterile, with air vent, etc. 15 liter size.
5. Culture media requirements

a. Trypticase Soy Agar

- Samples - 4/day x 3 dilns. x 2 plates/diln. x 7 days = 168 plates
- Microb. Samplers - 4 samples x 3 dilns. x 3 plates/diln. x 2 tests = 72
- Waste Pool = 2 samples/day x 3 dilns. x 2 plates/diln. x 7 days = 42

a. Trypticase Soy Agar (Continued)

- Urine Sterility Check = 10/day x 2  
plates/sample x 7 days

	= 140
Sub Total	<u>422</u> plates
+ 10%	42
Grand Total	<u>464</u> plates
ml/Plate	20
	<u>9280</u> ml

Grand Total = 10 liters media = 20 flasks with  
500 ml/flask

b. Dilution blanks - water

9 ml blanks = 80 tubes (2 racks)

99 ml blanks = 80 bottles

c. Trypticase Soy Broth - 250 ml screw-cap flasks with  
50 ml/flask = 25 flasks, sterile.

6. Test Organism - Escherichia coli (ATCC #15144)
7. Pipettes - 1 ml (Sterile) - As Required  
5 ml (Sterile) - As Required  
10 ml (Sterile) - As Required

8. Peracetic Acid - 2% Solution

Prepared as follows:

"Nacconol" - 0.5 ml  
Peracetic Acid - 50 ml of 40% solution.  
QS with H<sub>2</sub>O - 1 liter

9. Cold Sterilized rinse water reservoir for ABSS
  - 150 mm filter holder
  - 0.22 filter
  - Large Millipore SS pressure vessel filled with distilled H<sub>2</sub>O.
10. Large volume pipettes for sampling waste urine pool. 25 ml, sterile  
- 10 needed.
11. Sterile H<sub>2</sub>O, liter quantities, 1-liter flasks - need 5.
12. Sterilize Microbial Samplers - with gauze wick at 120°C for 24 hours  
- 5 needed.

PROCEDURE/TIME-LINE

Pre-Test Activities - (3 to 5 Days Before Start Date)

1. Connect filter system to ABSS, check for leaks and make preliminary assay of effluent rinse water sterility with TSB - 3, 10 ml samples.
2. Flush two 500 ml portions of 2% peracetic acid through ABSS.
  - Allow 2 re-circulate cycles to operate, then turn off ABSS.
  - Wait 15 minutes.
  - Turn on and allow complete cycle to operate (have sample receptacle ready).
  - Flush thoroughly with 4, 500 ml quantities of sterile H<sub>2</sub>O.
  - Check effluent water on both sample and dump ports for sterility on last flush - 3 x 10 ml samples in TSA.

Pre-Test - (1 Day Before Start Date)

1. Install waste jug to system.
2. Fill disinfectant reservoir with Betadine to provide a final flush concentration of 300 ppm.
3. Inoc. E. coli culture at 4 PM - in TSB at 37°C.

DAY 1 - WEDNESDAY

SAMPLE NO.

<u>0-1 Hour</u>	-Take pre-sample for Bacteria Check	1-1
	-Add <u>E. coli</u> to urine sample to ~ 10 <sup>6</sup> org/ml, i.e. approximately 1 ml undil. culture/100 ml urine.	
	-Add "Loaded" sample to ABSS and collect sample	1-2
	-Pre-sample for 2nd urine	1-3
	-Process 2nd urine sample	1-4
<u>1-10 Hours</u>	-Process each sample through ABSS and plate pre-sample only (2 x 1 ml)	1-5 1-6 1-7 1-8 1-9 1-10
<u>8 Hour</u>	-Inoc. <u>E. coli</u> flask	

		<u>SAMPLE NO.</u>
<u>10 Hour</u>	-Pre-sample - Sterility (Urine 9) Sample (Urine 9)	1-11 1-12
	-Disinfect cycle	
<u>11 Hour</u>	-Pre-sample - Sterility (Urine 10) Sample	1-13 1-14
<u>DAY 2 - THURSDAY</u>		
<u>0-1 Hour</u>	-Sample Waste Pool	2-15
	-Pre-sample (Urine 11) Sample (Urine 11)	2-16 2-17
	-Pre-sample (Urine 12) Add <u>E. coli</u> to Urine 12 as in Day 1	2-18
	-Insert microbial sampler MS in urinal and pour ~ 50 ml of loaded urine over MS, remove and label.	2-MS-1
<u>0-1 Hour</u>	-Continue with 3 samplers more, using ~ 50 ml portions each time.	2-MS-2 2-MS-3 2-MS-4
	-Re-cap MS-3 and MS-4 and place in freezer compartment at -18°C.	
	-Allow all accumulated "loaded" urine to process and collect sample.	2-19
<u>1 Hour</u>	-Pre-sample (Urine 13) Sample (Urine 13)	2-20 2-21
<u>1-10 Hour</u>	-Process additional urine with (14) Pre-samples only. (15) (16) (17) (18)	2-21 2-22 2-23 2-24 2-25
<u>8 Hour</u>	-Inoc. <u>E. coli</u> flask.	

		<u>SAMPLE NO.</u>
<u>10 Hour</u>	-Pre-sample (Urine 19)	2-26
	Sample (Urine 19)	2-27
	-Disinfect Cycle	
	-Pre-sample (Urine 20)	2-28
	Sample (Urine 20)	2-29
 <u>DAY 3 - FRIDAY</u>		
<u>0-1 Hour</u>	-Sample Waste Pool	3-30
	-Pre-sample (Urine 21)	3-31
	Sample	3-32
	-Pre-sample (Urine 22)	3-33
	-Add <u>E. coli</u> and process through sample	3-34
 <u>1 Hour</u>	 -Pre-sample (Urine 23)	 3-35
	Sample	3-36
 <u>1-10 Hour</u>	 -Routine urine processing (24)	 3-37
	Pre-samples only (25)	3-38
	(26)	3-29
	(27)	3-40
	(28)	3-41
 <u>10 Hour</u>	 -Pre-sample (Urine 29)	 3-42
	Sample (Urine 29)	3-43
	Disinfect Cycle	
	Pre-sample (Urine 30)	3-44
	Sample (Urine 30)	3-45
 <u>SHUT DOWN</u>	 -Leave as is over weekend.	

SATURDAY/SUNDAY - No Sampling

MONDAY - NOT AN OPERATIONAL DAY

SAMPLE NO.

0-1 Hour

- Sample Waste Pool,
- Remove Waste-pool Jug from system, autoclave, clean, and re-sterilize; then re-connect to system.
- Re-fill Betadine reservoir with TBD concentration of Betadine and re-insert into ABSS.

3-46

8 Hour

- Inoc. E. coli flask.

DAY 4 - TUESDAY

- Follow Schedule for DAY 1.

4-47  
thru  
4-60

DAY 5 - WEDNESDAY

- Follow Schedule for DAY 2 including Microbial

Sampler Expt. 5-MS-1  
thru  
5-MS-4

5-61  
thru  
5-76

DAY 6 - THURSDAY

- Follow Schedule for DAY 3.

6-77  
thru  
6-92

DAY 7 - FRIDAY

- Follow Schedule for DAY 3 - take last Waste Pool sample immediately after last urine sample - not Saturday morning.

7-93  
thru  
7-108

#### 4.1.9 Quantitative Recovery Of Bacteria From Microbial Samplers

##### Purpose

To study the quantitative recovery of urinary bacterial contaminants captured by the wick in the Microbial Samplers (MS) during operational use.

##### Objectives

Determine within the limits of the techniques, the capability of the MS to provide quantitative bacterial recoveries from urine streams containing representative bacterial contaminants.

##### Materials Required

1. Microbial Samplers containing standard amounts of gauze wicking and sterilized at 120°C for 20-24 hours - 5 needed.
2. Bacterial recovery flasks - 500 ml screw-cap flasks containing 47 ml of Trypticase Soy Broth (TSB) - 10 needed.
3. Dilution Blanks - water
  - 3 ml blanks - 10 needed
  - 9 ml blanks - 25 needed
4. Agar - Trypticase Soy Agar (TSA)
  - 8 Samples x 3 Dilutions/Sample x 3 Plates/Dilution = 72 Plates
  - Agar/Plate                    20
  - 1440 ml
  - 1.5 liters of TSA required
5. Pipettes
  - 1 ml - sterile as required
  - 5 ml - sterile as required
6. Urine sample containing typical urine contaminant - at least 250 ml urine containing approximately  $10^6$  organisms/ml of E. coli.

7. Urinary test organism - E. coli (ATCC #15144).

Timeline/Precedures

Test No. 1

Thursday - Capture of Bacteria

- Add fresh 18 hour E. coli culture to urine sample to provide  $\sim 10^6$  organism/ml as per directions in Main Protocol under Day 1.
- Insert first MS sampler into urinal.
- Pour 50 ml ( $\pm$  2-3 ml) portions of the "Loaded" urine over MS and into phase separator of ABSS.
- Remove MS, replace with fresh, sterile MS and repeat with another 50 ml portion.
- When all 4 MS have been employed as described above titrate immediately as per following instructions.
- Place wire mesh and gauze wick into 50 ml TSB, place flask on Eberback shaker and secure firmly on tray. Operate shaker at  $\sim$  40-50 excursion/minute for 15 minutes.
- Remove 1 ml of resulting suspension and place in 3 ml blank - this will provide a dilution of approximately  $10^{-2}$ .
- With this dilution, place 3, 0.1 ml portions into 3 petri dishes and add melted agar (TSA).
- Repeat with  $10^{-3}$  and  $10^{-4}$  dilutions using the 9 ml blanks. This will provide a titration at  $10^3$ ,  $10^4$ , and  $10^5$ .



- Incubate at ambient temperature over weekend and count following Monday.

Test No. 2

Wednesday

- Repeat as in Test No. 1.

#### 4.1.10 Protocol For Determining Efficacy Of Betadine For Use In ABSS Urine System

##### 1.0 INTRODUCTION

Betadine, the Purdue Frederick Company's<sup>1</sup> water-soluble complex of iodine and polyvinylpyrrolidone (Povidone) which when mixed with water possesses the well-known antiseptic properties of iodine, has been considered for use as a disinfectant for the ABSS Urine Sampling Subsystem.

##### 2.0 OBJECTIVES

The following objectives have been established to determine the capability of Betadine as the ABSS disinfection agent:

- 1) To disinfect the ABSS hardware, under typical or normal use conditions.
- 2) To "hold in check", the microbial population, in the collected pooled waste urine.

##### 3.0 DISCUSSION

The overall objectives are to determine, by test, if the ABSS Urine Sampling Subsystem hardware (hard, non-porous surfaces) can be disinfected and that concentration of Betadine germicide which can be used to reliably disinfect them. Since the 7-Day usage test will employ daily additions of typical urinary bacteria, this protocol is designed to provide basic data on the efficacy of Betadine, both diluted in water and Betadine diluted in freshly collected urine, to inactivate a viable test culture of E. coli.

The protocol also designates a method for chemically assessing the level of freely available iodine in the test solutions of Betadine and the residual Betadine in the pooled waste urine.

<sup>1</sup> Purdue Frederick Company and Affiliates, 99 Saw Mill River Road, Yonkers, N. Y. 10701; Executive Offices 15 East 62nd Street, N. Y., N. Y. 10021

## 4.0 PROCEDURE

### 4.1 Efficacy of Betadine in water and urine.

4.1.1 Prepare in duplicate the following dilutions of Betadine, in both water and urine, using 250 ml wide-mouth Erlenmeyer flasks, so that each flask contains ~ 100 ml:

	<u>Concentration Of Betadine</u>	<u>Available Iodine (ppm)</u>
1)	Undiluted (10% as supplied by manufacturer)	1% or 10,000 ppm
2)	1:1 (5% Betadine)	0.5% or 5,000 ppm
3)	1:10 (1% Betadine)	0.1% or 1,000 ppm
4)	1:30 (0.30% Betadine)	0.30% or 300 ppm
5)	1:100 (0.1% Betadine)	0.010% or 100 ppm
6)	1:1000 (0.01% Betadine)	0.001% or 10 ppm

4.1.2 Place each set of flasks in constant temperature both at 25°C and hold until stable temperature is obtained (minimum of 20 minutes hold time).

4.1.3 Prepare similar flasks containing:

- 99 ml of sterile phosphate buffer dilution water. (See Section 5.5 & 5.6)
- 99 ml sterile neutralizer solution. (See Section 5.3 & 5.4)

4.1.4 Add 1 ml of culture suspension (~  $10^8$  organisms per ml) to each test flask as follows: Swirl flask, (avoid foaming) stopping just before suspension is added, creating enough residual motion of liquid to prevent pooling of suspension at point of contact with test germicide. Add suspension midway between center and edge of surface with tip of pipet slightly immersed in test solution. Avoid touching pipet to neck or side of flask during addition.

4.1.5 Transfer 1 ml portions of the exposed culture to neutralizer blanks exactly 1 and 30 minutes after addition of suspension. Mix immediately after transfer.

4.1.6 For "numbers control" (assay of suspension of test organism), add 1 ml of culture suspension to 99 ml sterile phosphate dilution water.

4.1.7 For "toxicity check" on neutralizer solution, add 1 ml of culture suspension to 99 ml sterile neutralizer solution.

4.1.8 Plate from neutralizer tube to agar, using subculture medium (Trypticase Soy Agar) for germicide test dilutions and Trypticase Soy Agar and/or Eosin Methylene Blue Agar for numbers control. For dilutions to give countable plates, use 99 ml sterile phosphate buffer dilution water as follows:

4.1.9 Transfer 1 ml exposed culture (1 ml culture suspension transferred to 99 ml phosphate buffer dilution water in H<sub>2</sub>O bath) to 99 ml phosphate buffer dilution water (dilution A). Shake thoroughly and transfer 1 ml dilution A to 99 ml phosphate buffer dilution water (dilution B).

4.1.10 Shake thoroughly and plate three 1.0 ml and three 0.1 ml aliquots from dilutions A and B to individual sterile petri dishes. Add approximately 10-15 ml of the molten sterile culture medium to each plate, swirl and allow agar to harden. Incubate plates at 35°C in inverted position. Record the number of colony-forming units at 24 and 48 hours.

#### 4.2 Efficacy Of Betadine In Pooled Waste Urine

Sample of urine collected in the sterilized waste receptacle will be removed at appropriate intervals and aliquots added to dilution solution containing the antimicrobial neutralizer. These dilutions will be assayed for viable organisms (according to Section 4.1.2 through 4.1.10).

### 4.3 Determination Of Available Iodine In Betadine Solutions

Schmidt and Winicov (1967) have summarized the results of assessing various detergent/iodine systems for the iodine present and clarify the various terms as follows:

Total Iodine  
Volhard Iodine  
Available Iodine  
Complexed Iodine  
Free Iodine ( $I_2$ ) or Uncomplexed Iodine.

The technique for best determining that portion of the available iodine which is not complexed is by determining the distribution of iodine between the aqueous (product) phase and an organic solvent such as heptane. The following Distribution Coefficient (D.C.) procedure can be used to determine the free iodine.

#### The D.C. Method

The Distribution Coefficient (D.C.) is determined by adding 1.00 ml of standardized test solution containing between 0.05 and 5.0% iodine to a 50 ml graduated cylinder containing 25 mls purified n-heptane. The temperature of the heptane is brought to  $25^\circ \pm 1^\circ\text{C}$ . The cylinder is stoppered and shaken vigorously by hand for one minute during which time the aqueous solution suspends in the heptane as a uniform haze. The solution is then allowed to stand a minute or two, and the temperature adjustment and shaking are repeated. For best results the solution should settle for an hour before the iodine determination.

The amount of iodine in the heptane layer is determined colorimetrically at 520 m $\mu$ , the absorption peak; the relationship between light absorption and iodine concentration in this solvent is linear throughout the range of one to 25 mg per 100 mls. The distribution coefficient is calculated by the following formula:

$$\text{D.C.} = \frac{\text{mg I remaining in aqueous phase}}{\text{mg I in heptane}} \times \frac{\text{mls heptane}}{\text{mls aqueous phase}}$$

Using the Beckman DU spectrophotometer with 1.00 cm cells, an absorption of 0.142 corresponds to 1.00 mg iodine extracted by 25 mls heptane. Values so obtained are readily reproducible to within 10% and frequently to within 1%.

The D.C. as defined here parallels iodine complexing in that the greater the complexing, the higher the D.C. number. For a given available iodine concentration the D.C. values are dependent upon the iodine and detergent levels.

A satisfactorily complexed iodine product is one which is characterized by a D.C. number in the range of about 150 to 300 for 1.75% available iodine content.

## 5.0 Materials And Reagents

### 5.1 Culture Medium

- a) Trypticase Soy Broth - Prepare as indicated by manufacturer on bottle. Place 50 ml in 250 ml cotton stoppered Erlenmeyer flasks and autoclave 20 minutes at 121°C. Use for daily transfer of test culture.

## 5.2 Subculture Media

- a) Trypticase Soy Agar - Prepare as indicated by manufacturer on bottle. Place 250-300 ml in 500 ml screw-capped Erlenmeyer flasks and autoclave 20 minutes at 121°C. Use for all subculture and plating operations.
- b) EMB (Eosin Methylene Blue) Agar - Prepare as indicated by manufacturer. Place 250-300 ml in 500 ml screw-capped Erlenmeyer flasks and autoclave 20 minutes at 121°C. Use as subculture plating medium to identify E. coli.

## 5.3 Neutralizer Stock Solution

Sodium Thiosulfate Solution - Dissolve 40 grams of  $\text{Na}_2\text{S}_2\text{O}_3$  in screw-capped water blank bottles and autoclave 20 minutes at 121°C. Use concentration is 1 ml of this stock solution to 99 ml phosphate buffer blanks.

## 5.4 Neutralizer Blanks

Add 1 ml of Sodium Thiosulfate neutralizer stock solution to each 99 ml phosphate buffer dilution water.

## 5.5 Phosphate Buffer Stock Solution

0.25 M - Dissolve 34.0 gram  $\text{KH}_2\text{PO}_4$  in 500 ml  $\text{H}_2\text{O}$ , adjust to pH 7.2 with 1 N NaOH and dilute to 1 liter.

## 5.6 Phosphate Buffer Dilution Water

Add 1.25 ml of 0.25 M phosphate buffer stock solution to 1 liter of water and dispense in 104 ml amounts in 99 ml screw-capped water blank bottles. Autoclave 20 minutes at 121°C.

## 5.7 Test Organism

Use Escherichia coli AtCC No. 15144. Maintain stock cultures on nutrient agar at refrigerator temperature.

## 5.8 Apparatus

### a) Glassware

TBD

### b) Petri Dishes

TBD

### c) Water Bath

TBD

## 6.0 REFERENCES

Horwitz, W. (ed)., 1970. Germicidal and Detergent Sanitizers pp 66-68: Disinfectants. Chapter 4 in Official Methods of Analysis of the Association of Official Analytical Chemists. Eleventh Edition, Association of Official Analytical Chemists, P. O. Box 540, Benjamin Franklin Station, Washington, D. C. 20044.

Schmidt, A. M., 1974. Guidelines for Safety and Efficacy Testing of OTC Topical Antimicrobials Federal Register; Part II, Volume 39, Number 179, Friday, September 12, 1974, Washington, D. C.

Bruch, M. K. 1975 Personal Communication.

Schmidt, W., and Winicov, M., 1967. Detergent/Iodine Systems. Soap and Chemical Specialties 43, 61-64.



## Phosphorus (Inorganic) in Serum or Urine

### PRINCIPLE

A trichloroacetic acid filtrate is treated with ammonium molybdate solution (molybdic acid), which combines with phosphate to form phosphomolybdate. The molybdate thus formed is reduced with ferrous sulfate and the blue color produced is measured photometrically and is proportional to the amount of phosphorus originally present.

### PROCEDURE, (Serum)

1. To a test tube containing 3.5 ml. of 12% trichloroacetic acid add 0.20 ml. of serum, mix, allow to stand for 10 minutes and then centrifuge rapidly for 10 minutes. Include a blank containing 0.20 ml. water and 3.0 ml. of 12% trichloroacetic acid.
2. Transfer 3.0 ml. of the clear supernatant filtrate to a cuvette and add 2.0 ml. of ferrous sulfate-molybdate reagent.
3. After 1 minute (or within 2 hours) read the unknown against the blank at a wavelength of 660  $m\mu$  using the red filter (33-29-18). It is recommended that the red sensitive phototube (33-29-72) be installed for these measurements.
4. Refer to the table below to determine the concentration of phosphorus as mg. P. 100 ml. serum.

### PROCEDURE, (Urine)

1. Dilute 1.0 ml. of the urine specimen to 100 ml. This dilution will suffice with most urines. If, however, this dilution factor yields a final color too light or too dark, another appropriate dilution is selected.
2. Transfer 2.0 ml. of the diluted urine to a cuvette and add 1.0 ml. of 34% trichloroacetic acid. (If the urine contains proteins a turbidity will be produced after the addition of the trichloroacetic acid. In this case, proceed as follows: Pipette 4.0 ml. of diluted urine from step 1 into a test tube, add 2.0 ml. of 34% trichloroacetic acid, mix and allow the mixture to stand at room temperature for about 10 minutes. After centrifuging, pipette 3.0 ml. of the supernatant fluid into a cuvette and proceed with the following.)
3. Add 2.0 ml. of the ferrous sulfate-molybdate reagent and read as with serum (step 3 above).
4. Refer to the table below to determine the concentration of phosphorus as mg. P 1 ml. urine.

### REAGENTS

1. *Trichloroacetic acid, 12.0% (w/v).*—Dissolve 120.0 gm. of trichloroacetic acid in water and dilute to exactly 1 liter.
2. *Trichloroacetic acid, 34.0% (w/v).*—Dissolve 340.0 gm. of trichloroacetic acid in water and dilute to exactly 1 liter.
3. *Sulfuric acid, 10 N.*—Add slowly to about 700 ml. of distilled water 278 ml. of concentrated sulfuric acid. Cool, and dilute to 1 liter with distilled water.
4. *Ammonium molybdate, stock solution, 10%.*—Add 50 gm. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  into a liter beaker and add 400 ml. of 10 N sulfuric acid with constant stirring to prevent caking. When completely dissolved, transfer the solution to a 500 ml. volumetric flask and wash in quantitatively with 10 N sulfuric acid to the mark.
5. *Ferrous sulfate-ammonium molybdate reagent.*—Prepare just prior to using. Transfer 10.0 ml. of ammonium molybdate stock solution to a 100 ml. volumetric flask and dilute to about 70 ml. Add 5.0 gm. of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , make up to volume with water and shake until the crystals are dissolved. Transfer to a brown glass bottle.

### STANDARDIZATION

Prepare a stock standard solution by dissolving 0.1196 gm. of anhydrous potassium dihydrogen phosphate in water and diluting to exactly 100 ml. This solution is stored in the refrigerator. Two working standard solutions containing respectively, the equivalent of 5.0 and 10.0 mg. P per 100 ml. of serum are prepared by appropriate dilution and acidification of the aqueous stock standard solution in the following way: Dilute 1.0 and 2.0 ml. of the stock standard solution to exactly 100 ml. with 11.5% (w/v) trichloroacetic acid. These solutions are stable for about 3 weeks when kept in a refrigerator. Exactly 3.0 ml. aliquots of these working standard solutions are analyzed by the same procedure that is used for serum filtrates (steps 2 and 3). Record the A or % T and prepare a calibration table or curve as desired. A typical calibration table is given below.

### REFERENCE

1. Taussky, H. H., and Shorr, E., *J. Biol. Chem.*, **202**, 675-685 (1953).

## 4.2 Thermoelectric Cooling Design Details

### • DESIGN REQUIREMENTS

TEMPERATURE -  $10^{\circ} \pm 0.5^{\circ} \text{C}$

THERMOELECTRIC DEVICES REQ'D  
PHASE SEPARATOR & COLLECTION BAG COOLING

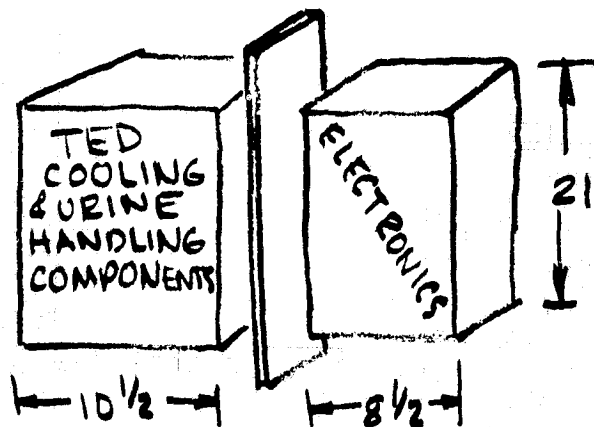
### • DESIGN APPROACH

TEMPERATURE  $7^{\circ}\text{C}$  ( $45^{\circ}\text{F}$ )  
COOL ALL URINE HANDLING COMPONENTS  
DESIGN POINT FOR MAXIMUM EFFICIENCY  
MODULAR CONFIGURATION COOLING PACKAGE  
THERMOSTAT TEMPERATURE CONTROL

### • THERMOELECTRIC DESIGN

DEFINE COOLING COMPARTMENT ENVELOPE }  
DETERMINE HEAT LOSSES }  
USAGE REQUIREMENTS, INSULATION  
MATERIAL & THICKNESS, AMBIENT TEMP,  
HEAT TRANSFER METHOD  
THERMOELECTRIC SELECTION  
POWER SUPPLY REQUIREMENTS  
HEAT SINK DESIGN  
BLOWER SELECTIONS  
MECHANICAL & ELECTRICAL INTEGRATION

## • CONFIGURATION / PACKAGING DESIGN



### COOLING SECTION

PHASE SEPARATOR  
ACCUMULATOR  
SAMPLE BAG  
URINE RECEPTACLE  
TED COOLING MODULE  
THERMOSTAT

### ELECTRONICS SECTION

LOGIC & SIGNAL CONDITIONING  
SWITCHES & FUSES  
DISPLAY  
BLOWER & FILTER ASS'Y  
RELAY PACKAGE  
PHASE SEPARATOR MOTOR  
SERVO AMPLIFIER

## • DESIGN APPROACH & FEATURES

COMPONENT / ASSEMBLY MODULAR CONSTRUCTION  
MAINTENANCE  
REPLACEMENT  
TEST BED FOR ADDITIONAL HARDWARE

ELECTRICAL - CABLE INTERFACE WITH EACH  
COMPONENT

ISOLATION & SHIELDING OF POTENTIAL EMI  
SOURCES

## • THERMAL ANALYSIS

HEAT LOSS ( $Q_L$ )

$$Q_L = K A \Delta T / dx$$

X = INSULATION THICKNESS  
A = COOLING SECTION SURFACE AREA

$$K = .025 \text{ BTUH} / \text{°F} / \text{ft} \\ \Delta T = 75\text{°F} - 45\text{°F} = 30\text{°F}$$

$$Q_L = 137.8 \text{ BTUH} = \underline{\underline{40.4 \text{ WATTS}}}$$

URINE PROCESSING ( $Q_P$ )

$$Q_{\text{URINE}} = Q_{\text{USCS PHASE SEPARATOR}} \\ W CP \Delta T_{\text{URINE}} = W CP \Delta T_{\text{PHASE SEPARATOR}}$$

ASSUME 2 LB. URINE PROCESSED (900ML) MAX  
URINE FINAL TEMP = 90.6°F = PHASE SEP. TEMP

$$Q_P = W CP (90.6 - 45\text{°F}) = 54 \text{ BTU} \\ \text{ASSUME } 1/2 \text{ HR. ALLOWABLE TIME TO COOL URINE} \\ \therefore Q_P = 108 \text{ BTUH} = \underline{\underline{31.6 \text{ WATTS}}}$$

IF UNIT IS COOLED BEFORE USAGE: -  
 $T_{\text{FINAL}} = 80\text{°F}$        $Q_P = 40.8 \text{ BTU} = 81.6 \text{ BTUH} = \underline{\underline{24 \text{ WATTS}}}$

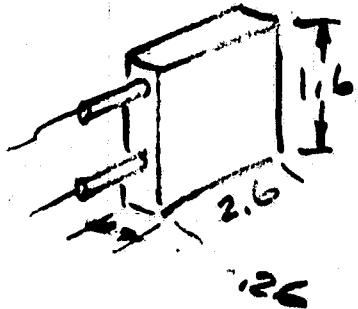
$$\text{TOTAL HEAT LOSS} = Q_L + Q_P \\ = 40.4 + 31.6 = 71.6 \text{ WATTS}$$

$$\text{SAFETY FACTOR} = 1.5$$

$$\text{COOLING REQ'D } (Q_C) = 1.5 \times 71.6 = 108 \text{ WATTS}$$

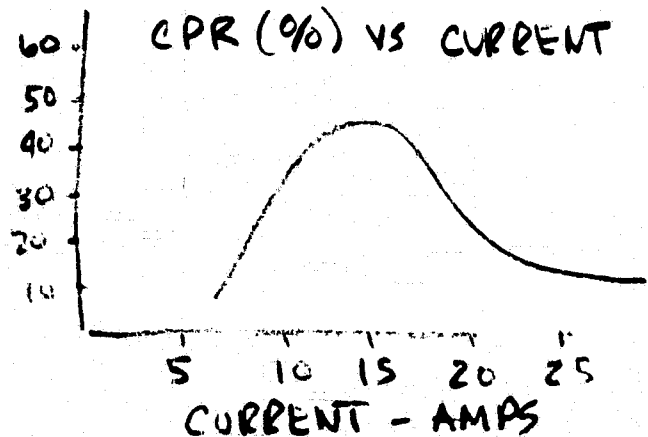
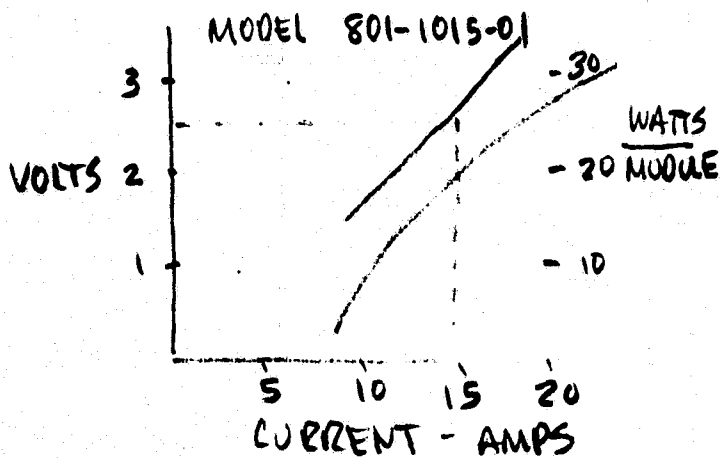
• THERMOELECTRIC SELECTION / POWER SUPPLY

TEC SELECTION - CAMBION 801-1015-01



WATTS/MODULE  $108/6 = 18$   
 VOLTS REQ'D  $2.68 \times 6 = 16.08$

PERFORMANCE & SELECTION



POWER SUPPLY

ABBOTT - DC TO DC CONVERTER

INPUT - 28 VDC, 17A

OUTPUT - 15.7 VDC, 15.36

ACTUAL OPERATION (FROM CHART)

VOLTS / MODULE  $15.7 \div 6 = 2.61$  VOLTS

CURRENT = 14.7 AMPS

WATTS/MODULE = 17.4 WATTS

TOTAL TEC COOLING (QC) = 104.4

• BLOWER SELECTION

$$Q = W C_p \Delta T$$

$$Q = 230.8 \text{ WATTS} \quad C_p = .24 \text{ (AIR)} \quad \Delta T = 15^\circ\text{C}$$

$$\text{FLOW REQ'D} = 25.3 \text{ CFM}$$

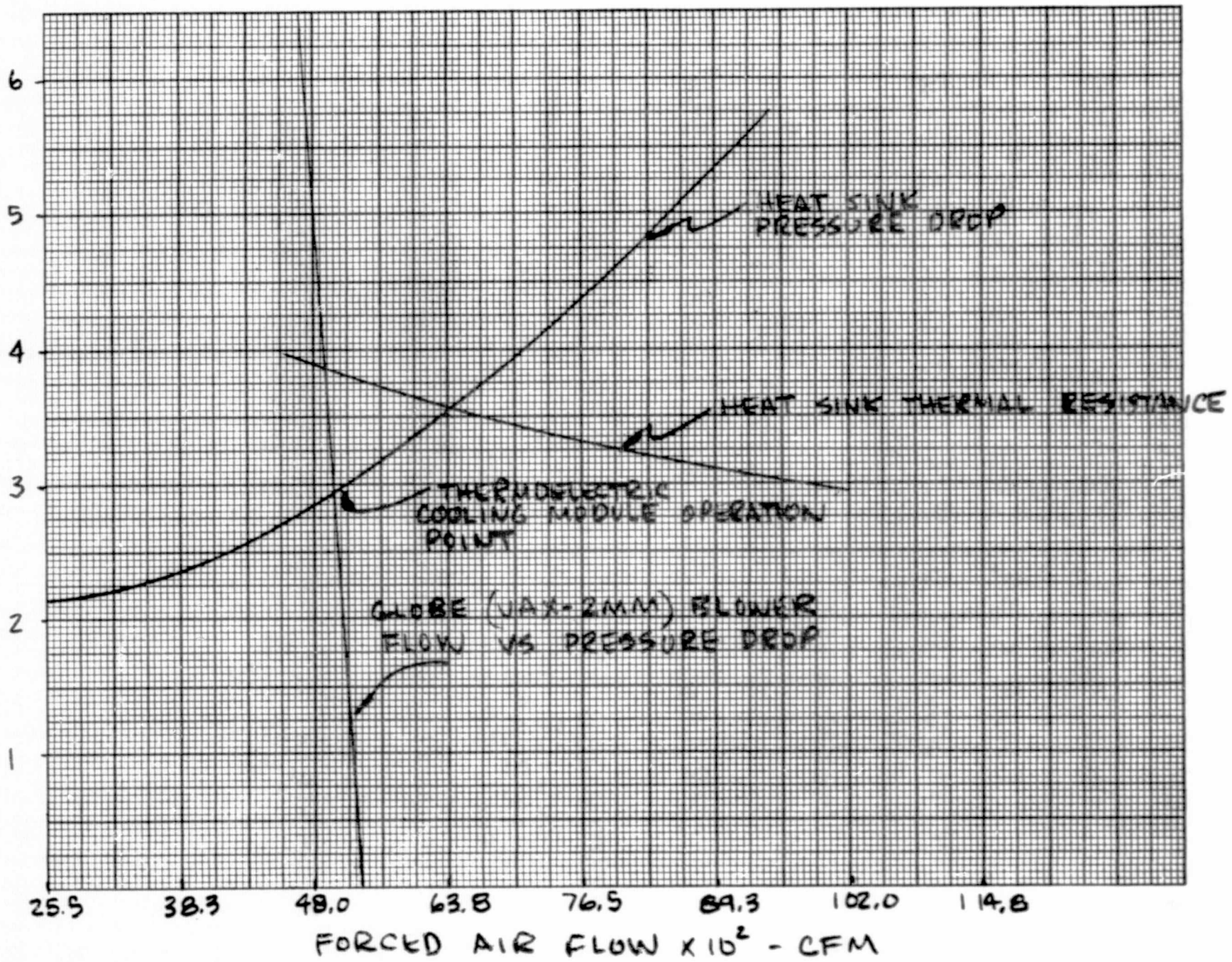
• HEAT SINK SELECTION

$$\text{THERMAL RESISTANCE} \quad 15^\circ\text{C}/230 \text{ W} = .065$$

$$\text{FLOW (CHART)} = 48.7 \text{ CFM}$$

$$\text{THERMAL RESISTANCE} = .0385 \text{ }^\circ\text{C/W}$$

PRESSURE DROP  $\times 10^{-1}$  - IN. WATER



HEAT SINK PRESSURE DROP

HEAT SINK THERMAL RESISTANCE

THERMOELECTRIC COOLING MODULE OPERATION POINT

GLOBE (VAX-2MM) BLOWER FLOW VS PRESSURE DROP

25.5 38.3 48.0 63.8 76.5 89.3 102.0 114.8

FORCED AIR FLOW  $\times 10^2$  - CFM

• THERMOELECTRIC POWER REQUIREMENTS

POWER SUPPLY - OPERATING AT FULL OUTPUT

INPUT POWER	476 WATTS
OUTPUT POWER	<u>241 WATTS</u>
POWER SUPPLY LOSS	235 WATTS *

← REGULATED SUPPLY - NOT REQUIRED FOR THIS APPLICATION

THERMOELECTRICS

INPUT POWER	230.8 WATTS
COOLING POWER ( $Q_c$ )	<u>104.4 WATTS</u>

$$\text{CPR (\%)} = Q_c / \text{INPUT POWER} = 45.3\%$$

• OPTIMIZED FOR FLIGHT APPLICATION

REDUCE VOLUME ( $\approx 35\%$ )  $\approx 21.2$  WATTS

INCREASE INSULATION THICKNESS

COOL SPECIFIC COMPONENTS

INCREASE MECH COMPLEXITY

LIMIT REPLACEMENT FEATURES

• SYSTEM WEIGHT CONSIDERATIONS

TEO MODULE & BLOWERS

19.9 LBS

POWER SUPPLY

11.0 LBS

30.9 LBS