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FINAL REPORT

No. 1102F

DEVELOPMENT OF A TAPE TRANSPORT BACTERIAL DETECTION SYSTEM

To

NASA/MANNED SPACECRAFT CENTER

Houston, Texas 77058

Contract NAS 9-11644

25 February 1972

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AEROJET MEDICAL AND BIOLOGICAL SYSTEMS

A DIVISION OF AEROJET-GENERAL

9200 EAST FLAIR DRIVE

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Section 1

SUMMARY

The feasibility of a tape transport chemiluminescence system for bacterial monitoring of regenerated water was demonstrated using a manually operated laboratory breadboard.

The principle of detection is based on measuring the increase in chemiluminescence produced by the catalytic action of bacterial porphyrins on a luminol-hydrogen perioxide mixture. Viable organisms are distinguished from non-viable by comparing the signals of incubated and unincubated water samples.

Using optimized protocols, sensitivities attained with 400 ml suspensions of E. coli and Cl. sporogenes, the two test organisms, were as follows:

	Unincubated Cycle (Total-Viable + Non-Viable)		Incubated Cycle (Viable)	
	Sens.	Processing Time	Sens	Processing Time
E. coli (aerobe)	30-35 cells/ml	37 min	7-8 cells/ml	2.3 hrs
Cl. sporogenes (anaerobe)	10^3-10^4 cells/ml	37 min	10^3 - 10^4 cells/ml	4 hrs

The lower sensitivity toward Cl. sporogenes is attributed to several factors, namely the lower cytochrome content, the tendency to sporulate, long lag periods and the lower growth rate of Clostridia in general.

The operational procedures used for processing the incubated and unincubated samples involved the following sequence:

1. concentrating the sample by filtration through a membrane filter,
2. washing with Dextrose-Thioglycollate Broth (DBT),
3. incubating (0 to 4 hrs as required),
4. washing with 4M Urea,
5. reacting with reagent in front of a photomultiplier tube (PMT).

The signal output from the PMT was recorded on a strip chart recorder.

In a study of the effect on sensitivity of exposing E. coli to distilled water at 160°F, whereas a 4 hour immersion had no significant effect, a 24 hour immersion resulted in almost a complete loss in signal. The evidence indicates that bacterial porphyrins are discharged into solution as a result of cell lysing and leaching.

In an evaluation of the ability of established protocols to operate in the presence of various bactericides which may be present in regenerated water, a decrease in signal ranging from 22 to 67% was observed, depending on the bactericide. Protocol modifications which might be used to minimize these effects are presented.

In a summary of the various parameters which might be used to determine water quality, the ability to detect viable and non-viable bacteria as well as viruses, toxins and lysed organisms was singled out. Chemical and physical properties which should also be monitored as an indication of water potability, include total organic carbon, ammonia, hexavalent chromium, nitrate, nitrite, silver ions, specific conductivity, pH, turbidity and color.

Apart from the chemical and physical sensors suggested for monitoring these parameters, the recommended methods for the biological agents include:

1. Porphyrin (Capsule) Chemiluminescence - for total cell populations and lysed organisms.
2. Colony Counter - for viable cell count.
3. Bead Agglutination - for virus and toxin.

A detailed program projection for these three system is presented together with sketches and outline drawings of possible flight prototype configurations.

Section 2

INTRODUCTION

This comprises the final report of progress in "Development of a Tape Transport Bacterial Detection System", and is submitted in accordance with the requirements of Contract No. NAS 9-11644, as well as Modification No. 2S.

The overall objective of the present effort was to demonstrate the feasibility of the chemiluminescence method for monitoring the sterility of reclaimed water using a tape sample transport system. A manually operated laboratory breadboard was to be utilized for optimizing process variables and to obtain preliminary design information for a fully-automated flight-rated prototype system.

The principal advantages of a moving tape over a liquid flow* for transporting the sample through the processing sequence are 1) recycling of the water sample without re-purification would be possible since a fresh, sterile filter surface can be utilized in the initial sample concentration step, 2) clogging and filter maintenance would be minimized and 3) the problem of incomplete recovery of organisms off a filter is eliminated since the reaction of deposited organisms and reagent is carried out directly on tape.

The detection principle used as a basis for the present design is to measure the increase in chemiluminescence produced by the catalytic action of bacterial prophyrins on a luminol-hydrogen peroxide mixture**. The most active bacterial prophyrins are the hemoproteins such as catalase or the cytochromes found associated with the respiratory sequence of all aerobes and some faculative anaerobes. The

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* In the liquid flow system, an indexing filter concentrator employing eight individual filter surfaces on a cylindrical disc is employed for concentrating the organisms in the water sample. In contrast to the moving tape approach which presents a fresh sterile filter surface to each new sample, the filters in the filter concentrator are reused after passing through a clean-up cycle between samples.

** Luminol is 5-amino-2, 3-dihydro - 1-4 - phthalazinedione.

reaction of these porphyrins with the liquid reagent is virtually instantaneous and occurs immediately on passing the luminol-H_2O_2 reagent over the deposited organisms. Mixing is carried out within view of a photomultiplier which monitors the light emitted by the reaction. The generated signals are directly proprotional to the number of bacteria present. Dead as well as live organisms initiate this luminescence. To distinguish between the two, chemiluminescent signals are compared for both incubated and unincubated bacterial samples. A higher signal for the incubated sample indicates the presence of viable organisms and a cause for rejection of the water supply.

The manually operated tape transport system developed under the current contract and the results obtained toward E. coli and Cl. sporogenes, the two test organisms, are described in detail below. The effects on sample signal of immersion in water and the presence of various chemical interferents, are also presented.

In conformance with the requirements of Modification No. 25 to the present contract, the results of a Systems Analysis are presented in Appendix D. This analysis describes the various parameters which should be monitored to evaluate water quality and the methods best suited for making these measurements.

Section 3
EXPERIMENTAL RESULTS

3.1 INSTRUMENTATION

The major components of the manually-operated tape transport system developed under the current program are shown in the layout drawings in Figures 1 and 2. Photographs of the unit are shown in Figures 3 and 4.

The unit shown can be used for processing either an unincubated or incubated sample for detection of total (viable + non-viable) or viable organisms, respectively. For determination of the later, it is first necessary to obtain a baseline value on an unincubated sample processed in the same manner as the incubated sample except that the actual incubation has been omitted. The signal obtained in this manner reflects the total cell population of viable and non-viable which may be present. If the incubated sample produces a higher signal than the first, the presence of viable organisms is indicated.

3.1.1 Composite Tape

Whereas a continuous tape feed is indicated by the phantom lines in Figure 1, in the laboratory breadboard employed on the present program, precut composite tape segments (approximately one inch long) were used and were transported manually from one station to the next. The composite tape (shown in insert of Figure 1) consists of a standard 13 mm diameter Acropor AN 450* membrane filter cemented** over a 10 mm diameter hole*** in 35 mm Mylar leader film. The individual segments are cut from roll tape and treated as prescribed below before being introduced into the unit.

3.1.2 Tape Preparation

In order to insure removal of surface finishes and prophyrin-containing particulates**** from the membrane filter which might produce spurious chemiluminescence signals, the filter was subjected to a Urea-presoak followed by a sterile water rinse prior to its use in the laboratory device.***** While this procedure was found to be adequate for determination of total (viable + non-viable) cell populations in an

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* Gelman, 0.45 u pore size

** Millipore MF XX 7000-00 Cement.

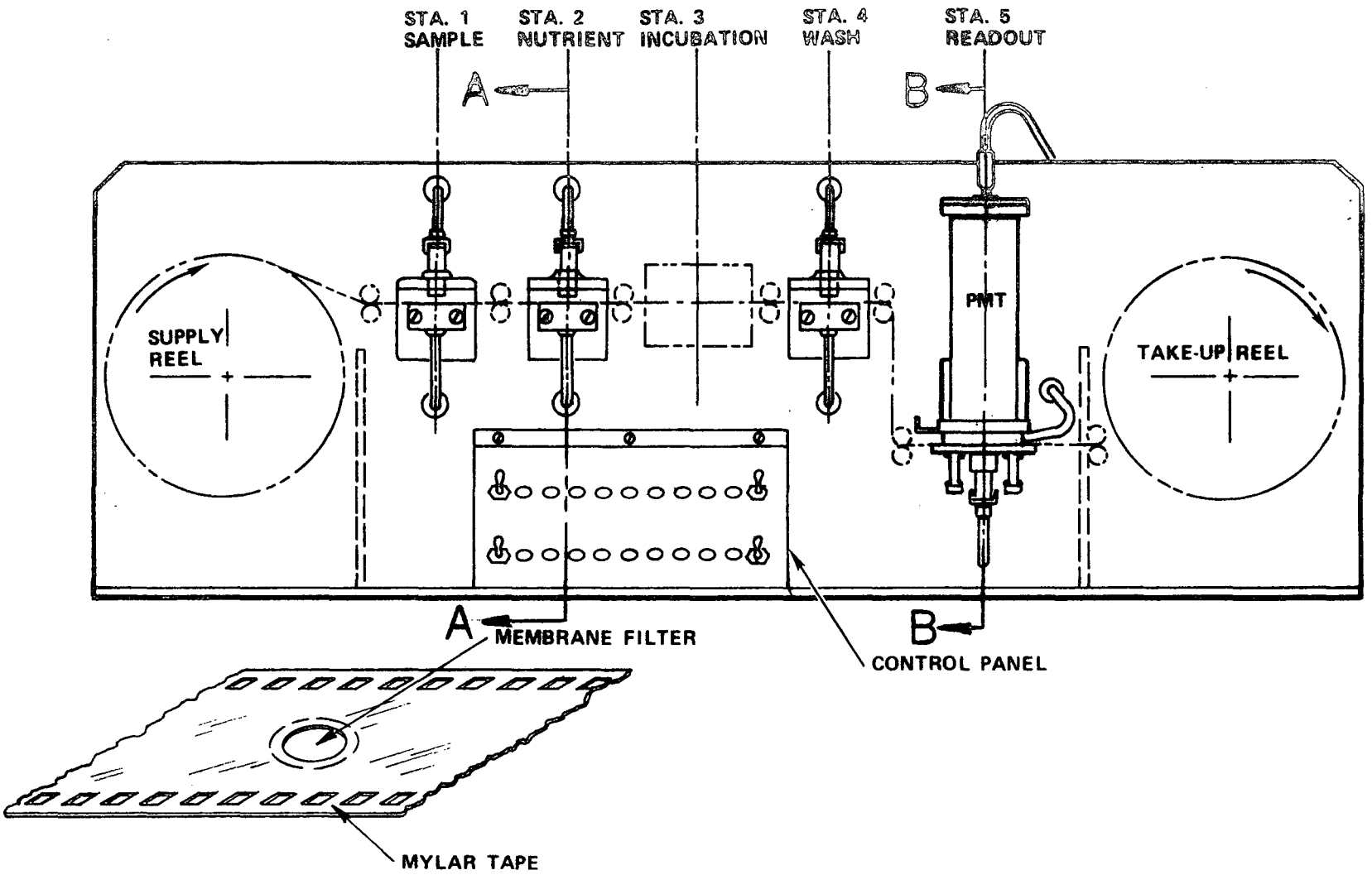
*** 10 mm diameter hole punch is shown in Figure 5.

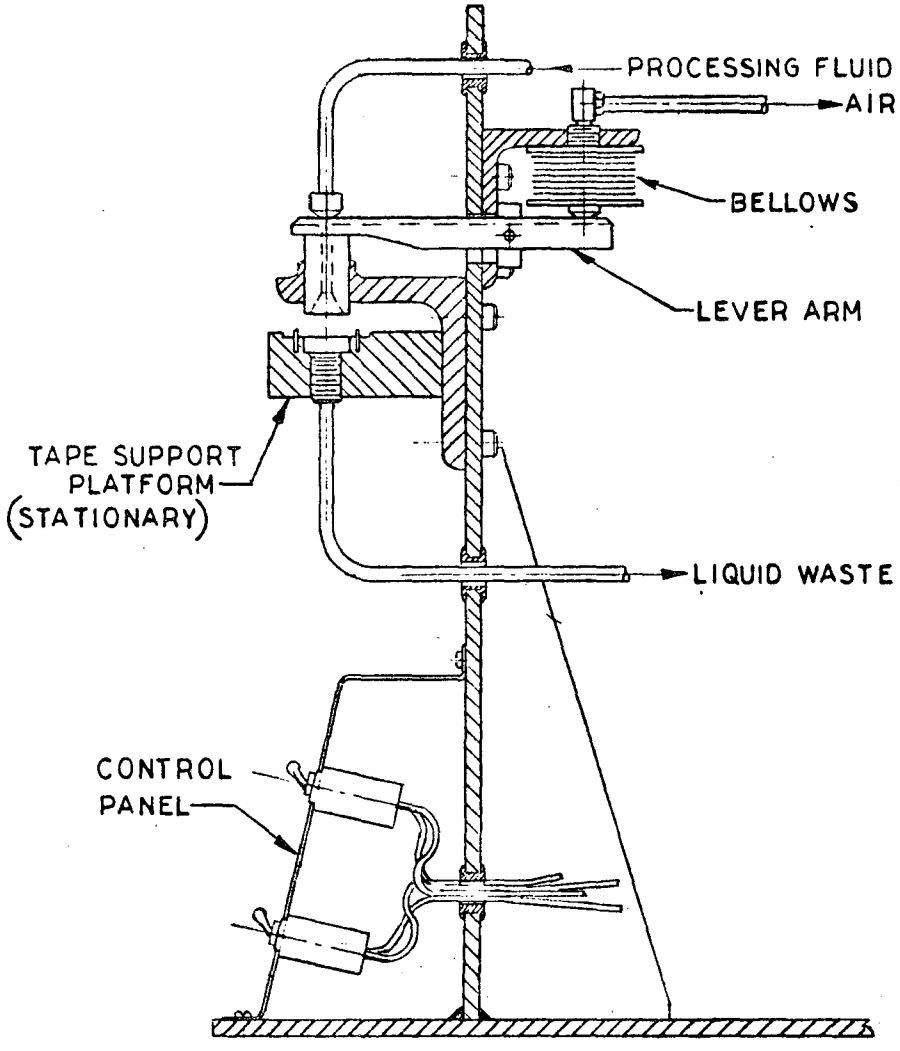
**** Bacterial debris or shedded skin fragments deposited on the filter during manufacture.

***** Details of this pretreatment procedure are contained in Section 4, Laboratory Support Studies.

Figure 1

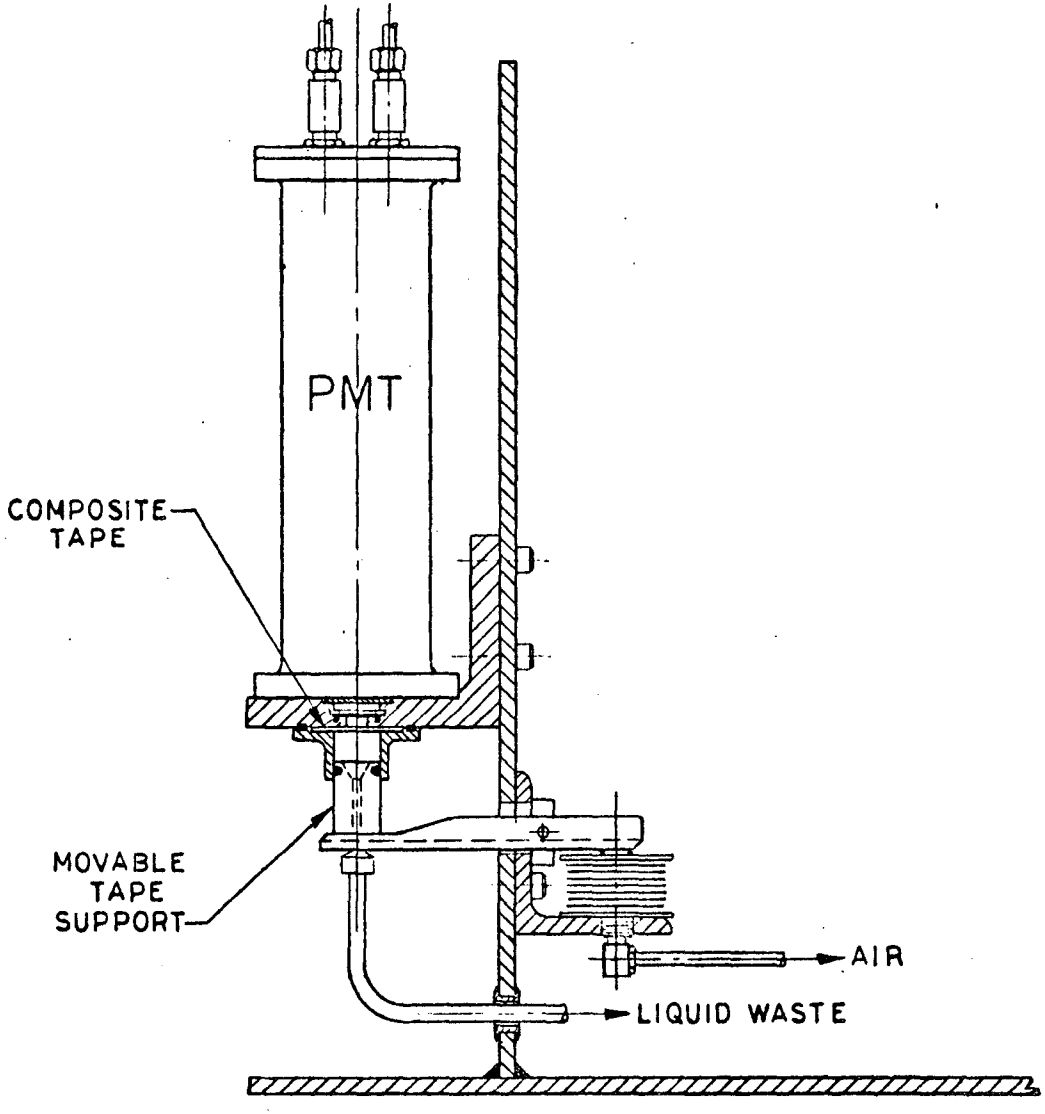
AMB WATER MONITOR (MANUAL LAB PROTOTYPE)





SECTION A-A

PROCESSING STATION



SECTION B-B

READ-OUT STATION

FIG. 2

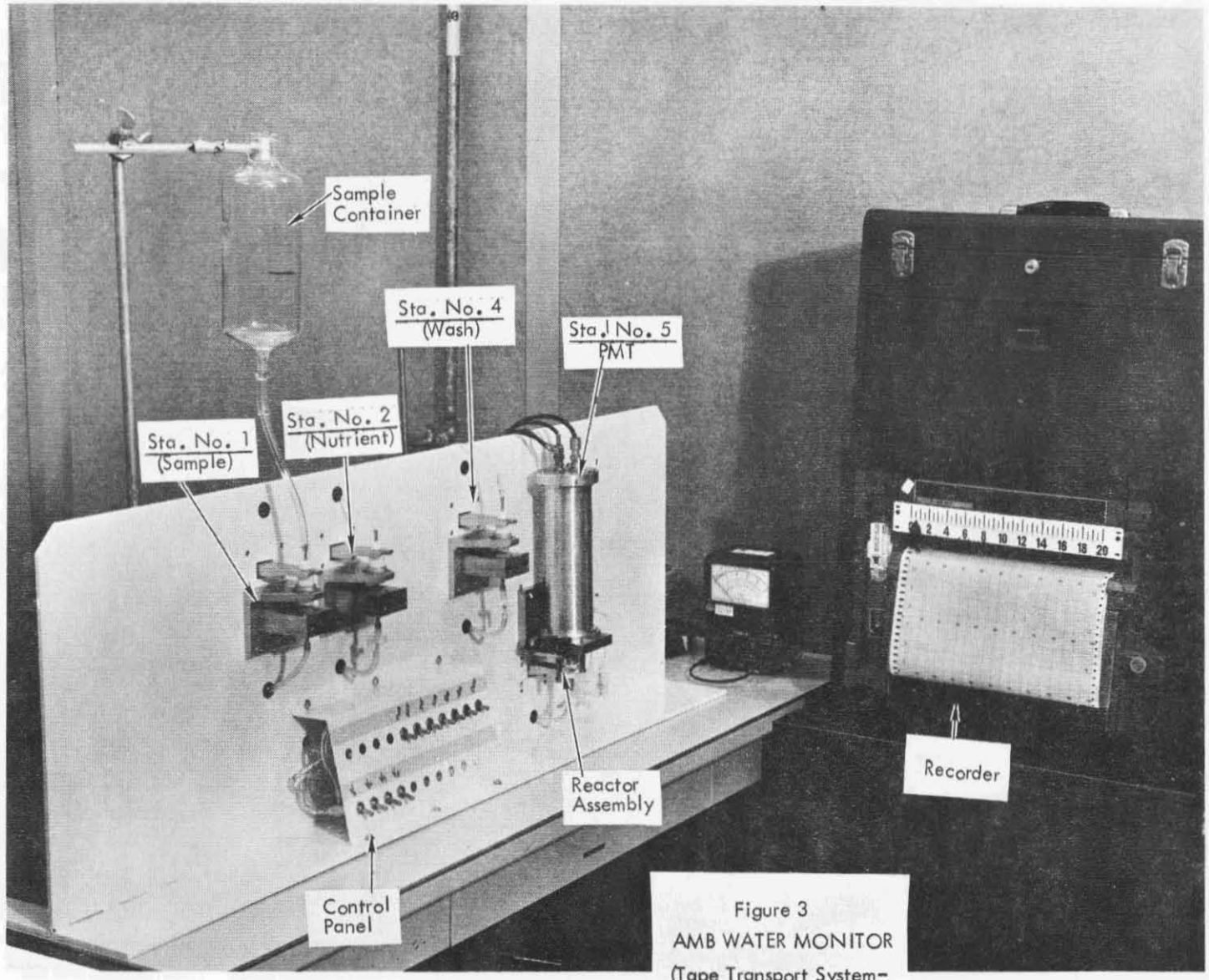


Figure 3
AMB WATER MONITOR
(Tape Transport System-Manually-Operated)

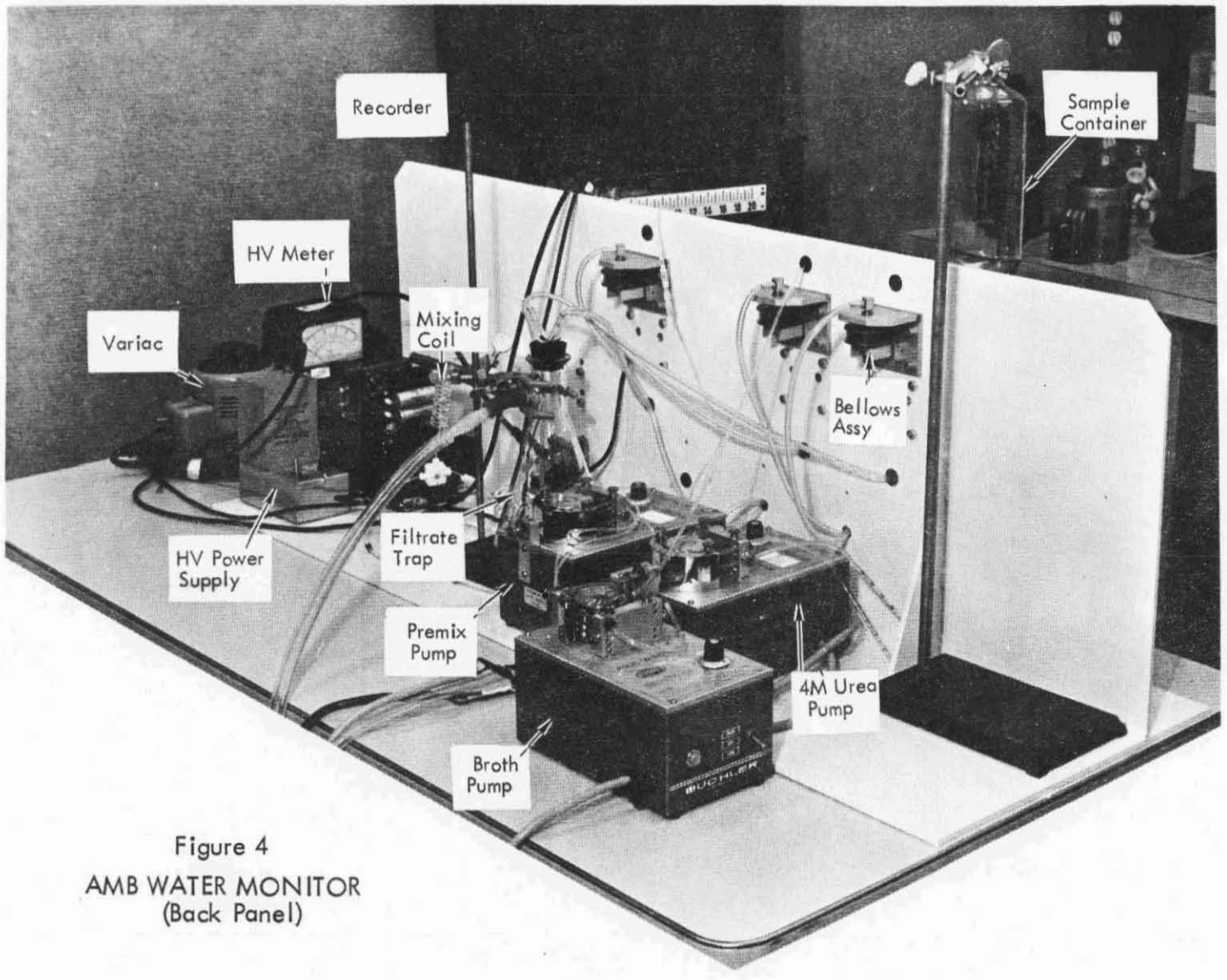


Figure 4
AMB WATER MONITOR
(Back Panel)

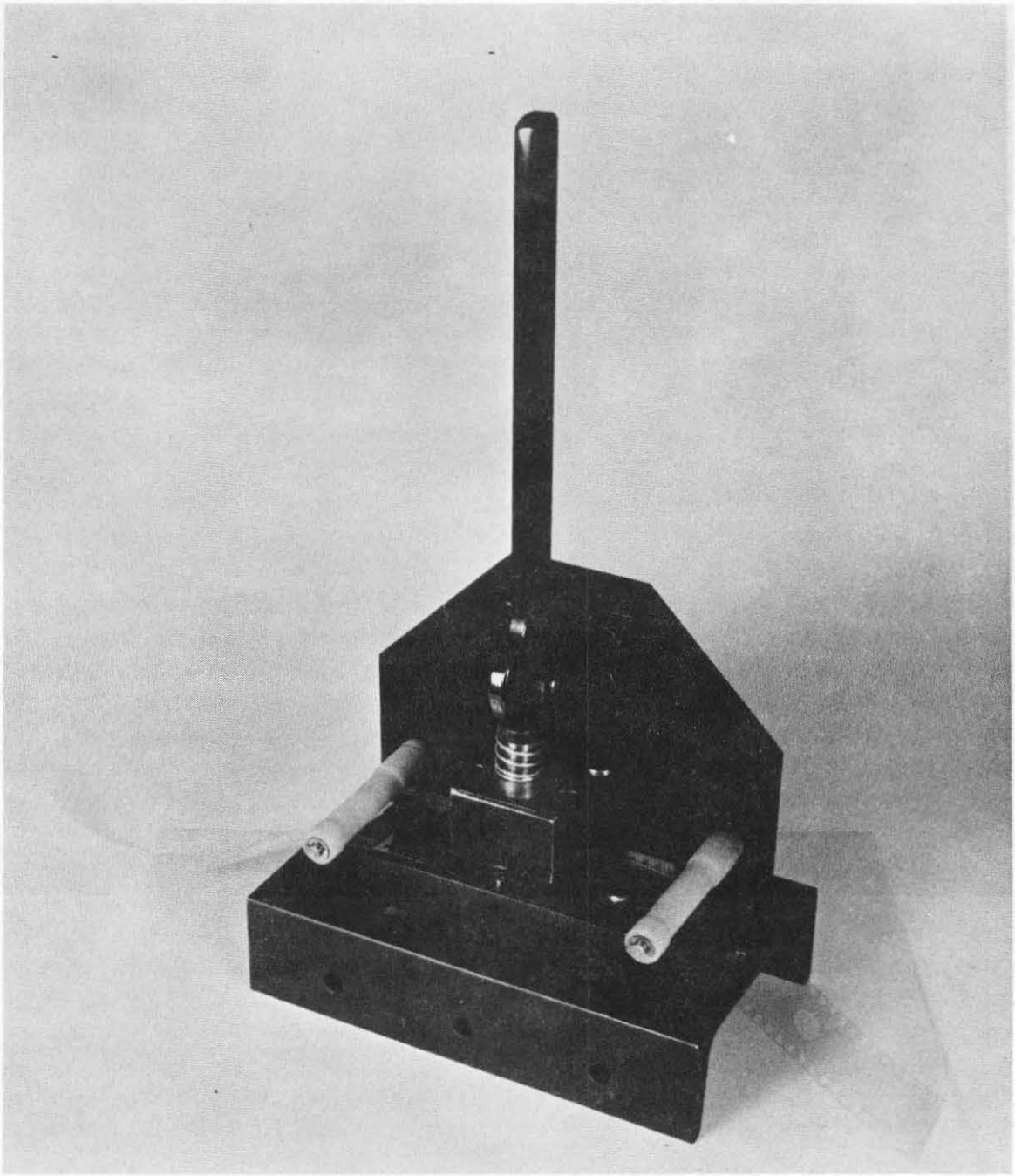


Figure 5
10 mm Diameter Hole Punch

unincubated cycle, with incubated cycles of 2-4 hour duration, a small signal (i.e., 1-2 volts) was sometimes observed for a blank incubated control (indicating that the tape was not completely sterile). However, the magnitude of this background signal was considered small or insignificant compared to the signal observed for an incubated bacterial challenge (see Table 6). A preliminary investigation of other techniques (autoclaving and ethylene oxide) for rendering the composite tape sterile, indicated that eithylene oxide (16 hours at ~ 70% Relative Humidity) appears to be the most effective. One problem associated with the ethylene oxide treatement is that liquid flow through the tape appears to be reduced by 20-25% and the effect of residuals on bacterial growth has not been fully evaluated. Consequently, unless otherwise indicated, the Urea-presoak followed by a sterile water rinse (described in Section 4.1.4.1) was used for obtaining the data for total and viable in this report.

3.1.3 Sample Processing Stations

Referring to the processing sequence in Table 1, the major functions performed at each of the stations shown in Figure 1 are the following:

a. Sample Concentration (Station 1)

A 400 ml water sample is concentrated in about 10 minutes by vacuum filtration (minimum of 28" vacuum) through the composite filter (Gelman Acropor 0.45 μ pore size; deposition surface, 9 mm diam.).

b. Nutrient Addition (Station 2)

5 ml of dextrous-thioglycollate broth (DTB) are pumped through the tape (partial vacuum ~15" on lower station) containing the deposited organisms. DBT was selected on the basis of a survey of several general purpose media (see Section 4, Laboratory Support Studies) and is suitable for growing both E. coli and Cl. sporogenes.

c. Incubation (Station 3)

Tape segments are placed on a Millipore filter pad saturated with dextrose-thioglycollate broth and incubated in a closed container with a controlled atmosphere* (90% N_2 + 10% CO_2, RH ~90% for Cl. sporogenes; ambient air, RH ~ 90% for E. coli) and incubated 2-4 hours at

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*Actual incubation is done in a thermostatically controlled oven which is not an integral part of the breadboard unit shown in Figure 1.

Table 1

Processing Sequence*

Station			Unincubated Cycle Duration, Min	Incubated Cycle Duration, Min
1		Sample Concentration (400 ml)	10	10
2		Nutrient Added (1 ml/min)	5	5
3		Incubation	0	120
4		Wash (4M Urea) (5 ml/min)	10	10
5	a.	Readout (Premix 1.35 ml/min)	10	10
	b.	Water Flush (5 ml/min)	2	2
		Total	37 Min	157 Min

*Reagent preparation is described in Appendix A.

37°C (see Laboratory Studies, Section 4). The actual incubation step is omitted for determination of "total" (viable + non-viable) cell population.

d. Wash Station (Station 4)

Nutrient is washed from the tape with 4M urea (latter pumped in at 5 ml/min; partial vacuum ~ 15" on lower station).

e. Readout Station

Luminol-H_2O_2 reagent is pumped over the surface of the tape in front of an EMI photomultiplier tube.* The amplified signal is processed either as a direct (analog) or integrated signal on a strip chart recorder.**

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* Luminescence is initiated virtually instantaneously (i.e., < 1 sec) on contact of the bacteria with reagent. Luminescence decay is a function of the bacterial concentration; for a total bacterial deposit of ~ 10^4 bacteria about 2 minutes would be required for the luminescence intensity to drop to ~10% of its initial value.

At the reagent flow rate employed (i.e., 1.35 ml/min) the maximum residence time that dislodged bacteria might spend in the viewing zone would be about 22 seconds. Thus, if bacteria were detached by flowing the reagent across the filter surface, some decrease in total luminescence would occur. However, considering the high flow velocities at which the bacteria are deposited on the tape (~51 ml/min/cm^2) and the low reagent flow rate employed, the probability of dislodging bacteria from the surface is small. Flowing reagent across the filter rather than through it, is preferred because it produces more consistent results.

** The recorder was calibrated at the inception of the program by attaching a DC power supply to the amplifier input (from the PMT) and adjusting the voltage until a full scale deflection was observed (value noted). This was repeated for all range/gain settings of interest.

Since the sensitivity of detection was a function of the PMT voltage, the latter was continually monitored by means of a voltmeter.

Overall sensitivity and functionability of the system was evaluated several times a week by challenging the system at a known bacterial (E. coli) level and noting the signal response.

After the sample signal has returned to a reagent baseline value, the reagent flow is shut off and the reaction chamber washed free of reagent with filtered distilled water.

Air valves mounted on a control panel are used to operate each of the processing stations. Movement of jaws on each station are controlled by air actuated metal bellows (see Figures 2 and 4). All processing stations are mounted on a single baseplate. A more detailed description of the operational sequence is presented in Appendix B.

Using the above laboratory breadboard, a number of parameters were investigated for optimizing the sensitivity and reliability of the tape transport system. Process variables evaluated included 1) the effect of flowing the reagent across the surface of the tape rather than through it, 2) effect of reagent concentration and flow rate on signal strength, 3) effect of PMT voltage on sensitivity and 4) effect of prestaining the organisms with a porphyrin-containing derivative to enhance the signal. Based on these studies, (described in detail in the Laboratory Support Studies section), the most optimum set of conditions (shown in Table 2) were utilized to derive the data presented below for unincubated and incubated samples of E. coli and Cl. sporogenes.

3.2 UNINCUBATED CYCLE

Typical traces obtained using the protocol and conditions described in Tables 1 and 2 for an unincubated sample of E. coli (3×10^5 cells/400 ml suspension) and a water control (400 ml filtered distilled water) are shown in Figures 6A and 6B. The overall net signal (S-N) due to the bacteria is derived by substrating the net signal of the water control from the net signal of the bacterial suspension, thus

Table 2

Recommended Conditions for Operation of AMB Water Monitor

1.	Sample Size	400 ml
2.	Filter Material	Gelman Acropor AN 450 (0.45 μ)
3.	Sample Processing Protocols (Incubated and Unincubated Cycles)	See Table 1 (For aerobic organisms such as E. coli, 2 hr incubation is sufficient; for anaerobic Cl. sporogenes, a minimum of 4 hrs is required.
4.	Nutrient	Dextrose-Thioglycollate Broth with added Hemin*.
5.	Wash Station	4M Urea*
6.	Luminol-H_2O_2 Reagent*	Luminol Solution @ 0.11 ml/min. H_2O_2 Solution @ 0.24 ml/min. Sterile Distilled H_2O @ 1.00 ml/min.
7.	PMT Voltage	1450 volts (EMI, 9635A PMT)

*See Appendix A for reagent composition.

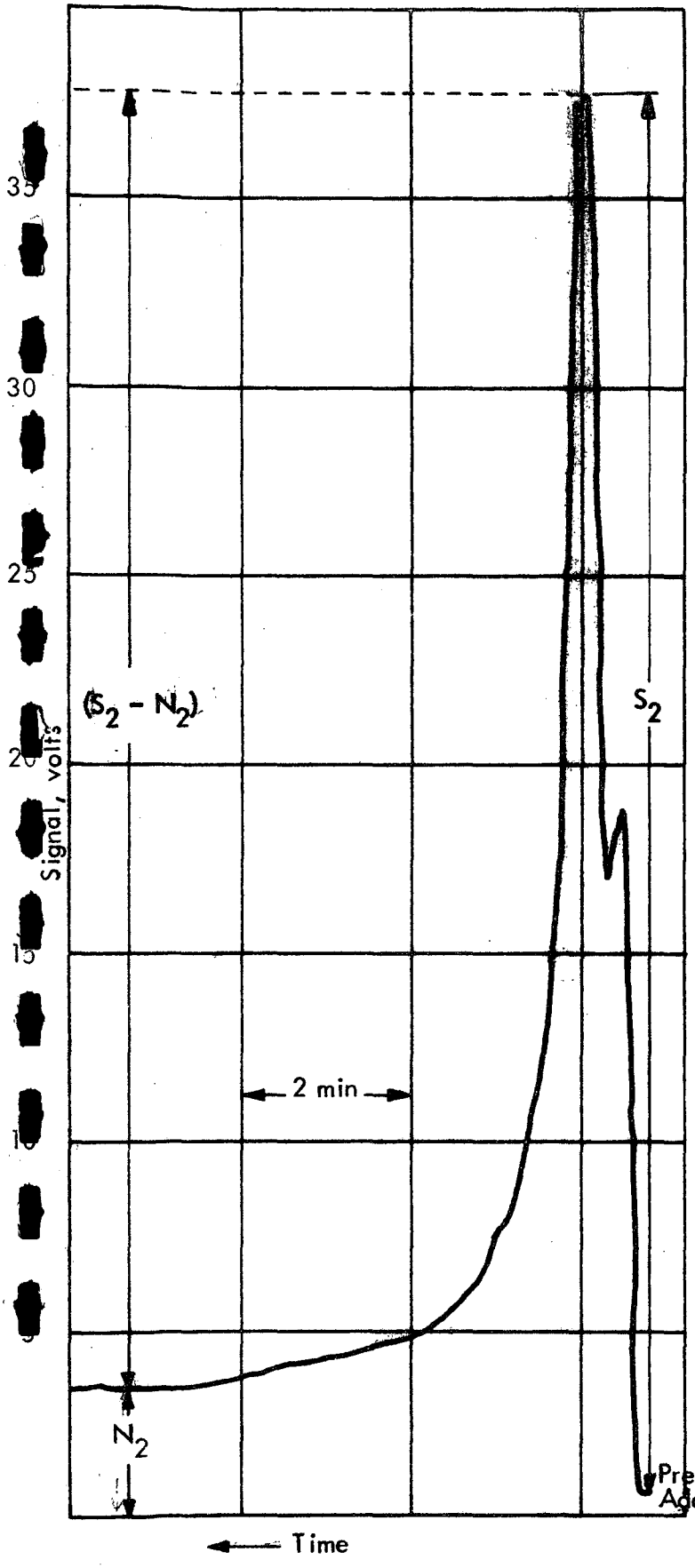

Figure 6 A

Signal Response 3×10^5 E. coli*/400 ml

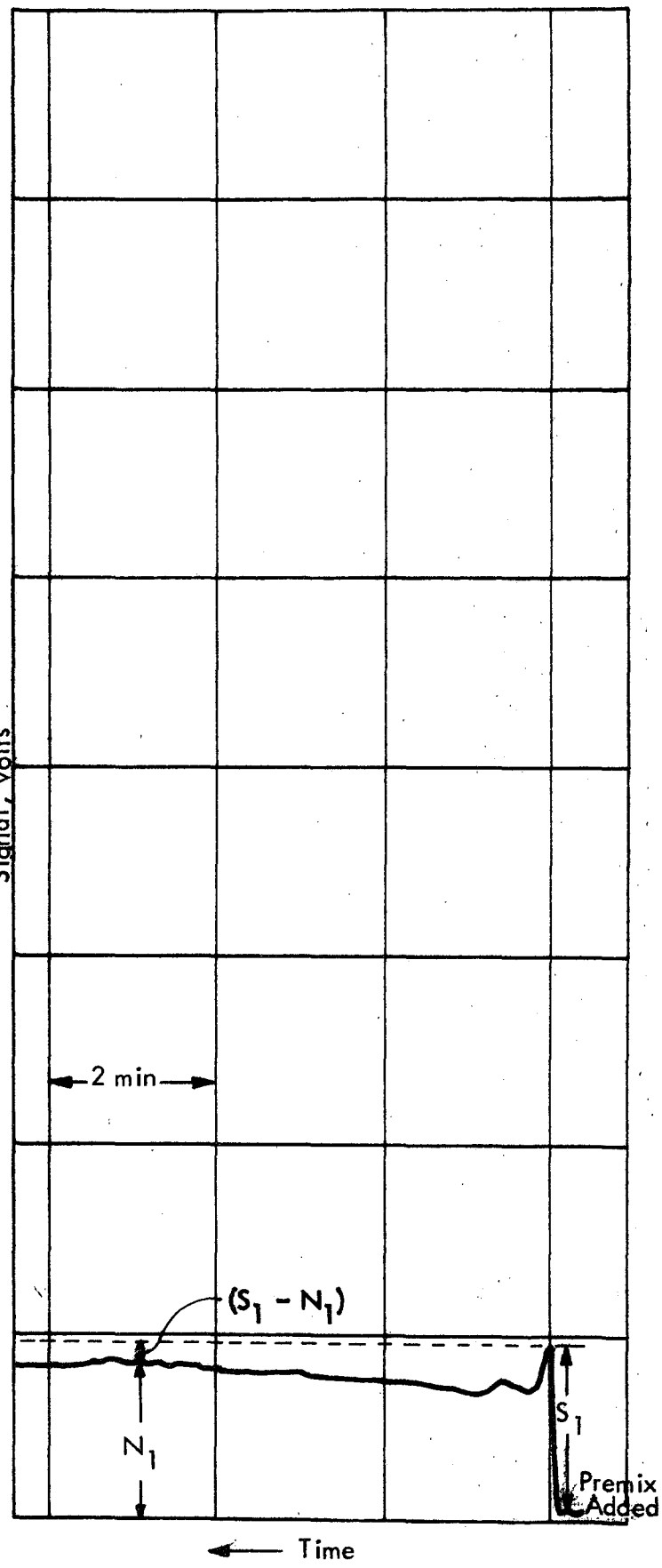

Figure 6.B

Signal Response Water (400 ml) Control

*Hemin Stained

Overall Net Signal (S-N)	=	(S_2-N_2)	-	(S_1-N_1)
(Due to Bacteria)		Net Signal of Sample		Net Signal of Water Control
33.5 volts*	=	34.5*	-	1.0*

As indicated by a comparison of N_2 with N_1 in Figures 6A and 6B, some variation in the reagent baseline is observed between different tape segments, probably as a result of variations in the tape or tape preparation procedures.

With reference to the shape of the sample signal, a double peak is evident, the first appearing 20 seconds after initiating the reagent flow and the second about 20 seconds later. The double peak may be the result of a diffusion phenomenon. Because of the plumbing arrangement, the first trace of liquid reaching the filter is Premix diluted with water (small amount of distilled water remaining in the section of tubing from the previous rinse cycle). The initial contact of the bacteria with this diluted reagent may give rise to the first peak, and subsequent contact with the more concentrated reagent, the second higher peak. A distinct double peak is rarely observed on flowing reagent through the tape probably because the flow rates used are generally much higher.

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*Refer to Data Sheet of 30 September 1971, Runs 1 and 2, contained in Appendix C.

The signal response as a function of bacterial concentration is given in Table 3. A plot of log (S-N)vs log bacterial concentration (Figure 7) indicates deviation from linearity, particularly at the higher bacterial levels. If area under the curve rather than signal amplitude is used in making this plot (Figure 7), linearity is still not achieved. Non-linearity may arise from scattering or absorption of the generated light due to overlapping of the deposited organisms, an effect which would be expected to increase with increasing bacterial concentration. To demonstrate that the PMT output was linear over the range of interest, the PMT was exposed to varying levels of light over the range of interest. For each level, the voltage signal and PMT current were noted. The data shown plotted in Figure 8 indicate linearity of reponse over the range of interest. The data are in conformance with that supplied by the manufacturer, namely that the output is generally linear up to about 1 ma.

Referring to Figure 7, assuming a net signal (S-N) of 1.5 volts significant, then approximately 1.3×10^4 hemin-stained E. coli in a 400 ml sample can be detected. This would correspond to a bacterial level of approximately 30-35 cells/ml. When hemin-staining* is not employed, the net signals (Table 4) are substantially lower and the detection threshold is then of the order of 50-75 cells/ml (400 ml sample).

Since the intensity of the generated signal drops off as the square of the distance, one way of improving the sensitivity of the present system would be by re-designing the reaction chamber to bring the tape surface closer to the face of the photomultiplier tube. (Distance is currently about 3/4" compared to about 1/8 - 1/4" for chemiluminescence monitoring systems employing fluid sample transport.) As few as 10 E. coli/ml (400 ml sample) can be detected by one of the latter systems**.

The signal response of the system toward unincubated samples of Cl. sporogenes (hemin-stained and unstained) were also evaluated with the results shown in Table 5. The data for Cl. sporogenes indicate a decidely lower sensitivity in addition to non-proportionality between bacterial concentration and signal response. The lower sensitivity exhibited by Cl. sporogenes is probably due to the absence of cytochromes, one of the iron-porphyrin proteins responsible for chemiluminescence. While all aerobes and some faculative (e.g., coliforms) and obligate (e.g., Desulfovibrio desulfivricans) anaerobes contain cytochromes in their respiratory system, Clostridia generall do not. The fact that Cl. sporogenes show some response indicates that small amounts of cytochromes are probably present.

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*To achieve hemin-staining, 5×10^{-7} grams of hemin are added to the DBT broth at the second station; see Laboratory Support Studies for details.

**Development of a Laboratory Prototype Water Quality Monitoring System Suitable for use in Zero-Gravity", Langley Research Center, Contract NAS 1-0382 in progress.

Table 3

Signal Response of Hemin-Stained *E. coli*

E. coli (cells/400ml)	Signal Response, volts: Sample S_2	Sample N_2	Sample S_2-N_2	Water Blank S_1	Water Blank N_1	Water Blank S_1-N_1	Overall Net Signal S-N	Reference: Data Sheet	Reference: Runs
3×10^5	32.5	3.5	29.0	3.5	3.5	0	29.0	9/16	1,2
3×10^5	32.5	4.0	28.5	3.5	3.5	0	28.5	9/16	1,3
3×10^5	39.0	3.0	36.0	3.5	2.5	1	35.0	10/3	1,2
3×10^5	38.0	3.5	34.5	5.0	4.0	1	33.5	9/30	1,2
							Avg. 31.5 ±3.3*		
1×10^5	29.0	3.5	25.5	5.0	4.0	1	24.5	9/30	1,3
3×10^4	12.0	4.5	7.5	5.0	4.0	1	6.5	9/30	1,4
2.5×10^4	8.5	3.5	5.0	5.5	3.5	2	3.0	8/10	2,4
1×10^4	5.0	3.0	2.0	5.0	4.0	1	1.0	9/30	1,6
1×10^4	7.0	6.0	1.0	4.5	4.5	0	1.0	8/19	1,3
5×10^3	6.0	3.5	2.5	5.0	2.5	2.5	0	8/13	2,3

*Standard Deviation

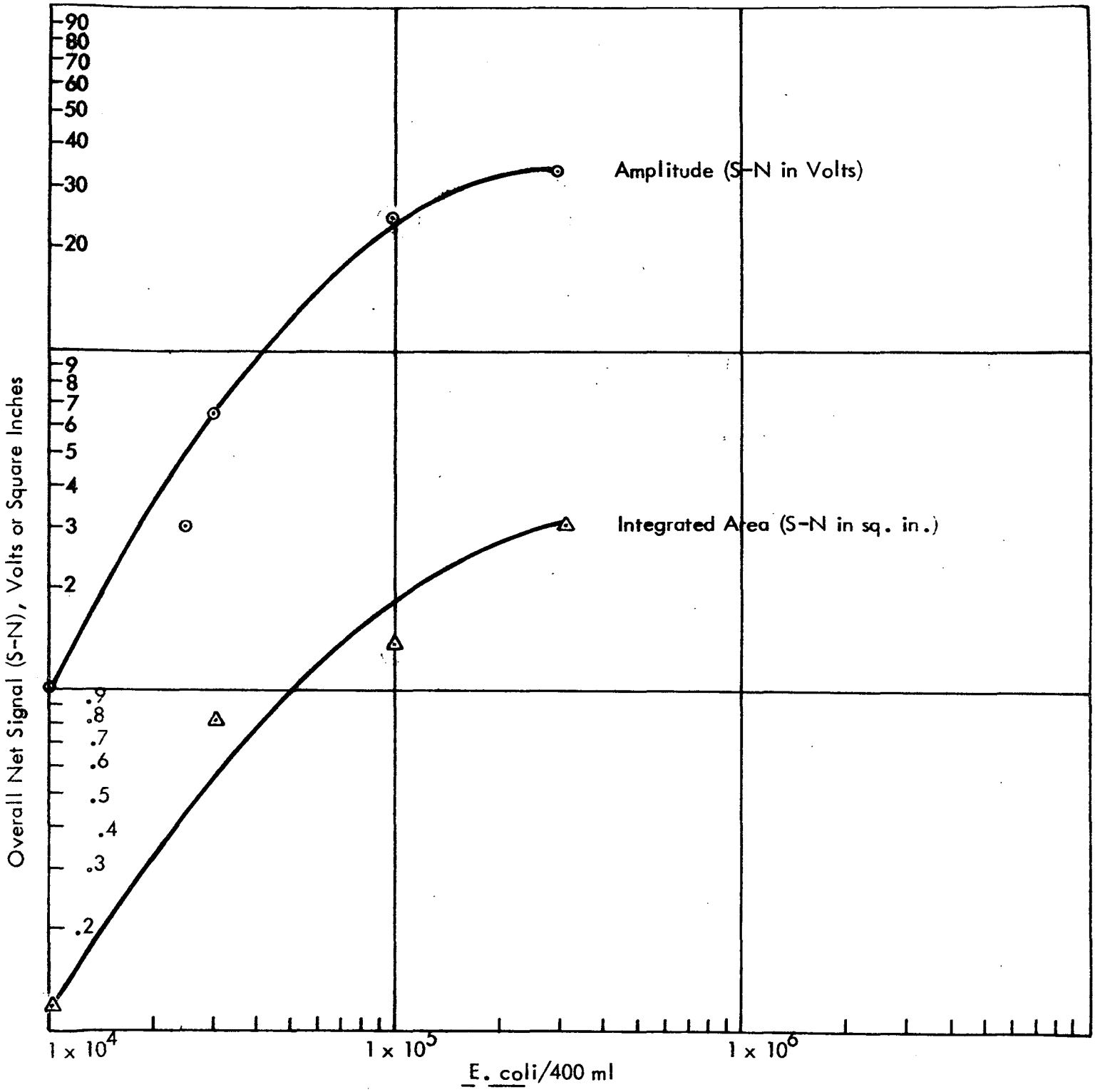

Figure 7 Signal Response for Hemin-Stained E. coli

PMT (EMI 9635A) LINEARITY

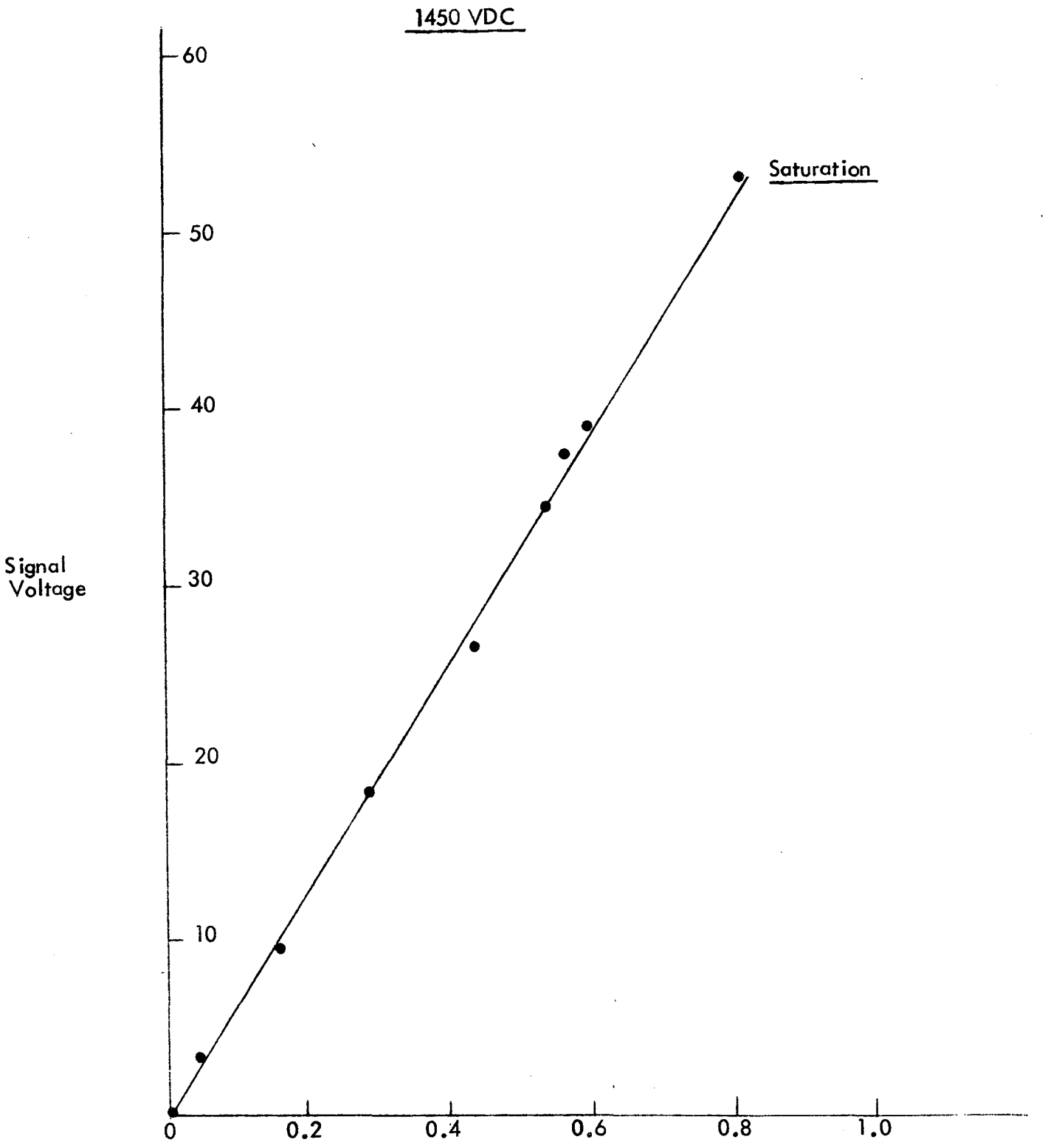

Figure 8

Table 4

Signal Response of Unstained E. coli*

E. coli/400 ml	Overall Net Signal (S-N), volts	Reference Data Sheet	Reference Runs
5×10^4	2.5	7/22	1,3
7.5×10^4	5.0	7/27	2,3
5×10^5	21.5 ±1**	7/9 7/13 7/20 7/22	1-3 1-4 1,4 1-2

*Standard protocol except that DBT broth did not contain hemin.

**Standard deviation; average of 7 points (20,21,22,23,23,20,21.5); others are single runs.

Table 5

Signal Response Toward an Unincubated Suspension of
Cl. sporogenes

Cl. sporogenes	Overall Net Signal (S-N), volts	Reference Data Sheet	Reference Runs
1×10^6 cells/400 ml (unstained)	1.0, 0, 3.0 (Avg. = 1.3)	9/10 9/17 7/23	1,5 1,2 1,5
5×10^6 cells/400 ml (unstained)	6.0	7/23	1,2
5×10^6 cells/400 ml (hemin-stained)	8.0	7/23	1,4

Reference to the data in Table 5 indicates that hemin-staining of Cl. sporogenes by the tape procedure improved the signal strength by about only 30% (i.e., from 6 to 8 volts for 5×10^6 cells of Cl. sporogenes). However, data presented in the Laboratory Support Studies section indicate that at least a one log improvement in sensitivity can be achieved by hemin-staining of these organisms in solution. Staining in solution rather than on tape provides more effective staining of the organisms since higher concentrations of hemin can be employed. In the solution staining technique excess hemin can be conveniently removed from the stained organism by centrifugation and washing. In adapting this procedure to the tape system, the hemin concentration has to be reduced 10,000-fold because of the nonspecific adsorption of the hemin by the membrane filter. The latter would tend to raise the background tape luminescence to an objectionably high value. By reducing the hemin concentration to the low levels required to provide an acceptable baseline signal, the effectiveness of hemin-staining by the tape procedure is markedly reduced. Additional studies would be required to further optimize the hemin-staining technique for use with the tape transport system.

3.3 INCUBATED CYCLE

The ability of the system to detect viable organisms by comparing the signals of incubated and unincubated samples was determined with the results shown in Table 6. A typical set of traces obtained for E. coli is shown in Figure 9. Traces (a) and (b) are unincubated controls, with (a) being sterile distilled water used for preparing the bacterial suspension and (b) the unincubated bacterial suspension. Trace (c) is the same bacterial suspension which had been incubated for 2 hours at 37°C and then processed in an identical fashion to the other two. The number of viable cells in this suspension was actually determined by the standard pour plate method at the time

Table 6

Detection Sensitivity for Incubated Suspensions of *E. coli* and *Cl. sporogenes**

Total Challenge	Viable*** Cell Count	Hours Incubation @ 37°C	Overall Net Signal, volts	Reference Data Sheets	Reference Runs
5×10^3 *E. coli*/400 ml (with hemin)	2.7×10^3/400 ml (7 cells/ml)	0	0	8/20	2,3
"	"	2	16	8/20	3,4
5×10^3 *E. coli*/400 ml (with hemin)	3.3×10^3/400 ml (8 cells/ml)	0	0	8/25	3,5
"	"	2	9	8/25	4,11**
5×10^3 *E. coli*/400 ml (with hemin)	3.3×10^3/400 ml (8 cells/ml)	0	0	8/13	2,3
"	"	3	~26 (broad peak)	8/13	4,5
1×10^4 *E. coli*/400 ml (with hemin)	6×10^3/400 ml (16 cells/ml)	0	1	8/19	1,3
"	"	3	> 40 (PMT saturation)	8/19	6,7
2.5×10^4 *E. coli*/400 ml (without hemin)	not taken	0	3	8/10	1,2
"	"	4	~ 33 (broad peak)	8/10	4,5
"	"	4	~ 32 (broad peak)	8/10	4,6
7.5×10^4 *E. coli*/400 ml (without hemin)	not taken	0	5	7/27	2,3
"	"	2	7	7/27	4,5
"	"	4	~ 30 (broad peak	7/27	6,7
1×10^6 *Cl. sporogenes*/400 ml (with hemin)	not taken	0	0	9/17	1,2
"	"	2	0.5	9/17	5,6
1×10^6 *Cl. sporogenes*/400 ml (with hemin)	not taken	0	1	9/10	1,5
"	"	4	4	9/10	2,4

*Standard protocol; with or without hemin in DBT broth as indicated.
**ETO treated filter
***By standard pour plate.

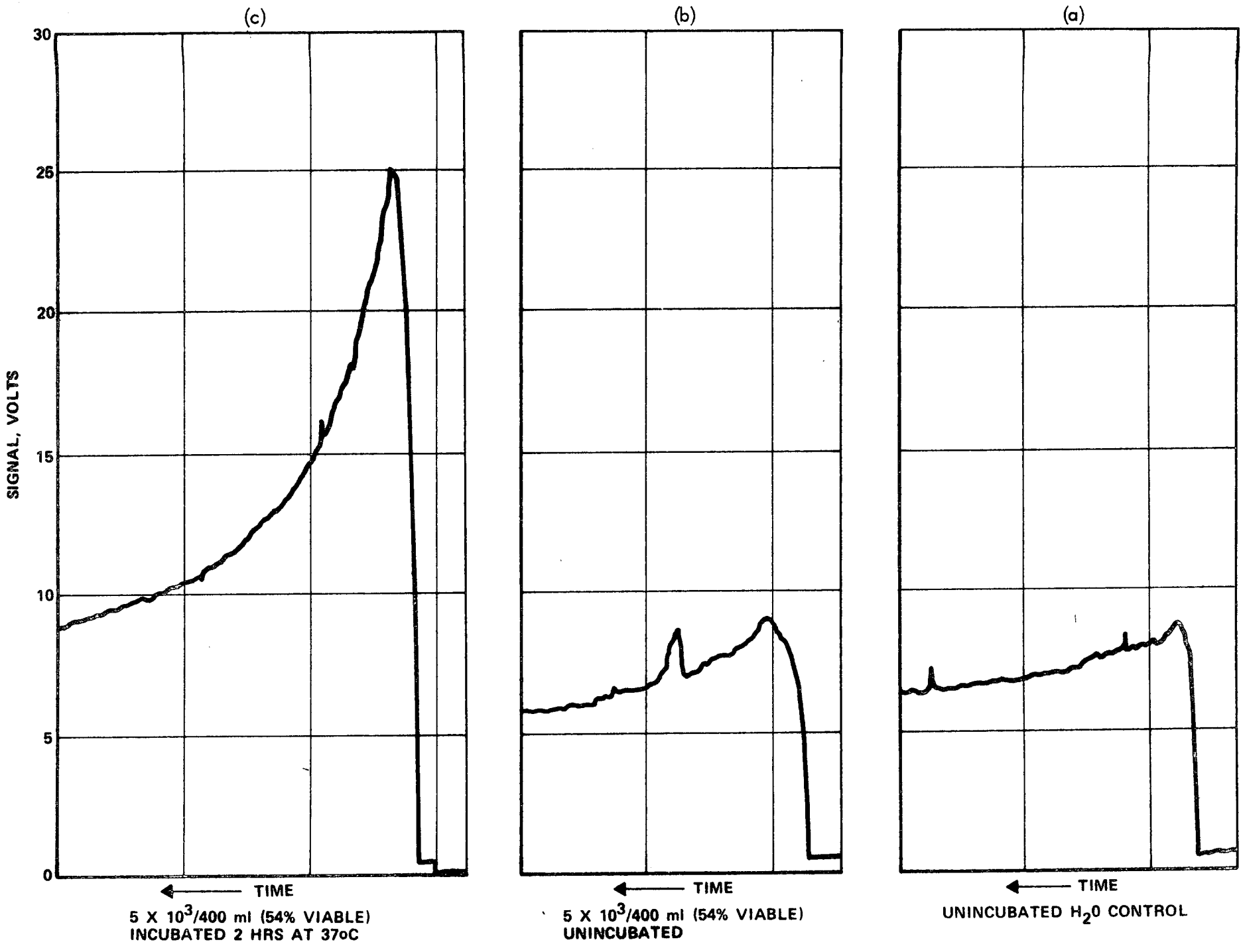

Figure 9 Incubated Cycle (E. coli)

this test was performed. The data for this run indicate that 54% of 5×10^3 E. coli or 2700 viable cells (in a 400 ml sample) can be readily detected after a 2-hour incubation period. Whereas a net signal of 1.5 volts is considered significant for an unincubated sample, probably twice that which would be required for an incubated cycle. Some increase in background signal occurs on incubation probably as a result of partial staining by hemin of the non-bacterial particulates in the water sample. Additional testing of the viable cycle at the lower detection level is required to establish the true level of significance. Assuming a net signal (incubated minus unincubated control) of 3 volts as significant, then based on the data shown in Table 6, 1 to 2 viable cells/ml would be detectable in a two-hour incubation for a 400 ml sample. The sensitivity can be further improved either by extending the incubation period or increasing the sample size.

Whereas < 10 viable cells of E. coli/ml (400 ml sample) can be detected in a two-hour incubation, approximately $10^3 - 10^4$ cells/ml (400 ml sample) and a 4-hour incubation are needed for detection of the anaerobe*, Cl. sporogenes. Apart from the lower porphyrin (i.e., cytochrome) content of the

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*Tape segment (from Station 2) with deposited organisms was placed over DBT saturated Millipore pad and incubated aerobically (for E. coli) or anaerobically (for Cl. sporogenes) for 2 or 4 hrs, respectively, at 37°C. See Section 4 for details.

anaerobes, the tendency to sporulate and the long lag times required for germination as well as the lower growth rate*, all contribute to a lowered sensitivity toward Cl. sporogenes. More effective hemin-staining may improve the sensitivity toward anaerobes such as Cl. sporogenes. The probability of anaerobes appearing as contaminants in a regenerated water supply is discussed in Appendix D, Systems Analysis.

3.4 EFFECT OF WATER STRESS ON SIGNAL STRENGTH

The effect on signal strength of immersing E. coli in distilled water at pasteurization temperature for extended periods was investigated. Immersions of 4 and 24 hours duration at 160°F were selected because this covers the range at which reclaimed water is stored prior to actual use. A decrease in signal might be expected if appreciable cell lysis or leaching of bacterial porphyrins occurred during immersion. The soluble porphyrins discharged into solution would then be lost on subsequent filtration and concentration of the water sample. The results summarized in Table 7 indicate the following:

a. A 4 hours immersion at 160°F reduces the signal strength an insignificant amount (i.e., from 33.5 volts to 31.0 volts or about 7%; the experimental error (1 σ) in determination at this bacterial level is $\sim \pm 10\%$).

b. Immersion of E. coli for 24 hours at 160°F causes a virtually complete loss of signal. Microscopic examination of the sample after immersion indicated extensive cell lysis and perfuse amounts of cellular debris suggesting that the loss in signal was due to cell lysis and/or leaching of the bacterial porphyrins. Assuming no destruction of the porphyrins on heating, the present chemiluminescence device, suitably modified to monitor soluble porphyrins, should be able to detect $\sim 2 \times 10^4$ lysed E. coli** in a 1 ml sample volume.

Qualitative evidence that soluble porphyrin signals increase with time of immersion was obtained by utilizing another chemiluminescence device, developed at AMB, which is suitable for measuring soluble porphyrins. The soluble signal shown in Table 8 refers to the signal generated on reacting the sample filtrate (filtration through a 0.41 μ membrane filter) with luminol-H_2O_2 reagent in the AMB chemiluminescence device. Although the net signals obtained for a particular challenge with this unit are not directly comparable to the signal obtained with the tape transport system (because of differences in sensitivity of the two instruments and the fact that the former is an integrated signal over a finite period as opposed to an amplitude signal for the tape system), the results summarized in Table 8 indicate that the soluble signal increases with time of immersion at 160°F. These results point up the importance of monitoring soluble bacterial porphyrins as well as discrete bacterial cells as an indication of bacterial contamination in the reclamation system.

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*Growth studies for Cl. sporogenes are described in Section 4.1.
**Based on ability to detect 30-35 E. coli/ml in a 400 ml sample.

Table 7

Effect of Water Stress on Signal Strength of *E. coli*

Immersion of 3×10^5 *E. coli*/400 ml	Signal, volts						Overall Net Signal, Volts
	Sample			Water Blank*			
	S_2	N_2	(S_2-N_2)	S_1	N_1	(S_1-N_1)	
0 hrs at 160°F (control)	38	3.5	34.5	5	4	1	33.5**
4 hrs at 160°F	35.5	3.0	32.5	4	2.5	1.5	31.0***
24 hrs at 160°F	8.0	3.0	5.0	7	3	4	1.0****

*Water blanks maintained at 160°F for 0, 4 and 24 hours respectively.

**Reference: Data Sheet 9/30, Runs 1,2

***Reference: Data Sheet 9/30, Runs 9,10

****Reference: Data Sheet 10/3, Runs 3,4

Table 8

Effect on Soluble Porphyrin Signal of Immersing E. coli in Distilled Water at 160°F

Procedure: 2×10^8 E. coli/20 ml held at 160°F for time indicated, filtered through a 0.45 u membrane filter, and filtrate reacted with luminol-H_2O_2 in a special AMB chemiluminescence device.

Sample	Chemiluminescence Signal (volts)
Water Blanks (control)	2.78, 2.88, 2.98, 2.92 (Avg = 2.89)
2.8×10^8 E. coli/20 ml (0 hr immersion)	3.58, 3.46, 3.43, 3.64 (Avg = 3.80)
2.8×10^8 E. coli/20 ml (19 hrs @ 160°F)	5.24, 5.13, 4.91, 5.42, 5.44 (Avg = 5.23)*

*Net signal of 1.43 volts (i.e., 5.23 - 3.80) corresponds to 2.6×10^5 E. coli on this instrument.

High soluble porphyrin signals were observed in reclaimed water samples taken at the conclusion of the test series at McDonnell Douglas on September 11, 1970*. Evidence that this soluble fraction was due principally to porphyrins from lysed organisms was the observation that samples with high soluble porphyrin signals exhibited relatively low total cell counts (determined microscopically) as well as low chemiluminescence signals (generated on reaction of backwashed organisms (off a filter) with luminol-H_2O_2 reagent). Bacteria present elsewhere in the water reclamation system were being lysed on reaching the storage tanks maintained at pasteurization temperature. Even though bacteria are destroyed in the storage tank, their growth elsewhere in the system and subsequent lysis can result in contamination of the water supply by bacterial toxins. Some of these toxins are heat stable (i.e., would survive pasteurization temperature) and thus present a health hazard. The absence of lysed organisms (i.e., soluble porphyrins) in a water supply would indicate that bacterial buildup was not occurring elsewhere in the reclamation system.

3.5 CHEMICAL INTERFERENCE STUDIES

The extent of interference which might be produced by various chemicals being considered for use as bactericides in water generation systems was investigated with the results shown in Table 9**. Concentration levels chosen were those normally employed in water sterilization. Samples consisted of

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* AMB, "Development of Bacterial Sensing Instrumentation for Spacecraft Water Systems", to NASA/MSC, Contract NAS 9-10432, 13 November 1970, pp. 3-36 and 3-50.

** The data shown in Table 9 represent the results of only a single determination for each of the parameters indicated. Additional data (including experimental details) are contained in the Laboratory Support Studies Section".

Table 9

Effect of Bactericides on Signal Strength of E. coli

	Signal, volts						Overall Net Signal, Volts
	Sample			Water Blank			
	S_2	N_2	(S_2-N_2)	S_1	N_1	(S_1-N_1)	
1×10^5 E. coli/400 ml (Control)++	29.0	3.5	25.5	5	4	1	24.5
+30 ppm "Available Chlorine" * +	14.5	4.0	10.5	4.5	2	2.5	8.0
+20 ppb Ag^+ (as AgBr)**	22.5	3.0	19.5	4	2.5	1.5	18.0
+6 ppm I_2***	17.5	3.0	14.5	3.5	2.5	1	13.5

*from $CaOCl_2$

+Reference Data Sheet 10/3, Runs 5,6

**Reference Data Sheet 9/30, Runs 7,8

***Reference Data Sheet 10/3, Runs 7,8

++Reference Data Sheet 9/30, Runs 1, 3

acqueous suspensions of E. coli with or without added bactericide. The water blank for the control bacterial suspension was simply filtered distilled water which had been used for preparing the bacterial suspension. The water blanks for the other samples contained the same level of bactericide as the respective samples. Sample and water blanks (400 ml of each) were all processed the same using the standard proctocol shown in Table 1. Bacteria were in contact with the bactericide for two hours before processing.

The data in Table 9 indicate that the presence of the bactericides at these levels produce a decrease in signal ranging from 22 to 67%. Three possible explanations for the decrease are 1) residual bactericide still present after sample processing is producing quenching of the chemiluminescence or 2) the bactericide is causing lysing of the bacterial cell (with subsequent loss of bacterial porphyrins on filtration), or 3) the bactericide is inactivating the bacterial porphyrins. It cannot be determined, without further testing, which factor is producing the observed effect. If the first factor is responsible, additional washing with urea (at Station 4) should eliminate this problem. If the second is the reason for the drop-off, monitoring of soluble porphyrins (released by the lysed cell) would be the solution. If the last one, on approach would be to place the bacterial monitor upstream of the bactericide addition.

Additional studies are recommended to establish the reason for the observed decrease in signal so that appropriate corrective action can be taken.

Section 4

LABORATORY SUPPORT STUDIES

A number of studies were conducted in order to establish the optimum conditions for processing the incubated and unincubated sample. Among these were a) a survey of several general purpose media for growing Cl. sporogenes and E. coli, b) the effect of various parameters (i.e., oxygen tension, incubation temperature and pH) on the growth of these test organisms, c) a comparison of different filter materials and tape pretreatments to minimize interference with the chemiluminescence reaction, d) the effect of process variables (i.e., direction, concentration and rate of flow of reagent, PMT voltage, and hemin staining) on signal strength.

Based on these results, the optimum protocol (shown in Table 1) for determining viable and non-viable cell populations was established and utilized to obtain the data presented in Section 3 of this report. The details of these laboratory support studies follow:

4.1 GROWTH OPTIMIZATION OF AEROBES AND ANAEROBES

4.1.1 Screening of Broth Media

Several general purpose media were compared to determine which provided the highest growth rate for both Cl. sporogenes and E. coli. To save time and effort, the preliminary studies were made using broth in culture tubes with anaerobiasis for growth of Cl. sporogenes being provided by a BBL Gaspack*. The most promising medium was then selected for further evaluation on tape. Of those media examined, Detrose broth with added (0.05%) sodium thioglycollate was chosen and found to provide good growth for Cl. sporogenes as well as E. coli. The details are presented below.

For optimal growth of Cl. sporogenes,** the growth media must contain a low oxidation - reduction potential. This may be obtained by the addition of sodium thioglycollate (0.05%) and 0.1% agar to the medium. Not only does the thioglycollate maintain the E_h of the media, but it nullifies the

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* BBL Gaspack is a disposable anaerobic growth chamber utilizing a CO_2 generator and an oxygen consumer. The cultures are placed in the chamber, water added to the generator pocket and the unit sealed prior to incubation.

** Stock cultures of Cl. sporogenes, the test organisms, were supplied by NCDC, Phoenix, Arizona.

inhibitory effects of heavy metals on growth. The agar, by increasing viscosity and reducing convection currents in the media, minimizes air absorption by the latter during handling. Optimal growth is generally achieved in a 95% - 5% nitrogen-CO_2 atmosphere. Although 37°C is considered optimal for growth of this organism, NCDC indicated that 32°C was suitable. The initial studies were made using an incubation temperature of 34°C.

The results obtained on screening three general purpose media are shown in Table 10. FTM* (Fluid Thioglycollate Media) contains thioglycollate and agar, whereas the Dextrose and Trypticase Soy Broths only added thioglycollate. Since the viscosity of broth containing 0.1% agar is too high at ambient temperature for convenient use in a flow system, other general purpose media suitable for growing anaerobes without agar, were evaluated.

From the data shown in Table 10, it is apparent that the TSB and DB media perform as well as FTM under aerobic as well as anaerobic conditions. The surprisingly good growth observed even under aerobic conditions can be attributable to the anaerobiasis provided by the sodium thioglycollate.

4.1.2 Effect of Air and Temperature on Growth Rate

Additional studies, the results of which are summarized in Table 11, indicate that for maximum growth of Cl. sporogenes, the volume of the air space above the broth should be kept to a minimum regardless of whether aerobic or anaerobic conditions** are employed. This was further substantiated by studies (see Table 12) which showed that Cl. sporogenes grows better in an evacuated system than in the Gaspack, (at least for incubations up to 4 hours). This is probably due to the more effective removal by the vacuum of the dissolved oxygen in the nutrient.

The results of a comparison of the growth rates of Cl. sporogenes and E. coli in Dextrose Thioglycollate broth (DTB) as a function of temperature and incubation time are summarized in Table 12. The data indicates that:

a. DTB is a suitable medium for growing E. coli as well as Cl. sporogenes.

b. Whereas complete anaerobiasis is optimum for growth of Cl. sporogenes, aerobic conditions are preferred for E. coli.

c. Based on the limited range investigated, the best single temperature for growing both organisms is 37°C.

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*Commercially available from Difco - not available without agar.

**DBT is dextrose broth containing added sodium thioglycollate.

Table 10

Growth of Cl. sporogenes in Various Media

Media	Atmosphere	Net Optical Density**
FTM	Aerobic	0.60
FTM	Anaerobic	0.62
DB + 0.5% Thioglycollate	Aerobic	0.74
DB + 0.5% Thioglycollate	Anaerobic	0.65
TSB + 0.05% Thioglycollate	Aerobic	0.56
TSB + 0.05% Thioglycollate	Anaerobic	0.62

*Growth in 20 ml volume culture tubes containing 8 ml of nutrient broth. Each tube received the same size inoculum (i.e., one loopful) and incubated for 16 hours at 34°C. Anaerobic growth in BBL Gaspack.

**Net optical density at 540 m μ; average of 3 separate runs (unincubated sample used for zero reference).

Table 11

Effect of Air Volume on Growth* of Cl. sporogenes

Nutrient	Volume of Nutrient	Ullage	Net O.D. at 540 mμ** Aerobic	Anaerobic
TSB + 0.05% Thioglycollate	8 ml	12 ml	0.56	0.52
	12 ml	8 ml	0.73	---
	16 ml	4 ml	0.70	---
TSB + 0.1% Agar	8 ml	12 ml	0.08	0.62
	12 ml	8 ml	0.35	---
	16 ml	4 ml	0.48	---
TSB	8 ml	12 ml	0.0	0.0
FTM	8 ml	12 ml	---	0.60

*Growth in 20 ml volume culture tubes with screw caps. Each tube inoculated with one loopful of fresh-grown Cl. sporogenes and incubated for 16 hours at 34°C.

**Net optical density (i.e., unicubated sample used for zero reference).

Table 12

Growth of Cl. sporogenes and E. coli in Dextrose-Thioglycollate Broth*

Incubation Time	Multiplication Factor					
	Cl. Sporogenes				E. coli	
	Aerobic		Anaerobic		Aerobic	
	2 Hrs	4 Hrs	2 Hrs	4 Hrs	2 Hrs	4 Hrs
Incubation Temp., °C						
34	2.0X	5.0X	4.3X	9.4X	8.6X	102X
37	---	---	---	10X (Gaspack)	---	337X
37			---	150X (Evacuated Medium)**		

*In culture tubes of 20 ml volume each containing 12 ml of nutrient.

**Medium evacuated and growth performed under partial pressure of 0.1 mm mercury.

d. At optimal growth, the multiplication factor for Cl. sporogenes was found to be 150X after 4 hours incubation at 37°C in DTB. The multiplication factor for E. coli, under comparable conditions was about twice as great.

4.1.3 Effect of pH and Incubation Time on Growth of Cl. sporogenes

The effect of nutrient pH on the growth of Cl. sporogenes is summarized in Table 13. The data indicate that over the range investigated a pH of 6.8 is the most optimum for growing Cl. sporogenes.

The effect of pH and incubation time on cell morphology is shown by the data in Table 14. It is evident that regardless of the starting pH of the nutrient, after 16 hours growth, the pH drops substantially and sporulation occurs, presumably the result of an unfavorable environment (i.e., depletion of essential metabolites, pH change, etc.). To ensure the absence of spores or deformed bacterial cells in the growing bacterial population, it will be necessary to either reduce the size of the inoculum or the incubation period. Since the chemiluminescence signal may be related to the cell morphology (i.e., vegetative vs spore), control of this important variable would be desirable, at least until the relationship is established.

The results of storing harvested Cl. sporogenes aerobically in distilled water are given in Table 15. The data indicate that significant spore formation and cell lysis begin to occur after two hours at ambient. This observation would indicate that distilled water suspensions of Cl. sporogenes that are to be used for test purposes should not be stored for more than two hours at ambient. These findings also suggest that if conditions are favorable for growth of anaerobes elsewhere in the water reclamation system, sporulation and lysis are bound to occur under the conditions of aerobic pasteurization maintained in the holding storage tanks for the reclaimed water. Aerobes would behave similarly also undergoing lysis in the storage tanks. Cell lysis may be reflected as an increase in soluble porphyrins and monitoring of the latter might be used as an indicator of bacterial buildup elsewhere in the reclamation system.

4.1.4 Tape Preparation Procedures

The following procedure developed for preparing the composite tape was found to be entirely satisfactory for determination of total (viable + non-viable) cell population in the unincubated sample. However, for determination of viable cells using an incubated cycle of 2 to 4 hours duration, a small signal

Table 13

Effect of Nutrient pH on Growth of Cl. sporogenes in DBT*

	Cell Count Cells/ml	Multiplication Factor
Control (initial)	3×10^5	0
After 4 Hrs. Incubation		
pH 6.8	1.6×10^7	53X
pH 7.5	6×10^6	20X
pH 8.0	6×10^6	20X
pH 8.7	3×10^6	10X

*DBT is dextrose broth containing 0.05% sodium thioglycollate growth in capped (full) culture tubes at 37°C for 4 hours.

Table 14

pH Shift in DBT From Clostridia and the Resultant Cell Growth

Incubation Time	0-Hours		4-Hours		16-Hours	
	pH	Cell Type	pH	Cell Type	pH	Cell Type
	6.8	Veg.*	6.4	Veg.	5.5	Veg + Spores**
	7.5	"	7.4	"	5.3	"
	8.0	"	7.7	"	5.3	"
	8.7	"	8.2	"	5.7	"

*Veg. = Vegetative

**Approximately 50% Spores

Table 15

Effect on Cell Morphology of Storing Cl. sporogenes in Water at Room Temperature

Conditions: Freshly harvested Clostridia diluted 1/1000 in water of pH 6.8 and 7.5 and stored aerobically at room temperature.

Time, hrs	Suspension	Appearance	Count Cells/ml
0	pH 6.8 pH 7.5	Good veg. cells, smooth edges, even staining, long chains.	2.2×10^5
2	pH 6.8	Less smooth edges, even staining, chains	2.1×10^5
	pH 7.5	Wrinkled edges, uneven staining	2.2×10^5
4	pH 6.8	Long cells, some wrinkled edges, even staining, some spore formation	1.7×10^5
	pH 7.5	Misshapen cells, definite lysis, uneven	1.7×10^5
16	pH 6.8	Considerable spore formation, reduced cell size, deformed cell shapes, lysis	1.2×10^5
	pH 7.5	Considerable spore formation, lysis, deformed cell shapes, uneven staining	1.2×10^5

(i.e., 1 to 2 volts) indicating non-sterility might sometimes be observed. To render the tape completely sterile, a number of techniques (autoclaving, ethylene oxide treatment (ETO) in addition to the urea treatment were evaluated. Of these investigated, treating the tape with ETO appears to be the most effective. Further studes are required, however, to establish that the ethylene oxide treatment does not leave any residuals which would affect subsequent bacterial growth. The method developed for preparing the composite tape used in determination of "total" cells and the various procedures evaluated for rendering the tape sterile for viable cells determination are described below. Once these tapes are sterilized, they should have an indefinite shelf life provided they are kept dry and clean.

4.1.4.1 Composite Tape Preparation for "Total" Cell Determination

a. Soak the 13 mm Acropor membrane filter (AN 450) in filtered* 8 M urea overnight.

b. Remove and soak in filtered distilled water for about 1/2 hour.

c. Wash with $\sim$ 75 ml filtered distilled H_2O on a Millipore frit that had been previously soaked in 8M urea and thoroughly rinsed with filtered distilled H_2O.

d. Place in a petri dish and allow to dry.

e. Cement the dry filter to the Mylar leader film (using forceps to handle the membrane).

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*Unless otherwise indicated, a 0.22μ pore size filter was used for filtering of the urea or distilled water.

f. Allow to dry in a covered sterile petri dish.

g. Place the composite tape, cut to individual filter units, deposition surface down, on a Millipore frit that had been soaked in 4M urea overnight and thoroughly rinsed with 0.22μ filtered distilled water.

h. Using this complete Millipore filtration assembly, filter approximately 40 ml of filtered distilled water through the backside of each filter.

i. Place the composite tape segments in sterile disposable petri plates with the deposition surface up and allow to air-dry. Tape is now ready for use in determination of "total" cell count.*

4.1.4.2 Composite Tape Sterilization Techniques

Of the following procedures evaluated for rendering the composite tape sterile, the ETO treatment is to be preferred since it is effective and has less of an effect on the filtering characteristics of the tape than either of the other two methods.

a. Autoclaving - The composite tape was autoclaved at 15 psi (121°C) for 15 minutes. After autoclaving, the tape had the following characteristics:

1) Flow rate through tape reduced $\sim$ 75%.

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*This sterilization technique was also employed for most of the runs of the viable cycle as well, unless otherwise indicated.

2) Tape appeared darker in color.

3) Tape incubated 4 hrs at 37°C and then processed with standard protocol yielded no signal, indicating no contamination.

b. Ethylene Oxide (ETO) Treatment - The composite tape was ETO sterilized for 16 hours at ~ 70% R.H. The treated tape showed the following:

1) Flow rate reduced 20-25%.

2) Tape unchanged in appearance.

3) Four-hour incubation blank yielded no signal indicating no contamination.

c. 4M Urea Treatment - The composite tape was soaked in 4M urea for 1/2 hour, removed, rinshed and backwashed with 50 ml of filtered distillled water and then air-dried in a sterile disposable petri dish. The treated tape possessed the following properties:

1) Flow rate unchanged.

2) Tape appearance unchanged.

3. Four-hour incubation blank yielded no signal; however, in attempting to grow deposited organisms on this tape, the growth was impaired. Take-up of 4M urea by the Mylar leader film with subsequent leaching was probably responsible for the inhibition of bacterial growth.

4.1.5 Bacterial Growth on Tape

After DBT nutrient had been passed through the deposited organisms at Station 2 (see Table 1), the tape segment is placed in a disposable petri dish on a Millipore pad saturated with DBT nutrient. Incubation was carried out for 2 to 4 hours at 37°C in a controlled atmosphere - anaerobic growth of Cl. sporogenes in an atmosphere consisting of 90% N_2 + 10% CO_2, 90-100% Relative Humidity, and aerobic growth of E. coli at ambient atmosphere and 90-100% Relative Humidity. A direct microscopic count of stained* organisms was made to derive the multiplication growth factor for each of the organisms. The results summarized in Tables 16 and 17 indicate that the multiplication factor for both organisms on tape is lower than it is in broth. Discrepancies of this order of magnitude between growth on tape and growth in broth or on agar nutrient have been reported before for other organisms and differences could generally be minimized by a more careful control of the relative humidity or by increasing the concentration of nutrient several fold. However, reference to the data in Table 16 indicates that doubling the nutrient concentration appears to lower the growth with a 4 hr incubation cycle.

*Aerojet Proprietary Protein Specific Dye.

Table 16

Growth of *E. coli** in Dextrose-Thioglycollate Medium at 37°C as a Function of Nutrient Concentration

(Broth vs Tape)

		Microscopic Count	Growth Multiplication Factor
A.	**Growth in Broth**		
	1. **Normal DBT Concentration****		
	Control (unincub.)	9 cells/field	-
	2 hr incubation	100/field	11X
	4 hr incubation	2800/field	311X
	2. **Twice Normal DBT Concentration****		
	Control (unincub.)	9 cells/field	-
	2 hr incubation	100/field	11X
	4 hr incubation	1000/field	110X
B.	**Growth on Tape**		
	1. **Normal DBT Concentration**		
	Control (unincub.)	11/field	-
	2 hr incubation	65/field	6X
	4 hr incubation	2000/field	180X
	2. **Twice Normal DBT Concentration**		
	Control (unincub.)	11/field	-
	2 hr incubation	22/field	2X
	4 hr incubation	1200/field	109X

*1×10^3 organisms deposited initially on composite tape.

**See Appendix A for concentration of ingredients.

Table 17

Growth of Cl. sporogenes and E. coli in Dextrose-Thioglycollate Medium at 37°C

	Cl. sporogenes (Anaerobic)		E. coli (Aerobic)	
	In Broth	On Tape	In Broth	On Tape
2 Hour Growth	--	--	11X	6X
4 Hour Growth	53X	13X	311X	180X
	150X (Outgassed Medium)	--	--	--

Poorer growth on tape has also been attributed to accumulation of toxic metabolic by-products on the filter and the poor diffusion of fresh nutrient to growing bacteria. A more thorough investigation of the effect of these parameters on growth of Cl. sporogenes and E. coli on tape would be recommended.

4.2 PROCESS VARIABLE STUDY (UNINCUBATED CYCLE)

Some of the parameters investigated for optimizing the sensitivity for detection of organisms included 1) the effect of flowing the luminol-H_2O_2 reagent across the filter containing the deposited organisms rather than through it, 2) the effect of reagent flow rate and concentration, 3) the effect of PMT voltage and 4) the effect of hemin staining. The results obtained in each of these areas are described below.

4.2.1 Reagent Flow Through vs Across the Tape

In comparing reagent flow across vs through the tape, the latter was found to produce highly variable results, principally because of the difficulty in achieving a uniform and reproducible reagent flow through the membrane filter. The magnitude of the signal appeared highly dependent on flow rate and variations in the latter could be produced by non-uniformity in filter porosity, fluctuations in the vacuum source used to draw the liquid reagent through the filter and variations in the effective filtering area (resulting from partial plugging of pores by Millipore cement used in sealing the filter membrane to the Mylar backing). Non-uniform coverage of the filter surface by reagent could also contribute to the observed variability in signal response.

It was found that the above problems could be minimized by passing the Premix (luminol-H_2O_2) reagent across the tape rather than through it. Further improvement in the consistency of the data was achieved by utilizing the full protocol shown in Table 1. Passing nutrient over the deposited organisms and washing with 4M urea (Stations 2 and 4 respectively) even in the "unincubated" cycle are essential if the signal from the "total cell count" sample is to serve as a baseline value for the incubated sample in the viable cycle.

Typical signals for a bacterial suspension and a water control using the final protocol are shown in Figures 6A and 6B.

Some of the pertinent experiments carried out in developing the above protocol are described briefly below.

a. Effect of Filter Variability on Reagent Baseline Signal

The composite Acropor AN filter prepared according to the procedure described in Section 4.1.4.1 (i.e., Urea presoak, wash, dry, etc.) produced in one series of tests, baseline variations of the magnitude shown by the data in Table 18.

b. Effect of Premix Dilution on Sample Signal

The reagent baseline and sample signals obtained with diluted and undiluted Premix reagent are summarized by the data in Table 19**. The results indicate that while the reagent baseline is reduced to approximately one-half its value on dilution, the net signal obtained for a given bacterial challenge remains about the same.

c. Effect of PMT Voltage on Signal

The data in Table 20 indicate that raising the PMT voltage increases not only the reagent baseline but the overall net signal (and S-N/N ratio) even more. A PMT voltage of 1450 was used in all subsequent runs.

d. Effect of Using Complete Protocol

The effect of passing Dextrose-Thioglycollate broth (without hemin) over the organisms deposited on the composite tape followed by washing with 4M urea prior to reaction with Premix was investigated with the results summarized in Table 21. The data indicate that the signals obtained show good day-to-day reproducibility. The average value of 21.5 ±1.2** volts for a challenge of 5×10^5 E. coli/400 ml sample is substantially higher than the 15.5 volts obtained with an abbreviated protocol in which washing with DTB and urea are omitted (see Table 20).

Data on the response of the instrument toward E. coli and Cl. sporogenes at several levels using the developed protocol (without hemin in the DBT) are shown in Table 22.

e. Effect of Hemin Staining on Signal Strength

Enhancement in bacterial chemiluminescence can be achieved in most cases by staining with a heme (iron-porphyrin) material such as hemin***. Heme is the active component in cytochromes responsbile for chemiluminescence.

*Standard Deviation

**The desired dilution was achieved by mixing the effluent from the first mixing coil (i.e., 0.11 ml luminol + 0.24 ml H_2O_2, Figure b1, Appendix B) with 1.0 ml of sterile distilled water.

***Available as hemin chloride from CalBiochem., Los Angeles, Calif.

Table 18

Effect of Filter-to-Filter Variability on Reagent Baseline Signal

Filter No.	Premix-Reagent Baseline, volts*
1	5
2	6
3	5
4	3.5
5	4
6	6
7	5
8	4
9	6
Average	5.0 ±0.9 (S**)

Conditions: (1) PMT Voltage 1300

(2) Premix flow (undiluted - 0.35 ml/min) across tape.

*Reference Data Sheet 6/29/71, Runs 1-9 incl.

**Standard Deviation

Table 19

Effect of Premix Reagent Dilution on Sample Signal*

	Premix Baseline, volts	Overall Net Signal S-N (volts)
Undiluted Premix (0.35 ml/min)**	5 ± 0.9**** (9 pts)	2.2 ±1.0 (3 pts)
Diluted Premix (0.35 ml Premix + 1.00 ml distilled water, 1.35 ml/min)***	1.9 ±0.6 (16 pts)	2.8 ±1.2 (4 pts)

*Conditions: (1) PMT Voltage 1300

(2) Bacteria 5×10^5 E. coli in 50 ml suspension deposited on tape in Millipore filtration apparatus; 50 ml sterile water control on same tape segment; reagent flow-across the tape.

**Reference Data Sheet 6/29/71, Runs 1-9 incl.

***Reference Data Sheet 6/30 71, Runs 1-7 incl.; 7/1/71, Runs 1-9 incl.

****Variation is expressed in terms of the standard deviation.

Table 20

Effect of PMT Voltage on Net Signal*

PMT Voltage	Reagent Baseline, volts	Overall Net Signal** (S-N), volts	$\frac{S-N}{N}$
1350	2	3, 3	1.5
1400	2.5	9	3.6
1450	4	15.5	3.9

Conditions:

(1) 5×10^5 *E. coli* in 50 ml water sample; 50 ml water control

(2) Deposition on Acropor AN 450 filter sandwiched between Mylar tape - concentration of organisms on Millipore filtration apparatus; no nutrient wash.

(3) Standard Premix reagent diluted with sterile distilled water in ratio of 0.35 to 1.00, respectively; reagent flow 1.35 ml/min. across tape.

*Reference Data Sheet 7/2/71, Runs 4-10 incl.

**Overall Net Signal = Sample Net Signal - Net Signal for Water Control.

Table 21

Day-to-Day Reproducibility Using Complete Protocol*

Total Challenge	Overall Net Signal (S-N), volts	Reference Data Sheet	Reference Runs
5×10^5 *E. coli* in 400 ml Water Sample	20 21	7/9	1-4
**			
5×10^5 *E. coli* in 400 ml Water Sample	23 23 22	7/13	1-4
5×10^5 *E. coli* in 400 ml Water Sample	20	7/20	1,4
5×10^5 *E. coli* in 400 ml Water Sample	21.5	7/22	1,2
Average =	21.5 ±1.2***		

* Same as shown in Table 1 (except that dextrose-thioglycollate broth does not contain hemin).

** Dotted line separates runs made on different days.

*** Standard Deviation

Table 22

Signal Response of *E. coli* and *Cl. sporogenes**

Total Challenge	Overall Net Signal (S-N), volts	Reference Data Sheet	Reference Runs
5×10^4 *E. coli*/400 ml	2.5	7/22	1,3
7.5×10^4 *E. coli*/400 ml	5.0	7/27	1,3
5×10^5 *E. coli*/400 ml	21.5 ±1**	(see Table 21)	
1×10^6 *Cl. sporogenes*/400 ml	3, 0,1 (Avg = 1.3)	7/23 9/17 9/10	1,5 1,2 1,5
5×10^6 *Cl. sporogenes*/400 ml	6	7/23	1,2

*Standard protocol (except that hemin has been omitted from DBT); PMT voltage 1450, Premix flow (1.35 ml/min) across tape.

**Standard deviation; average of 7 points; others are single runs.

2A

An illustration of the extent of enhancement which could be achieved with Cl. sporogenes is shown by the data in Table 23. The Cl. sporogenes were grown in a dextrose-thioglycollate broth containing 5×10^{-3} g/liter of hemin. The cells were harvested and washed by centrifugation in the normal manner. For a control Cl. sporogenes were grown in the dextrose-thioglycollate broth without hemin. An additional control consisted of hemin-containing broth without bacteria. All controls were processed in the same manner. The relative chemiluminescence signals were evaluated using an experimental chemiluminescence unit developed by Aerojet for commercial application. In this unit approximately 2 ml of sample is reacted with Premix reagent and the integrated light output monitored over a finite time interval. Although the absolute values for the signal shown would be different from those obtained with the tape chemiluminescence unit, the relative values obtained by the two instruments would be approximately the same. The data indicate that the detection threshold of Cl. sporogenes could be improved by at least one log by this treatment with hemin. While staining with hemin can be conveniently carried out in solution (with excess hemin being removed by washing and centrifugation), the problem in utilizing this in a tape process is the nonspecific adsorption of the hemin by the tape, resulting in a higher reagent baseline.

In adapting hemin staining to the present process, it was found necessary to reduce the hemin level in the broth to 5×10^{-7} g hemin/liter of broth. The DTB broth was passed over the deposited organism at Station 2 in the conventional manner, at 1 ml/min for 5 minutes. The excess hemin and broth were removed by washing with 4M urea at the next station prior to reaction with luminol-H_2O_2 Premix. Results obtained using this procedure, shown in Table 5, indicate a slight, if significant, improvement in the Cl. sporogenes signal (from 6 to 8 volts for a total challenge of 5×10^6 cells).

The most recent data obtained with E. coli which had been hemin-stained by this procedure is shown in Table 3. For optimum results, the hemin staining solution (5×10^{-3} g/l) should be stored at refrigeration temperature for not more than one week, and dilutions (5×10^{-7} g/l) in DBT prepared fresh daily.

It is felt that an even greater enhancement in chemiluminescence signal might be achieved with hemin staining by further optimization of the hemin concentration and urea wash cycle.

Table 23

Enhancement of Chemiluminescence Signal by Hemin Staining*

	Overall Net Signal (S-N), volts**
Cl. sporogenes (without hemin-control)	
5×10^5 cells/ml	~ 0
Cl. sporogenes (grown in presence of hemin)*	
5×10^5 cells/ml	8.76
2.5×10^5 cells/ml	2.76
5×10^4 cells/ml	0.36
Hemin-Broth (control)***	~ 0
E. coli (without hemin)	
5×10^5 cells/ml	2.76

* Cl. sporogenes grown in a dextrose-thioglycollate broth containing 5×10^{-3} g/liter of hemin; excess hemin removed by washing and centrifugation.

** With Aerojet Commercial Chemiluminescence Unit (set a 2 M ⌐∧¬ gain).

*** Processed same as sample but without added bacteria.

4.3 EFFECT OF WATER STRESS AND CHEMICAL INTERFERENCES ON SIGNAL RESPONSE

The initial evaluation of the effects of 1) Cl_2, I_2, Ag^+ and pH and 2) pasteurization temperature (160°F) on the signal response of E. coli was made at a bacterial concentration of 5×10^5 cells/400 ml. The results, summarized in Tables 24 and 25, appear to indicate that except for the presence of chlorine, none of the other parameters appeared to have a signficiant effect on the signal response of E. coli. Based on the shape of the response curves* (Figure 10 is typical), however, and the magnitude of the total signal (i.e., S_2) it appears that we are near the saturation limit of the PMT (saturation occurs at approximately 45-50 volts). Consequently the tests were repeated at a lower bacterial level (i.e., at 1×10^5 and 3×10^5 E. coli/400 ml) with the results shown in Tables 7 and 9. The data presented in Section 3 indicate significant effects of most of these parameters.

*Rounding out of peaks.

Table 24

Effect of pH and Chemical Interferents on Signal Strength***

	Sample Signal*, Volts		
	S_2	N_2	S_2-N_2
5×10^5 *E. coli* (pH 7.5) Control	43.5	4.5	39
5×10^5 *E. coli* (pH 7.5) Control	42.0	4.0	38
5×10^5 *E. coli* (pH 5)	43.5	4.5	39
5×10^5 *E. coli* (pH 9)	44.0	5.0	39
5×10^5 *E. coli* + 6 ppm I_2	41.5	4.0	37.5
5×10^5 *E. coli* + 30 ppm Cl_2**	33.5	4.0	29.5

*Does not include water blank.

**Available chlorine.

***Reference Data Sheet 9/13, Runs 1-7

Table 25

Effect of Water Stress on Signal Strength**

Sample Treatment	Sample Signal*, Volts		
	S_2	N_2	S_2-N_2
Control	40.0	2.5	37.5
2 hrs @ 160°F	39.5	3.0	36.5
4 hrs @ 160°F	40.0	2.0	38.0
4 hrs @ ambient	40.0	2.5	37.5

*5×10^5 E. coli in 400 ml sterile distilled water.

**Reference Data Sheet 9/14, Runs 1-5 incl.

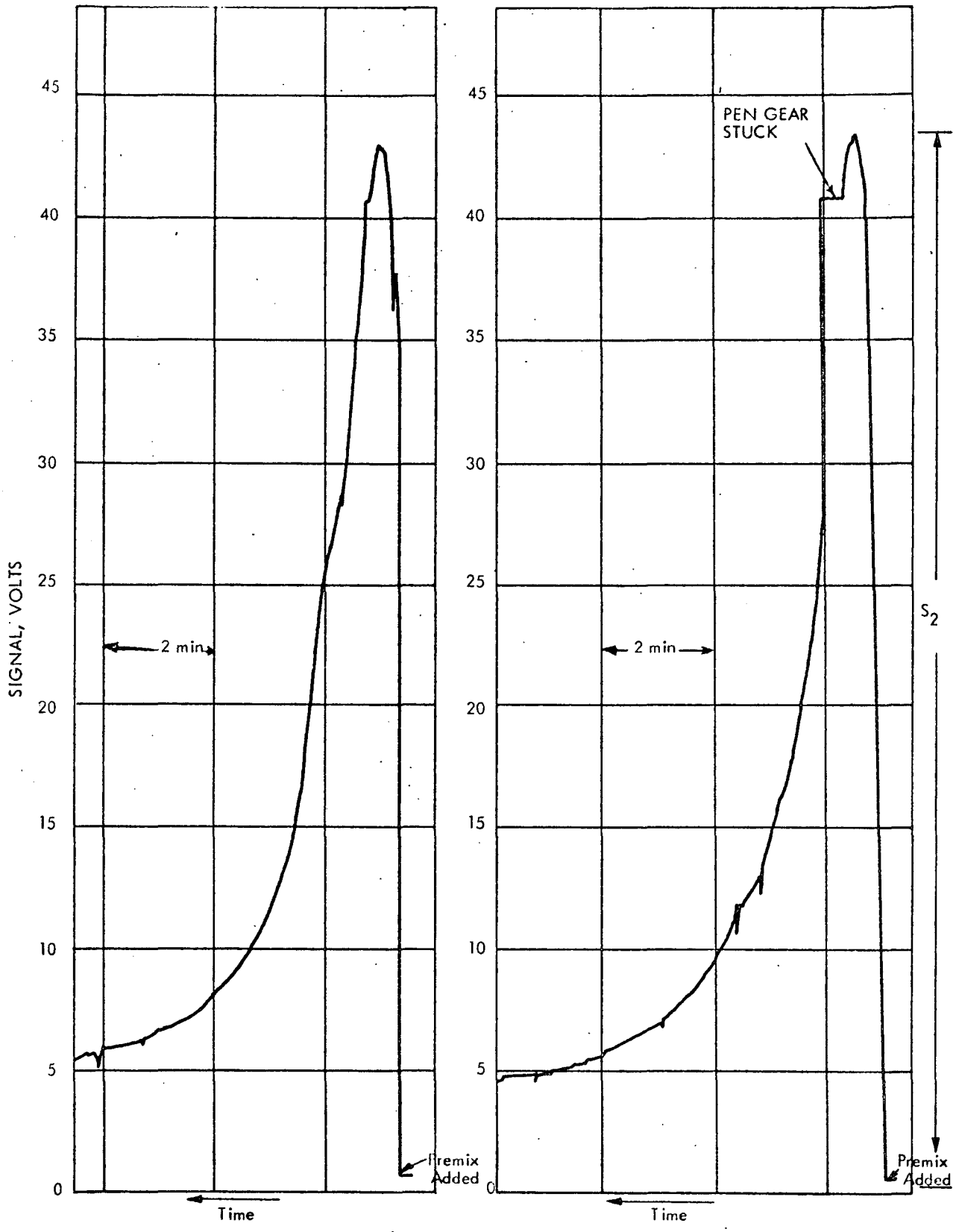

Figure 10B
Signal Response 5 x 10^5 *E. coli**/400 ml
(pH 5.0)

Figure 10A
Signal Response 5 x 10^5 *E. coli**/400 ml
(pH 7.5) (Control)

Figure 10

*Hemin-Stained

Section 5

CONCLUSIONS AND RECOMMENDATIONS

The feasibility of the chemiluminescence method for monitoring the sterility of reclaimed water using a tape sample transport has been demonstrated.

Sensitivities achieved with 400 ml suspensions of E. coli and Cl. sporogenes using developed protocols and a manually operated laboratory breadboard were as follows:**

	Unincubated Cycle (Total Viable + Non-viable)		Incubated Cycle (Viable)	
	Sens.	Processing Time	Sens.	Processing Time
E. coli (aerobe)	30-35 cells/ml	37 min	7-8 cells/ml	2.3 hours
Cl. sporogenes (anaerobe)	10^3-10^4	37 min	10^3-10^4	4 hours

Further improvement in sensitivity might be achieved by modifying the reaction chamber to bring the tape surface closer to the face of the photomultiplier tube.

The program objectives were to better the detection thresholds of 75 cells/ml (total) and 30 cells/ml (viable) achieved with 100 ml samples of E. coli using a chemiluminescence system employing a liquid sample transport*.

The decidedly lower sensitivity observed for non-viable Cl. sporogenes is probably due to its lower cytochrome content (one of iron porphyrins responsible for chemiluminescence), a feature common to Clostridia in general. Apart from the lower porphyrin content, the tendency to sporulate, long lag times required for germination and the lower growth rate, all combine to produce a lowered sensitivity for viable Cl. sporogenes. A more optimal procedure of hemin-staining may improve the sensitivity toward anaerobes such as Cl. sporogenes.

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*Aerojet Medical & Biological Systems, "Development of Bacterial Sensing Instrumentation for Spacecraft Water Systems", Contract NAS 9-10432, NASA/MSC Houston, Texas.

**The processing sequence used for both the incubated and unincubated cycles is summarized by the data in Tables 1 and 2. Water blanks were processed in an identical fashion to the samples. The sterilization technique employed for all runs except one, was the Urea prewash described in Section 4.1.4.1. One viable cycle (at a level of 8 cells/ml) was performed using the ETO sterilization technique described in Section 4.1.4.2.b.

In a supplementary section (Systems Analysis, Appendix D), the results of a survey of the various parameters (i.e., biological, chemical and physical) which should be monitored to determine water potability are presented. The methods and instrumentation best suited for making these measurements are also delineated.

APPENDIX A

REAGENT PREPARATION

REAGENT PREPARATION

A. LUMINOL-H_2O_2 REAGENT

1. Luminol Solution

a. 60.0 gms sodium hydroxide ("Baker Analyzed" Pellets Reagent) are dissolved in liter of tiltered distilled water and allowed to cool to ambient temperature.

b. 15.0 gms disodium ethylenedinitrilotetracetate dihydrate ("Baker Analyzed" Reagent Powder) are dissolved in above alkaline solution.

c. Dissolve 1.00 gm of luminol (3-aminophthalhydrazide, Aldrich Chemical) in above. Let solution stand at ambient temperature overnight before use.

d. The stock luminol solution may be stored in an unpigmented polyethylene or polypropylene container. The solution has a shelf life at ambient temperature of at least 4 months*. Freezing does not affect the luminol solution, however, elevated temperatures accelerate the decomposition (with loss in sensitivity). For maximum shelf life, storage temperatures should not exceed 95°F.

e. The usability of an aged luminol solution can be evaluated by determining the signal obtained with a fresh H_2O_2 and a fixed bacterial (E. coli) challenge. If the net signal is less than expected, the luminol would be suspect.

2. Hydrogen Peroxide Solution

a. Dilute 3% hydrogen peroxide ("Baker Analyzed" Reagent) to 1% with filtered distilled water.

b. The 1% H_2O_2 should be stored in an unpigmented polyethylene or polypropylene container and preferably at temperatures not exceeding 95°F (elevated temperatures accelerate decomposition). Previous studies on shelf life of this reagent indicated at least a 5 week stability at ambient temperature*. Freezing does not appear to affect this reagent.

c. The efficacy of an aged hydrogen peroxide solution may be checked by the procedure outlined above (par. 1.e) for luminol.

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*Reagent shelf life studies performed for periods indicated and reported under Contract NAS 9-10432, Final Report No. 1504F, "Development of Bacterial Sensing Instrumentation for Spacecraft Water Systems", 13 November 1970, pp. 3-77 to 3-82.

In actual use, the luminol and hydrogen peroxide are pumped initially (see Figure B-1)+ through a glass mixing coil (4 mm I.D., 84 cms long) of sufficient volume to provide a residence lag time of 30 minutes (the reagent reaches its maximum sensitivity about 1/2 hour after mixing and retains this sensitivity for about 2 hours). The resulting solution is then pumped through a second glass mixing coil (4 mm I.D., 110 cms long) where it is diluted with filtered distilled water to the proper concentration prior to entering the reactor.

The respective flow rates are:

Luminol	0.11 ml/min
1% H_2O_2	0.24 ml/min
H_2O	1.00 ml/min

Buchler pumps (with tygon tubing) were used for metering the reagents; a variability ot ±10% in the flow rates can be tolerated.

B. 4M UREA SOLUTION

Reagent grade ("Baker Analyzed") urea is dissolved in glass-distilled water to a final concentration of 4 Molar. The solution is filtered through a 0.1μ Ultripor filter and then passed through a mixed bed ion exchange column (equal quantities of Dowax AG 50W-X8 and Dowax AGI-X8, 200-400 mesh, 200 ml bed volume). The pH of the effluent from the column is adjusted to pH 7.0 to 7.5 with hydrochloric acid and then filtered through a 0.2μ (prewashed*) Millipore prior to use.

The 4M Urea may be stored in a glass, polyethylene or polypropylene container during use. The solution is stable for at least 5 weeks at ambient temperature**. The solution was introduced into the upstream with a peristaltic pump (Buchler) at the prescribed flow rate (5 ml/min).

To determine whether significant determination of a Urea solution has occurred on standing, the baseline signals obtained with the prescribed processing sequence (shown in Table 1) are compared for aged and unaged Urea solutions. If significant decomposition has occurred, the aged Urea solution would be less ettective in removing nutrient and so produce higher baseline values on reaction with luminol-H_2O_2.

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*Pass about 500 ml of distilled water through a Millipore filter prior to use to remove finishing agent from filter.

**AMB Final Report No. 1504F to NASA, 13 November 1970.

+Appendix B

C. DEXTROSE-THIOGLYCOLLATE BROTH (DBT)

1. Add 23.0 grams of Difco Dextrose broth to 1 liter of freshly distilled water.

2. Heat and stir until dissolution is complete.

3. Add 0.5 gm sodium thioglycollate to broth and dissolve.

4. Dispense into sterile glass bottles with screw-cap (cap loose), autoclave 15 psi for 15 minutes; remove and tighten cap.

5. A fresh DBT source is hooked up to the water monitor daily using the following procedure:

a. The tygon line used to connect the nutrient source with the water monitor has been filled with 4M Urea at the conclusion of the previous day's run to prevent bacterial buildup in the line; there is a peristaltic pump and in-line filter (Figure B-1)+ between the nutrient source and the water monitor. The in-line filter (0.45μ pore size) is replaced daily.

b. On startup the following morning, pump out broth line with filtered distilled water.

c. Assemble a new in-line filter and install.

d. Pump filtered distilled water to wet/prime the filter.

e. Remove filtered distilled water and pump line dry.

f. Connect the broth line to the sterile broth supply.

g. Pump/prime the broth line and filter*.

h. At the end of the day, remove broth in-line filter and soak in 4M Urea.

i. Pump 4M Urea through pump and lines.

j. Turn off pump and leave 4M Urea in lines overnight to prevent bacterial growth.

With regard to the shelf life of the DBT broth (with hemin), although studies have not been conducted on this particular formation, an evaluation of a similar composition (i.e., dextrose broth without added thioglycollate) on an earlier program**

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*NOTE: Between each run, a small volume of 4M Urea is pumped through the broth deposition head to prevent any surface contamination between runs. Before a run, a few ml of broth are pumped through the broth deposition head to wash out any residual 4M Urea.

**AMB Final Report No. 1504F, "Development of Bacterial Sensing Instrumentation for Spacecraft Water Systems, Contract NAS 9-10432, 13 November 1970.

+Appendix B

indicated some deterioration on storage for 5 weeks at ambient in the light (in glass bottles). This was reflected by a decreased growth rate for E. coli in aged versus unaged nutrient. Storing in the dark at perhaps 5-10°C may be useful in prolonging the shelf life of this nutrient broth.

Periodic checks of aged nutrient should be made to insure a) absence of bacterial growth (by microscopic examination) and b) that nutrient properties have not degraded (by comparing growth rate of E. coli in aged and unaged nutrient).

The most probable method for storing and feeding reagents to the biosensor under zero-g operation is in pressurized bladder containers. The bladder is required to maintain the liquid intact as fluid is being withdrawn from the reagent container. Under zero-gravity, liquids in a partially filled container tend to randomly disperse in globules throughout the container. The bladder prevents this from happening. Dry nitrogen gas is an acceptable pressurant. Container and bladder materials must be compatible with the reagents; Urea, Nutrient, Luminol and H_2O_2. Unmodified polypropylene bottles are currently used for reagent containers and would be acceptable for zero-g use. The bladder material must be flexible, and the most probable choice is Viton.

D. HEMIN SOLUTION

Add 0.1 gm hemin chloride (CalBiochem) to 100 ml of filtered distilled water (pH of latter, adjust to pH 10 with dilute sodium hydroxide). Let stand one hour at ambient temperature and then filter through a 0.45μ Millipore. Store this stock solution in the refrigerator. Prepare fresh stock weekly.

For use in the DBT broth, dilute 0.1 ml of the hemin stock solution in 20 ml of the DBT broth. Then dilute this 1/100 with filtered distilled water and then dilute this 1/100 with DBT to yield a final concentration of 5×10^{-7} gm/liter of hemin in DBT. Fresh dilutions of the latter are to be prepared daily.

APPENDIX B

PROTOCOLS

SETUP PROCEDURE

Power requirements: 117VAC
28" Vaccum (house or pump)

Preliminary Preparations (see Figure B-1).

1. Install appropriate plumbing.
2. Make necessary vacuum connections.
3. Make necessary electrical (power) connections (pumps, electronics, high voltage) - use common ground to avoid ground loops.
4. Make recorder connections (impedance matching, calibration, chart loading).
5. Check all switch functions for proper operations; i.e., pumps, bellows, electronics.
6. Prior to initial testing, flush all pump tubing and lines with 30% hydrogen peroxide (caution against skin contact) for 30 minutes; then flush for 1-2 hours of filtered distilled water.
7. Fill necessary reagent supply containers.
   a. Luminol - 0.11 ml/min
   b. Hydrogen Peroxide 1%-0.24 ml/min
   c. Premix diluent - filtered distilled water 1 ml/min
   4. 4M Urea - wash 5 ml/min
   5. Dextrose thioglycollate broth - growth media 1 ml/min
   6. Filtered distilled water - reactor-cell wash 5 ml/min

   Premix Reagents — Daily
8. a. Turn on power switch (PWR).
   b. Turn on electronics switch (DET).
   c. Turn on series mode (electronics) switch (SM).
   d. Turn on premix switch to start premix pump (at least one hour prior to test).
      1) Have Premix valve in premix-waste position.
      2) Occasionally open/close valve to flush it out.
      3) Leave in premix exhaust position.
9. Turn on bellows, vacuum pump and collection vacuum pump.
   a. Collection vacuum pump should have at least 28" vacuum.
10. Operate all bellows vacuum-switches to insure proper operation.
11. Install prewashed 0.22μ inline filter between media tubing and media station.

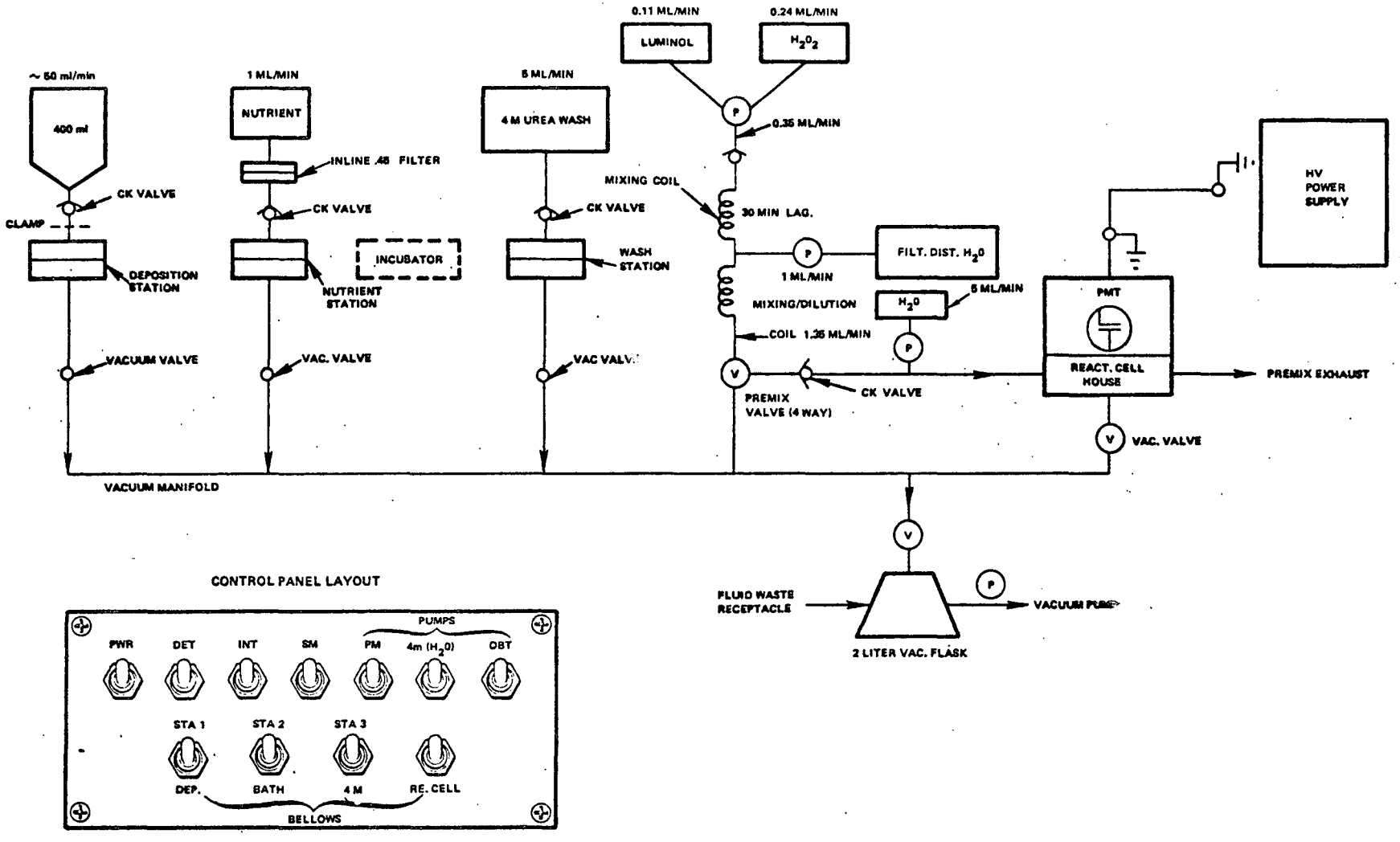


FIGURE B-1. AMB WATER MONITOR (SYSTEM SCHEMATIC)

12. Open all station vacuum valves except the deposition station valve.

13. Close all bellows switches and turn on all pump switches.

    a. 4M Urea pump and distilled water wash pump are on the same switch.

    b. Prime and flush all lines for $\sim$ 5 minutes with appropriate solutions.

14. Clamp the tubing at the deposition station and the supply vessel.

    a. Open the deposition vacuum valve.

    b. Alternately fill and flush the supply vessel $\sim$3-4 times with filtered distilled water.

    c. Keep the supply vessel opening covered at all times.

15. Liberally flush each station with filtered-distilled water from a heater; suck and wipe dry.

16. Close the deposition vacuum valve.

OPERATIONAL PROCEDURE (See Figure B-1)

1. Open the deposition bellows switch (Control Panel).
2. Place a composite filter on the deposition station.
3. Close the deposition bellows switch.
4. Clamp the tubing between the deposition station and the supply vessel.
5. Fill the supply vessel with the prescribed test solution and cover the opening.
6. Open the deposition vacuum valve and collect.
7. Following deposition, close the vacuum valve.
8. Open the bellows switch.

   Note: Always close vacuum valve before opening bellows switch to prevent contamination by impaction.

9. Remove and place the composite tape on the media station; close the bellows switch and open the vacuum valve.
10. Turn on the media pump for 5 minutes at 1 ml/min and then turn off.
11. Close the vacuum valve and open the media bellows switch.
12. Remove and place the composite tape:
    a. Incubate for viable test or
    b. Place on the 4M Urea station.
13. Place the composite tape on the urea station; close the bellows switch and open the vacuum valve.
14. Turn on the 4M Urea pump for 10 minutes at 5 ml/min (this also turns on the wash pump which flushes out the reactor cell with filtered distilled water); turn off the pump.
15. Close the vacuum valve and open the 4M Urea bellows switch.
16. Remove the composite tape from the 4M Urea station.
17. Lower the reactor cell pressure plate and insert the composite tape accordingly.
18. Release the plate and cover the assembly with a black cloth; close the reactor cell stations vacuum valve.
19. Open the reactor cell shutter and observe zero base line on the recorder.
20. Close the premix valve so premix enters the reactor cell and observe waveform on the recorder.

21. Following the test;
    a. Open the premix valve.
    b. Open the vacuum valve for the 4M Urea and reactor cell stations.
    c. Turn on the 4M Urea pump to flush the reactor cell.
22. Close the shutter and remove cloth.
23. Lower the pressure plate, remove the composite tape and release the plate.
24. After 2 minutes, turn off the 4M Urea pump and close the vacuum valve.

APPENDIX C

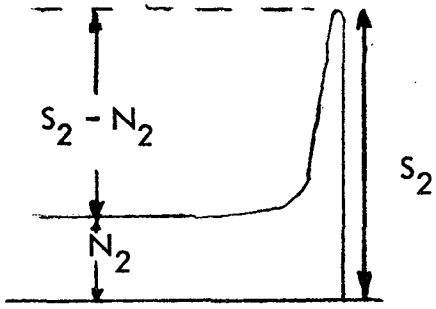
DATA SHEETS

APPENDIX C

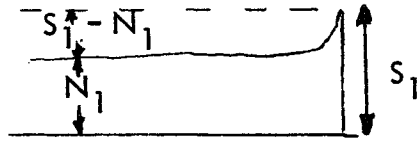
DATA SHEETS

Pertinent information with respect to the terminology used on the data sheets are as follows:

Challenge:	Total number of bacteria deposited on tape in sample volume indicated.
PMΦ:	Reagent baseline in volts (or N)
WB:	Water blank is net signal (S-N) of a water control, without bacteria, over reagent baseline.
Sample:	Overall Net Signal (due to bacteria) = Net Signal of sample - Net Signal of Water Blank



Sample



Water Blank

Overall Net Signal (S-N) (due to bacteria) = $(S_2 - N_2) - (S_1 - N_1)$

[illegible] Oil @ 0.35 ml/min Configuration MOD 2 ([illegible]) 50 v F.S. Deposition on MF
[illegible] DATE 6/28/71 Ep 1350 & silicone apparatus.
[illegible]
[illegible] Loose Vapor on Suit

Use of Neoprene Gasket for water seal over Filter – New Support Screen

Run	Agent	Challenge	Volume	[illegible] Φ	WB (S–N)	Sample (S–N)	Comments
1	WB		50	8.5			white SWINNEY silicone rubber GASKET; [illegible] Φ w/o WB peak
2	WB		50	5.5	~1	~~1~~ [illegible]	irregular [illegible]
3	~~WB~~ coli	2 × 10⁵	50	5	~1	0	no signal
4	WB		50	9	3		used black neoprene GASKET
5	coli	2X10⁵	50	8	3	0	" " " "
6	coli	2X10⁵	50	9	3	0	" " " "
7	coli	2X10⁵	50	~12	3	10	used dble mylar sandwich in reactor ASSY

Note: same filter used for WB & organism runs

Conclusion: gaskets still leak and not provide a good seal. dble mylar need further tests

REAGENTS
Premix CM @ 0.35 ml
Nutrient ____ @ ____
NaOH ____ @ ____

Configuration MOD 2 Reagent flow across filter

DATE 29 Jun '71 Ep 1300 V 50U FS

TAPE dble mylar sandwich ON MF FAIT

TEST PLAN Use of dble mylar sandwich w/ Reagent filters on one-shot basis first time

RUN	AGENT	CHALLENGE	VOLUME	CM Φ baseline	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	CM			5	—	—	filter blank
2	WB		50	6	1	—	slow increase but OK
3	WB		50	5	<1		OK
4	WB		50	3.5	1		OK
5	coli	2×10^5	50	4	1	1.5	low
6	coli	2×10^5	50	6	1	1	low
7	coli	5×10^5	50	5	1	2.5	
8	coli	5×10^5	50	4	1	1 } 2.2	low but dble peak
9	coli	5×10^5	50	6	1	3 } AVG	

Conclusion – filters reliable for one-shot basis
sensitivity still not reliable and too low.

AGENTS
Premix PM+H_2O @ 1.35 ml/min
Nutrient — @ —
WASH — @ —

Configuration MOD 2 - Flow Across Filter

DATE 6/30/71 Ep 1300 50CFS

TEST PLAN Increased Premix Flow (0.35 → 1.0 ml H_2O)
Larger Exhaust Tubing To Permit Better Flow

TAPE 1000 Acropor w/ dble Mylar sandwich

RUN	AGENT	CHALLENGE	VOLUME	PM Φ Baseline volts	WB (S-N) volts	SAMPLE (S-N) (volts)	COMMENTS
Φ	PM		—	1			flow check & new exhaust tubing smooth waveform. Good prime
1	WB		50	1.5	1		smooth
2	WB		50	1.5	~0.5		"
3	WB		50	1.5	0		
4	Coli	5×10^5	50	2.5	~1V	2.5 } av = 2.0	
5	Coli	5×10^5	50	1	~1V	2. }	
6	coli	5×10^5	50	1	~1V	4.5 }	
7	coli	1×10^6	50	2.5	~1V	14	high peak, but piston not up against filter so unreliable data

Conclusions: a) dble MYLAR setup not leak
b) higher pm flow and exhaust tubing yield better waveforms
c) av 3 V for 5×10^5 coli

REAGENTS Premix PM + H_2O @ 1.35 ml/min
Nutrient ____ @ ____
Wash ____ @ ____

Configuration Mod 2 - Flow Across Tape
DATE 7/1/71 EP 1300 V 50 UFS
TEST PLAN E. coli Sensitivity Study
TAPE loose Acropor w/ MYLAR SANDWICH

RUN	AGENT	CHALLENGE	VOLUME	PM Q	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		50	2.1	1		OK
2	WB		50	2	1.1		OK
3	coli	5×10^5	50	1	1	2	
4	coli	5×10^5	50	2	1	2	
5	coli	5×10^4	50	3	1	0.5	coli down 1 log $5 \times 10^4 \sim 0.5$ V
6	coli	1×10^6	50	2.1	1	14	high for 10^6
7	coli	1×10^6					line failure
8	coli	1×10^6	50	2	1	6	
9	coli	2×10^6	50	2	1	11	

Conclusion: E. coli sensitivity

5×10^4	0.5 V	1300 VAC
5×10^5	2 V	
1×10^6	6 V	
2×10^6	11 V	

Reagents
Premix PM + H_2O @ 1.35 ml/min
Nutrient ——— @ ———
WBSH ——— @ ———
TAPE ———

Configuration Mod 2 - Flow Across Tape 50 ml. volumes on MF Assy.
DATE 7/2 Ep Variable 5% VFS
TEST PLAN EFFECT OF PMT VOLTAGE ON SIGNAL

RUN	AGENT	CHALLENGE cells	VOLUME ml.	PM Φ V.	WB (S-N) V.	SAMPLE (S-N) V.	COMMENTS
1	WB		50	4.5	2.5		1350 VDC off ~1400 from prev test.
2	WB		50	2	1		1375 VDC
3	WB		50	2	<1		1350 "
4	Coli	5×10^5	50	~2	<1	~3	1350 "
5	Coli	5×10^5	50	3	<1	~3	1350 "
6	WB		50	2.5	3.5		1400 "
7	Coli	5×10^5	50	5	3.5	9	1400 "
8	WB		50	4	4		1450 "
9							Air bubbles and blown line
10	Coli	5×10^5	50	4	4	15.5	1450 VDC
			(conclusions: 1)		1450 VDC - max signal w/ Good PM Φ		

Reagents
Carrier 8M EtOH @ 1.35 ml/min — Configuration MOD 2 — END-END — Vacuum deposition 400 ml/~10 min
Nutrient DBT @ 5ml w/syringe — DATE 2/4/71 — Ep 1450 — 50 U F.S.
Wash 4M @ 5ml/min
Tape composite tape w/ mylar sandwich @ [illegible] (15 min)

END-TO-END TEST - FIRESTONE
DBT w/ Syringe

Run	Agent	Culture	Volume	PM Φ	WB (S-N)	Sample (S-N)	Comments
✓1	NB		400	10	2 ✓ ↓		ok — separate filter
✓2	coli	5 X 10^5	400	14	2 ↓	20	Good tail off — separate filter
✓3	coli	5 X 10^5	400	11	2 ↓	21	ok composite tape
✓4	WB		400	8	2 1		ok very clean

Conclusion: E/E test a) higher signals for E coli
b) due to 4M Urea wash?
c) higher PM Φ due to DBT background

REAGENTS
Carrier PM + H_2O @ 1.35 ml/min Configuration MOD Z - END TO END
Medium DBT @ syringe (5 ml) DATE 13 Jul Ep 1450
Wash 4M @ 5 ml/min/10 min 100 V F.S.
TARGET composite 13 mm scraper w/ sandwich @ reactor END TO END E. coli

RUN	AGENT	CHALLENGE	VOLUME	PM Φ	WB (S-N)	SAMPLE (S-N)	COMMENTS
0	—	—	—	12	F		12V PMΦ due to broth
1	WB	—	400	12	1		
2	coli	5X10^5	400	7		23	
3	coli	5X10^5	400	7		23	
4	coli	5X10^5	400	8		22	
5	WB		400				Abort - cloth like look

Conclusions 23 + 23 + 22 = 68; $\frac{68}{3}$ = ~ 23V Avg / 5x10^5 coli

tight group STD deviation ~1V

Reagents
Wash: DMH H_2O @ 1.35 ml/min
Wet: DBT 5ml @ 1ml/min
Work: 4M SO @ 5 ml/min

Type: lamp type w/ dble mylar E/E

Configuration MOD 2 E/E

DATE 7/20/71 Ep 1450 50 V. F.S.

TEST PLAN Effect of H/10000 DBT on DETECTION OF E. coli

Run	Agent	Challenge	Volume	PM ϕ	WB (S-N)	Sample (S-N)	Comments
1	WB		400	5	4.5		
2	coli	5×10^4	400				abort - WE piston not seated
3	coli	5×10^4	400	5.5	4.5 ↓	< 1 Volt	near detect.
4	coli	5×10^5	400	8	4.5 ↓	~ 20	one log up
5	coli	1×10^5	400	8	4.5 ↓	9	1/2 log down

Conclusions

coli @ 5×10^4 < 1 Volt
1×10^5 9 Volts
5×10^5 20 Volts

linearity takes heating @ higher [conc.]

2

Reagents
Premix PH+H2O @ 1.35 ml/min
Nutrient DBT 5ml @ 1 ml/min
Wash 4M (SB) @ 5 ml/min

Configuration MOD 2 E/E

DATE 7/24/71 EP 1K50

TEST PLAN E. coli bracket w/o Hamin

TAPE Comp. tape w/ dble mylar @ reader

RUN	AGENT	CHALLENGE	VOLUME	PM ℄	WB (S-N) V.	SAMPLE (S-N) V.	COMMENTS
✓ 1	WB		400	5	1V		
2	Coli	5X10^5	400	7	1V	21.5	
✓ 3	Coli	5X10^4	400	5	1V	~21.5	

AGENTS
MAX PMgHO @ 1.35 ml/min
TINAL DBT 5ml @ 1ml/min
AM 50M @ 5ml/min

Configuration

DATE 7/23 Ep 1450

SOU F.S.

TEST PLAN Detection of Clostridium w/o Hemin

TYPE: Comp type of dhb in hyb reactor

RUN	AGENT	CHALLENGE	VOLUME	RM Q	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	5.5	2V ↓		no hemin
2	Clostridium	5×10^6	400	4	2V	6	no hemin
3	Clostridium	5×10^5	400	5	2V ↓	0	no detection H/10,000
4	Clostridium	5×10^6	400	5	2V ↓	8	hemin yields 2V gain over no hemin H/10,000
5	Clostridium	1×10^6	400	5.5	2V ↓	3	H/10,000

Conclusion 1×10^6 lower limit detection of Clostridium w/ Hemin/10,000

Reagents
Reagent Orig H2O @ 1.35 ml/min
Nutrient DBT Sol @ 1 ml/min
Wash .1 M SO @ 10 ml/min

Type C.T.

Configuration E/E 1002

DATE 7/27 Ep 1450

TEST PLAN Coli Visible Study / No Heatin
2 & 4 Hour incubation

SDV F.S.

Run #	Agent	Cuvette	Volume	pH	WB (S-N)	Sample (S-N)	Comments
1	WB		400				ABORT PINCHED LINE
2	WB		400	4	1.5		
3	Coli	7.5x10⁴	400	7	1.5	5V	OK. ⊕ control
4	WB		400	7.5	~2.0		2 hr incubation WB
5	Coli	7.5x10⁴	400	4	2.0	~7V	2 hr incubation coli
6	WB		400	3	1		4 hr incubation
7	Coli	7.5x10⁴	400	7	1	~30V	4 hr incubation coli ~ 4X Gain

Good Coli Visible Data

R[illegible]NTS
[illegible] x [illegible] @ 1.35 ml
[illegible] 5 ml @ 1ml/min.
[illegible] 4M 5ml @ 10ml/min

Configuration E/E MOD 2

DATE 10 Aug   Ep 1950

TEST PLAN E/E with coli

TYPE C.T.

RUN	AGENT	CHALLENGE	VOLUME	PM Φ	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	~4V	6.5		too high for WB; do over.
2	coli	2.5x10^4	400	~4V	~2V	3V	2 time control
3	WB		400	~4V	~2V		
4	WB		400	~5V	~3.5		4 hr incubation control
5	coli	2.5x10^4	400	~4	~3.5	33	4 hr incubation
6	coli	2.5x10^4	400	~5	~3.5	32	4 hr incubation

Notes: a) deposited filters place on abs[illegible] pad w/ broth in petri dish; incubate 4 hrs

b) broth inoc & control after 4 hours
ZOX gain.

Conclusion – good signal for 2.5x10^4 – go lower

Agents
Matrix DMtH₂O @ 135 ml/min
Nutrient DBC Sol @ 1 ml/min
Wash 4M Sol @ 10 ml/min

Configuration E/E SOUFS

DATE 13 AUG Ep 1950

TEST PLAN coli viable cycle
H/10K

TIME

Run	Agent	Challenge	Volume	RM Φ	WB (S-N)	Sample (S-N)	Comments
1	WB		400	Short air in line			
2	WB		400	7.5	7.5		
3	coli	5×10^3	400	3.5	7.5	~0	unic control
4	coli	5×10^3	400	5.0	}	~26	inc 3 hrs
5	WB		400	6.5	7.5 }		inc 4 hrs
6	coli	5×10^3	400	4.5	7.5	~27	4 hr inc.

65% viable

Reagents
Buffer GATHD @ 1.35 ml/min
Nutrient DBTS ml @ 1 ml/min
Wash AN 80 ml @ 10 ml/min

Configuration E/E 50 V FS MOD 2

DATE 19 Aug 16 Ep 1450V

TEST PLAN E coli viable

TUBE Long tape

Row	Agent	Inoculum	Volume	AM Q	WB (S-N)	Sample (S-N)	Comments
1	WB		400	4.5	0		clean
2	coli	1×10^4	400				SAT - long tail off bottom
3	coli	1×10^4	400	6	0	1 V	low look.
4	WB		400	7	~8.5		2 Hr incubation
5	coli	1×10^4	400			> 40 V	SAT long tail off
6	WB		400	6	~5.5	> 40 V	3 Hr incubation
7	coli	1×10^4	400			> 40 V	SAT long tail-off bottom

60% visible

AGENTS
Carrier MMH.D @ 1.35 ml/min
Nutrient DBT Sol @ 1ml/min
NOSH Alt sol @ 10ml/min

Configuration MOD 2 QUFS E/E

DATE 8/20 Ep 1450

TEST PLAN E. coli viable

TYPE Loop type

Run	AGENT	CHALLENGE	VOLUME	Dm Φ	WB (S-N)	SMOKE (S-N)	COMMENTS
1	WB		400	6	3		
2	Coli	5×10^3	400	5	4p	~0	Unic. control
3	WB		400	5	4		inc 3 hrs
4	Coli	5×10^3	400	5	4	1k	inc 2 hrs.
							54% viable

Reagents
Buffer x gal H2O @ 1.35 ml/min
Nutrient DST 5ml @ 1ml/min
Water 4M SO @ 10ml/min

Configuration MOD 2 E/E

DATE 8/25 Ep 1450V 50 v F.S.

TEST PLAN Further sterilization - pre-incubation studies

Time Long Type H/10K

RUN	AGENT	CHALLENGE	VOLUME	AM Φ	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	6.5	3.5		2 Hr incubation 4M pre-test
2	filter			3.5	3.5		2 Hr incubation ETO control
3	WB		400	4	2		WB control 4M pre-test
4	WB		400	6	3.5		ETO 2 Hr incubation
5	coli	5×10^3	400	4.5	2	0	coli control 4M pre-test
6	WB		400	5	<1		WB 2 Hr incubation, 4M pre-test
7	WB		400	4.5	4.5		2 Hr incubation autoclaved filter
8	coli	5×10^3	400	4.5	1V	2V	2 Hr incubation 4M pre-test
9	coli	5×10^3	400	5	2		2 Hr incubation 4M pre-test no growth
10	coli	5×10^3	400	6	1V	2.5	3 Hr incubation 4M pre-test no growth
11	coli	5×10^3	400	5	3.5	9	2 Hr incubation ETO Good
12	coli	5×10^3	400	5	4.5	—	No growth 3 Hr incubation (Autoclaved filter)
						Conclusion - from data so far	
						a) 4M pre-test prior to incubation inhibitory	
						b) ETO looks best sterilization technique	
						* autoclaved accept - slower flow rate	

Viability 65%

AGENTS
[illegible] ______ @ ______
[illegible] ______ @ ______
[illegible] ______ @ ______

TYPE C.T. BM Pre treat

Configuration E/E 50 U.F.S.

DATE 10 Sep'71 Ep 1450

TEST PLAN Clostridium - Incubation H/10K

RUN	AGENT	CHALLENGE	VOLUME	BM Φ	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB	Control	400 ml	1.5V	1.5		
2	WB		400	2.5	3	~~[illegible]~~	4 Hr WB incubation Anaero
3	Closti	1X10[6]	400	2.5		4V	4 Hr Closti incubation Anaero
4	WB		400	2.5	1V		4 Hr WB incubation
5	Closti	1X10[6]	400	2	1.5	1V (2.5-1.5)	Closti Control

Conclusion

WB 1.5V Cont
1X10[6] Closti 1V over control

4Hr WB 3V
4Hr Closti 1X10[6] 3V over WB 4Hr

Configuration 50 U F.S. E/E

DATE 13 Sep '71 Ep 1450

TEST PLAN pH & Chemical Effects on Signal

TAPE Long tape 8 cc pretest

H/10K

RUN	AGENT	CHALLENGE	VOLUME	PM Q	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	5	2		control pH 7.5
2	coli	5X10^5	400	4.5	2	37	pH 7.5 control
3	coli	5X10^5	400	4	2	56 ✓	pH 7.5 control
4	coli	5X10^5	400	4	2*	37.5 **	pH 7.5 w/ 6 ppm Iodine control
5	coli	5X10^5	400	4	2*	27.5	pH 7.5 w/ 50 ppm Chlorine control
6	coli	5X10^5	400	5	2*	37	pH 2.0 control
7	coli	5X10^5	400	4.5	2*	37	pH 5.0 control

* Not run WB for pH/chemical

** 5X10^5 - saturated recorder-PMT.

AGENTS
[illegible] QM H₂O @ 1.135 ml/min
[illegible] DBT 5ml @ 1ml/min
[illegible] 4M 50ml @ 10 ml/min

Configuration E/E 50 V F.S. Mod 2

DATE 14 Sep Ep 1450

TEST PLAN Pasteurization

H/10K

TIME

RUN	AGENT	CHALLENGE	VOLUME	BM ⊄	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	2.5	2.5		pH 7.5
2	coli	5X10⁵	400	2.5	2.5	37.5	[illegible] SAT.
3	coli	5X10⁵	400	2	2.0	38	4 Hr Pasteur 160°F coli
4	coli	5X10⁵	400	2.5	2.5	37.5	Ambient 4 Hr coli
5	coli	5X10⁵	400	3	3.0	36.5	2 Hr 160°F coli

So. 0, 2, 4 Hr pasteur 160°F
no effect on [illegible] coli

[illegible]SENTS
[illegible] SMHHC @ 1.35 ml/min
[illegible] DBS sol @ [illegible]/min
[illegible] AM sol @ [illegible]/min

TYPE Lamp tape

Configuration E/E 50 UES.

DATE 16 Sep Ep 1450

TEST PLAN Chem. Interference, pH

H/10K DRIE CYCLE WATER

[illegible]	AGENT	CHALLENGE	VOLUME	PM Q	WB (S-N)	SAMPLE (S-N)	COMMENTS
4	WB		400	3	3		control 30 ppm Chlorine
5	WB		400	3.5	8.5		control 6 ppm iodine
6	WB		400	3.5	1		control pH 9.0
7	WB		400	3.5	3.5		control pH 5.0
1	WB		400	3.5	0		
2	coli	3x10^5	400	3.5	0 ↓	29.0	
3	coli	3x10^5	400	4.0	0 ↓	28.5	

REAGENTS
Sterox [illegible] @ 135 ml/min
Patient DET 5ml @ [illegible] ml/min
WASH [illegible] @ [illegible] ml/min

Configuration E/E 50 V F.S.

DATE 12 Sep Ep 1450

TAPE Lamp Tape

TEST PLAN [illegible] [illegible]
[illegible] distill. water

RUN	AGENT	CHALLENGE	VOLUME	RM Φ	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	3	~4.5		high
2	~~WB~~ Clostr.	$1X10^6$	400	5	~4.5	O~0.1-0.?	no peak @ all
3	WB		400				bad off scale, SAT
4	WB		400	2.5	1V		GOOD, control
5	Clostr.	$1X10^6$	400	3	~~[illegible]~~ 2.5	~0.5V	2 hr incubation Mano.
6	WB		400	3.5	2.5		Ep drifted 50V high but
7	WB		400	3.5	2.5		value apparently OK.

Reagents
Buffer CH4 H2O @ 1.25 ml/min
Nutrient DBT Soln @ 1ml/min
Wash All Soln @ ml/min

Washington E/E 50 U F.S. MOD 2

DATE 30 Sep   Ep 1450

TEST PLAN E coli STD Curve Ag+, Cl-

TAPE Long tape   H/10K   Glass distill

Run	Agent	Challenge	Volume	SM Φ	WB (S-N)	Sample (S-N)	Comments
1	WB		400	4	1		Control
2	~~[illegible]~~ coli	3×10^5	400	3.5	1	33.5	"
3	coli	1×10^5	400	3.5	1	24.5	"
4	coli	3×10^4	400	4.5	1	6.5	"
5	~~NoAT~~	~~1×10^4~~	~~400~~	~~3~~	~~+~~	~~+~~	
6	coli	1×10^4	400	3	1	1	barely seen
7	WB	Ag+	400	2.5	~1.5V		Ag+ control
8	coli	1×10^5 Ag+	400	3	~1.5	18	coli in Ag+
9	WB		400	2.5	~1.5		control 4 Hr 160°F } Pasteur.
10	coli	3×10^5	400	3	~1.5	~51	coli 4 Hr 160°F
11	WB		400	~7	~17.5		control 30 ppm Chlorine
12	~~[illegible]~~ coli	1×10^5	400	~7	~17.5	~~[illegible]~~	no signal for coli over WB

[illegible]
[illegible] H2O @ 635 ml/min
[illegible] DET 5ml @ 1ml/min
[illegible] 30ml @ 10ml/min

Configuration F/E

DATE 3 Oct 71 Ep 1450

TEST PLAN Pasteurization & Chemical Interference
Glass dist water (Special Test)

TYPE Long tape

RUN	AGENT	CONC	VOLUME	AM ⊄	WB (S-N)	SAMPLE (S-N)	COMMENTS
1	WB		400	2.5	1V		control
2	coli	3×10^5	400	3V	1V	35V	control
~~3~~ 4	coli	3×10^5	400	3V	3.5 ~~[illegible]~~	~1V	24 Hr pasteur. @ 160°F } all second lost pulse
3	WB		400	3V	3.5		24 Hr pasteur. @ 160°F
5	WB		400	2V	2.5		Chlorine 50 ppm control
6	coli	1×10^5	400	4V	2.5	8V	Chlorine 50 ppm control
7	WB		400	2.5V	1V		Iodine 6 ppm control
8	coli	1×10^5	400	3V	1V	14.5	Iodine 6 ppm control

APPENDIX D

SYSTEMS ANALYSIS

CONTENTS

Section 1

INTRODUCTION

The following is submitted in accordance with the requirements of Contract NAS 9-11644, Modification No. 2S, dated December 1, 1971.

The effort to be performed under this modification was to include:

1. A definition of water potability monitoring requirements pertaining to bacteria, toxins, viruses, molds and yeasts.

2. A survey of different techniques which might be used to monitor these agents including ATP, Partichrome, Colony Counters, light scattering, absorption, bead agglutination and prophyrin chemiluminescence.

3. A study effort to evaluate the sensitivity, response time, reagent requirements, zero-g adaptability, as well as weight, power and volume requirements. An estimate of the lead time required to reach a flight-rated status.

4. Recommendation for including other chemical and physical sensors.

5. After selection of three systems by NASA, AMB was then to prepare a detailed program projection of these systems to include man-hour and material cost estimates.

6. Sketches and outline drawings of possible flight prototype configurations were then to be prepared by AMB.

The study effort, described below, is presented in the following order.

1. Definition of the water potability requirements pertaining to biological monitoring.

2. A description of detection techniques which might be used to monitor the desired biological parameters.

3. A critical evaluation of the various methods.

4. Recommendations for including other chemical and physical sensors.

5. Preliminary sketches and outline drawings of the selected biological monitors.

6. A program projection for each of the three systems.

Section 2

DEFINITION OF WATER MONITOR REQUIREMENTS

In attempting to define the contaminants which could appear in water reclaimed from urine and other liquid wastes, one must consider the various microflora which might be introduced through a number of sources (e.g., the feces, skin and space cabin atmosphere).

Although urine, in the absence of any bladder infection is normally sterile, surface contamination from the penis or anal area could introduce fecal microflora into the regenerated water supply. There is also ample opportunity for contamination by fecal material suspended in aerosols or transmitted by the hands or utensils of the astronauts. The constant shedding of the surface layer of skin (10^4 particles/minute) and the microorganisms present on them can result in significant contamination of the space cabin atmosphere. Sneezing and coughing can produce anywhere from 10^4 to 10^6 infectious droplets; merely talking can disperse 10^3 infectious nuclei/minute into the air*. Where space cabin air is utilized in the water reclamation process (e.g., in connection with the wick evaporation), contamination of the water supply can result**. In a number of studies dealing with man in a closed environmental system, ample evidence has been obtained for bacterial buildup of coliforms or other pathogenic

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*Infectious Diseases in Manned Spaceflight, National Academy of Sciences, Washington, D.C. 1970.

**The 1 μ filter normally employed for filtering the space cabin air for use with the wick evaporator is only partially effective in removing the microflora shown in Table 1.

Table 1

MICROFLORA IN CONTAMINATED WATER

	PATHOGENIC	TOXIN PRODUCER
• AEROBIC BACTERIA		
Strep. facaelis	Secondary	-
Staph. aureus	Yes	Yes
E. coli	No	Yes
B. subtilis	No	-
B. cloacae	No	-
B. proteus	Secondary	-
Ps. fluorescens	No	-
Ps. aeruginosa	Yes	-
Alcal. facaelis	No	-
Salmonella (typhimurium, etc.)	Yes	Yes
Mycobacterium tuberculosis	Yes	-
Shigella dysenteriae	Yes	Yes
Proteus sp.	Yes	-
• ANAEROBIC BACTERIA		
B. welchii	Yes	Yes
C. botulinum	Yes	Yes
• VIRUSES		
Polio		
Infectious Hepatitis		
Influenza		
Adenovirus		
Herpes		
• FUNGI		
Histoplasma capsulatum		
Candida albicans		
Aspergillus		
Nocardia		
• PYROGENS		

microflora on interior surfaces of the spacecraft and in the drinking water supply*.

A list of some of the more likely microflora which could be present in reclaimed water if a malfunction occurred in the regeneration system, is shown in Table 1. The two most common types of infection likely to occur in a spacecraft environment are respiratory and gastrointestinal. A contaminated water supply can serve as a vehicle for transmission of both of these disorders with the respiratory pathogens in water finding their way into the respiratory tract by way of the mucociliary escalator.

Reference to Table 1 shows that potential contaminants include bacteria, virus, toxins, yeast, molds and pyrogens.

With respect to the bacteria, they may be viable or non-viable, aerobic or anaerobic. Whereas most of the enteric bacteria are normally aerobic, a shift to predominately anaerobic fecal organisms (1000:1) was observed as a result of an altered space diet. Some of these microorganisms are pathogenic, some produce lethal toxins which are thermally stable and would survive the pasteurization temperature used for storing the reclaimed water. The type of regeneration system employed would also affect the ratio of aerobes to anaerobes. If air distillation was employed and a N_2+O_2 mixture used to pressurize the regeneration water tank, aerobic organisms would probably predominate at first. However, with subsequent growth, the oxygen supply could be depleted to a level suitable for propagating anaerobes. In a regeneration system utilizing vacuum distillation and nitrogen for pressurizing the storage tanks, growth of anaerobes would be favored. It is interesting to note that in three studies

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* (1) K.R. Coburn, NASA Contractor Rept CR-708, February 1967.

(2) Integrated Life Support System, 28-day Manned Evaluations, Internal Langley Working Papers 1968-69, Applied Materials and Physics Division, Langley Research Center.

(3) P.E. Rielly et al, AMRL-TR-66-33, April 1966.

(4) Manned Environmental System Assessment, NASA CR-134, November 1964.

(5) 7-Day Closed-Door Test of the Langley Integrated Life-Support System, J.R. Wilkens, Langley Working Paper, LWP-431, August 31, 1967.

involving evaluation of water management subsystems at Langley, the only organisms isolated from reclaimed water were either aerobes or facultative anaerobes*. No anaerobes (comparable to Clostridium) were ever found (see Table 2).

Virus infection either latent or transmitted through the water route could be severe and poses a major threat.

With the exception of Histoplasma, and some species of Candida and Actinomyces, most fungi likely to be present are of minor concern except for the mechanical fouling which can result from their uncontrollable growth.

Pyrogens, resulting from autolysis of most Gram-negative bacteria, are mainly of concern if injected into the blood stream (resulting in fever). Ingestion of pyrogens are of little consequence and generally harmless with large quantities being consumed daily in our food and drink. A notable exception are the endotoxins released from the pathogenic coliform bacteria belonging to the genera Salmonella, Shigella and Escherichia. These endotoxins apart from being pyrogenic are also toxic and can produce gastrointestinal illness if consumed in sufficiently high quantities.

Summary and Conclusions

The criteria which one must use in evaluating the relative importance of each of these contaminants are how they affect the health of the astronaut.

1. Bacteria

The presence of any bacteria, viable or non-viable, should be cause for concern since it would indicate some breakdown in the reclamation system and the possibility that bacterial toxins might be present. The latter are capable of producing serious illness or death.

The presence of viable organisms particularly those pathogens which produce respiratory or acute gastrointestinal illness are of primary concern. Infections of the skin appear to be of secondary importance.

As to the need for monitoring anaerobic as well as aerobic bacteria, from the standpoint of numbers (i.e., enteric anaerobes outnumber aerobes 1000/1), the possibility of them appearing in reclaimed water is certainly good. The fact that only aerobes have been isolated from regenerated water supplies todate may be due to the stringent anaerobiasis required for growth of these anaerobes, conditions which apparently do not prevail in present water reclamation systems. If one wished to cover all contingencies, the ability to monitor aerobic and anaerobic bacteria would be desirable.

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*Infectious Disease in Manned Spaceflight, National Academy of Sciences, Washington, D.C. 1970, pp. 181, 185, 193.

Table 2

REFERENCE*	ORGANISMS RECOVERED FROM RECLAIMED WATER
1	Pseudomonas sp. - aerobic, facultative
	Achromobacter sp. - aerobic, facultative
2	Proteus mirabilis - aerobic, facultative
	Pseudomonas sp. - aerobic, facultative
	Aerobacter sp. - aerobic, facultative anaerobe
	Streptococcus sp. - aerobic, facultative
	Alcaligenes sp. - aerobic
	Klebsiella - facultative anaerobe
3	Pseudomonas - aerobic, facultative
	Proteus - aerobic, facultative anaerobe
	Alcaligenes - aerobic, facultative
	Chromobacter - aerobic, facultative
4	Staphylococcus - aerobic, facultative anaerobe
	B. subtilis - aerobic, facultative anaerobe

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* (1) 7-Day Closed-Door Test of the Langley Integrated Life-Support System. J.R. Wilkens, Langley Working Paper, CWP-431, August 31, 1967.

(2) Microbiological Studies in a Water Management Subsystem for Manned Space Flight, J.R. Wilkens and D.C. Grana, National Aeronautics and Space Engineering Meeting, Los Angeles, Calif. October 7-11, 1968.

(3) Integrated Life Support System, 28-Day Manned Evaluations. Internal Langley Working Papers, 1968-1969.

(4) Studies of the Naval Facilities Engineering Command Protective Shelter, NRL Report 6656, March 29, 1968.

2. Toxins

Since these can prove incapcitating or even lethal (e.g., the exotoxins of Cl. botulism, Cl. welchii, Staph aureus, Shigella dysenteriae), the ability to monitor toxins should be a real requirement.

3. Virus

There are a number of latent virus infections which can persist undetected in the astronaut for long periods of time. These could be transmitted to the others via a contaminated water supply and cause serious illness. Viral infections can also increase susceptibility to bacterial illness. A detector which monitored viral agents would be desirable.

4. Fungi

Certain fungi capable of causing serious illness (e.g., Histoplasma capsulatum) can persist at relatively high concentrations and for long periods of time in a spacecraft environment. The ability to detect certain pathogenic fungi in the drinking water supply would be desirable.

5. Pyrogens

Monitoring this parameter is of minor importance in terms of its effect on the astronaut's health. Depending on the amount consumed, endotoxins of the pathogenic coliform bacteria can produce gastrointestinal illness.

6. Soluble Porphyrins

The ability to detect lysed organisms by the prophyrins released on lysis would be another important parameter to measure. Conditions may exist in the reclamation process where a major breakdown has occurred in the reclamation system and bacteria are being generated upstream. On reaching the storage tanks, which are generally maintained at pasteurization temperature, lysis of these cells occur. A system which responded only to bacterial particulates would miss this completely. However, the chemiluminescence monitor which responds to porphyrins contained in the organism or released into solution, could be used to detect this situation. Results of evaluations by AMB of regenerated water samples, supplied by MACDAC indicated that this was actually taking place*.

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*Aerojet Medical and Biological Systems, Final Report No. 1504F, NASA/MSC Contract NAS 9-10432, 13 November 1970, p. 3-51.

In conclusion, the ideal monitoring system would be one capable of detecting total and viable organisms, virus, toxin and fungi in water. In the section which follows, the ability of various devices to detect one or more of these parameters is described.

Section 3

DESCRIPTION OF BIOLOGICAL MONITORING SYSTEMS

PARTICHROME DETECTION SYSTEM

Principle of Operation

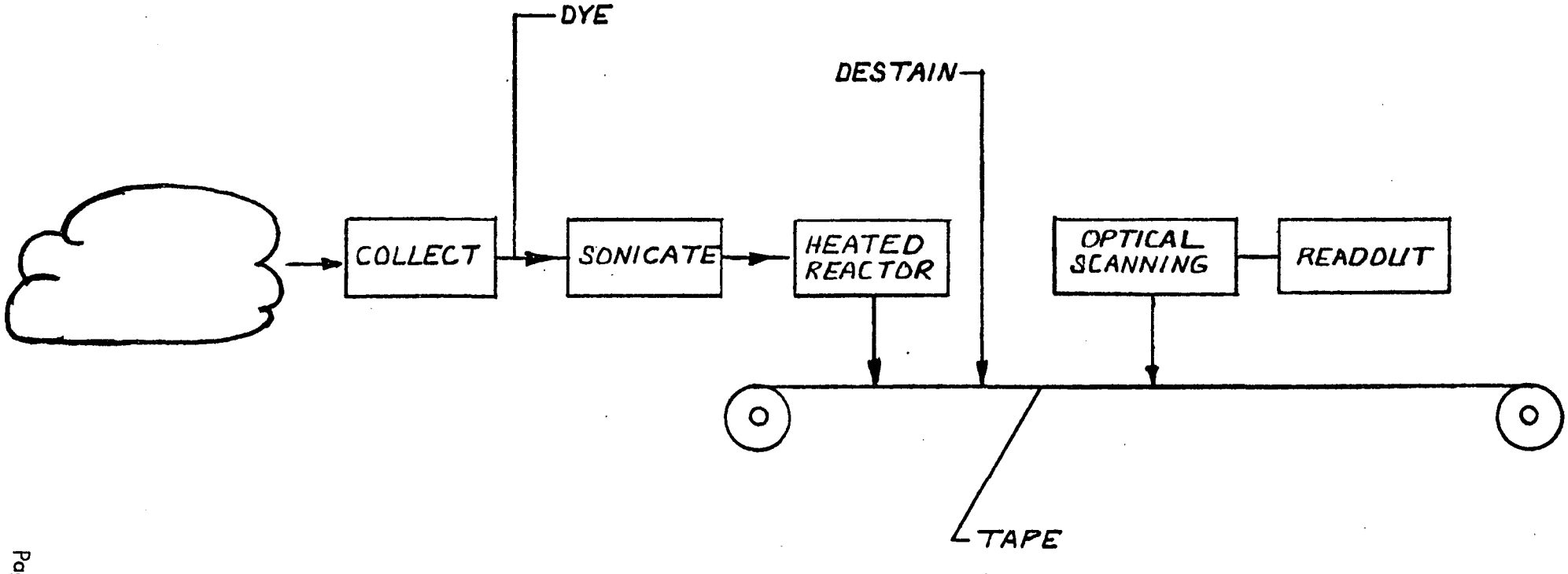
The Partichrome System, developed and successfully field tested for the U.S. Army by AMB, was designed to detect aerosolized biological particulates, more specifically proteinaceous material between 0.5 to 10 microns in diameter. With slight modification, this system can be readily adapted to monitoring the cell count (of bacteria and fungi) in a liquid stream.

The overall system illustrated in Figures 1 through 3 consists of an air sampler, liquid processor, optical scanner and alarm logic. As shown schematically in Figure 1, air particulates are continuously drawn into the system and concentrated in a small volume of collection fluid by an air sampler. The fluid is then pumped through a sonication unit which breaks up clumped particles. These are then stained and deposited as a continuous narrow (100μ wide) stripe on a moving microporous tape. The tape then moves to a destain station where excess stain is removed by application of a solvent. Immersion oil is applied automatically to the moving tape to clarify it for optical scanning. The staining process causes protenaceous material including bacteria to have low optical transmittance in the green portion of the spectrum and high optical transmission in the red portion of the spectrum. An electromechanically generated flying spot scans the tape as it passes through the Scanner Subsystem. Discrimination between bacteria and other material is achieved by signal ratio measurements, above a threshold setting, between a green and a red photodetection channel. In contrast to a stained bacteria, an opaque (i.e., non-proteinaceous) particle will attenuate light in both channels. The electronic logic discriminates between stained organisms and background particulates and causes an alarm to sound when the alarm level, set on the basis of background characteristics, is exceeded.

Figure 4 is a schematic of how this system might be utilized for monitoring total cell count in a regenerated water supply. After initially concentrating the water sample by filtration through a porous tape, the deposited organisms are advanced to the next station where they are stained (and then the background destained) before passing on to the flying spot scanning system for readout.

FIGURE 1

PARTICHROME SYSTEM SCHEMATIC



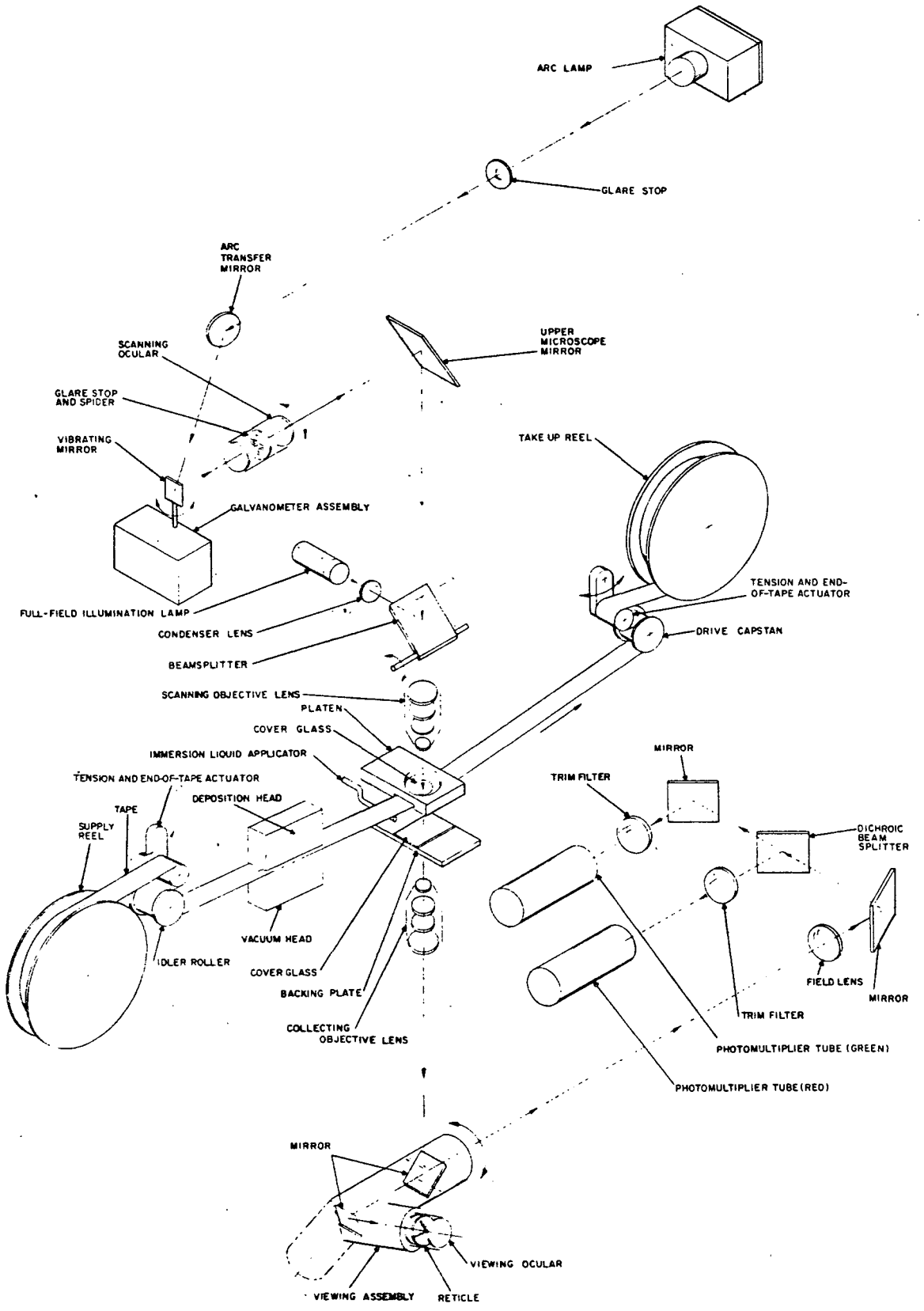


FIGURE 2 SCANNER SUBSYSTEM, EXPLODED VIEW

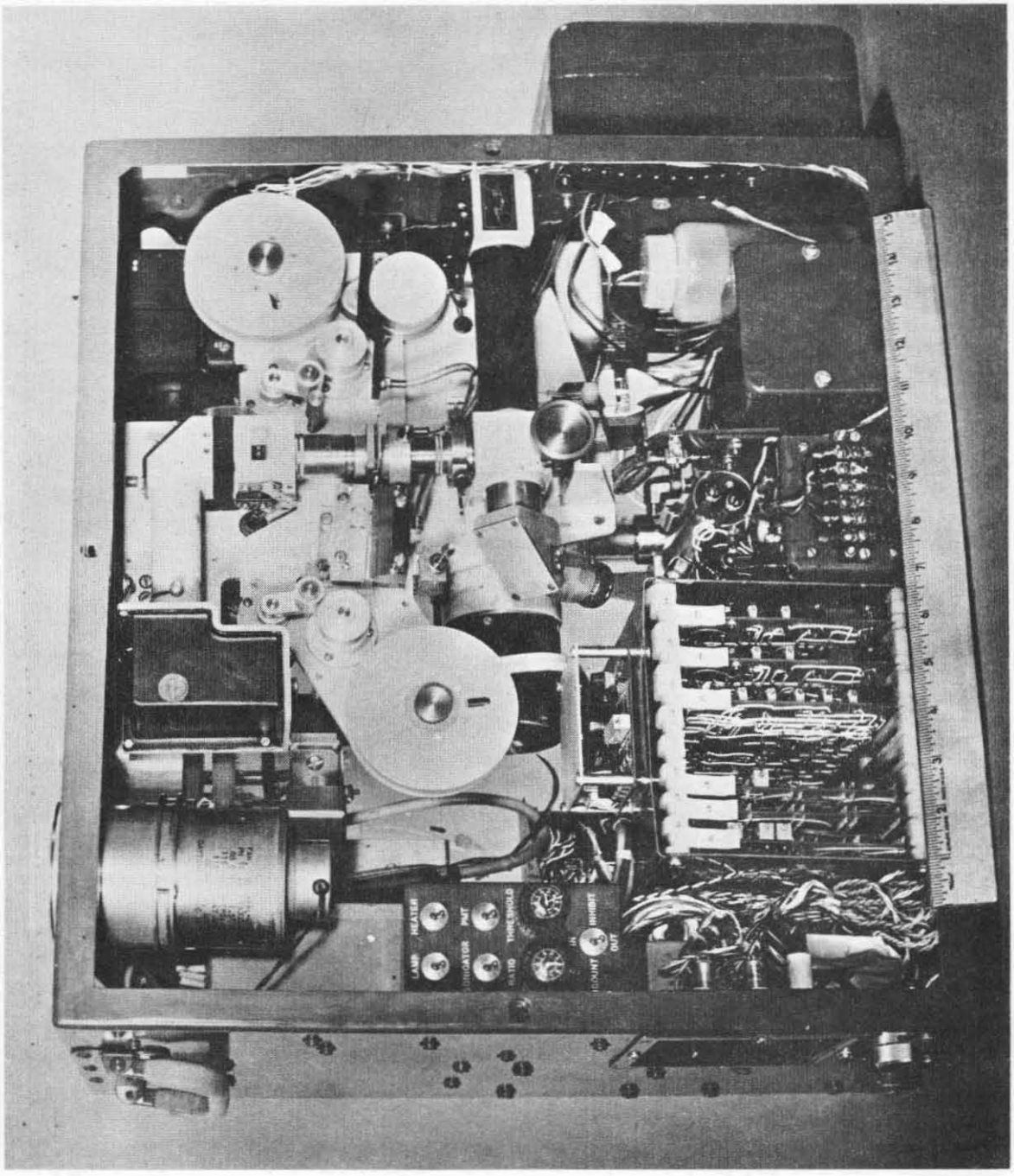


Figure 3 Partichrome Detection System

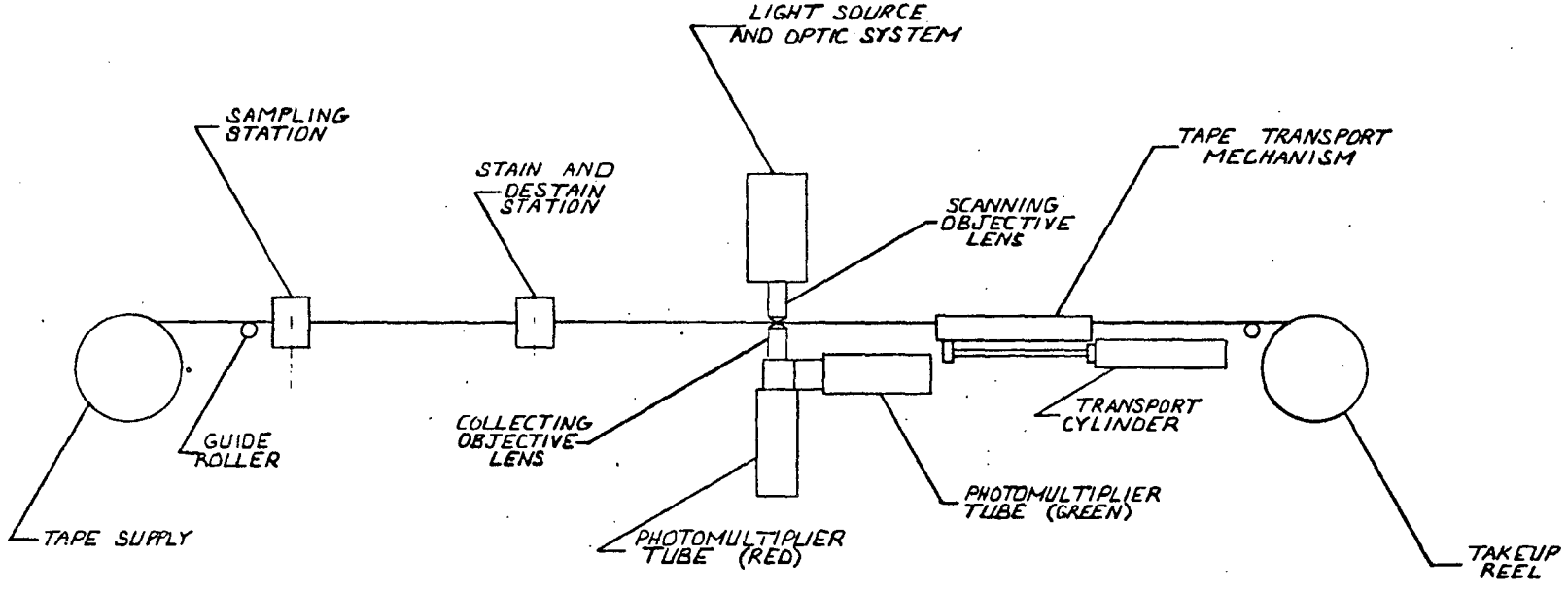


Figure 4

FLYING SPOT TOTAL COUNT SYSTEM

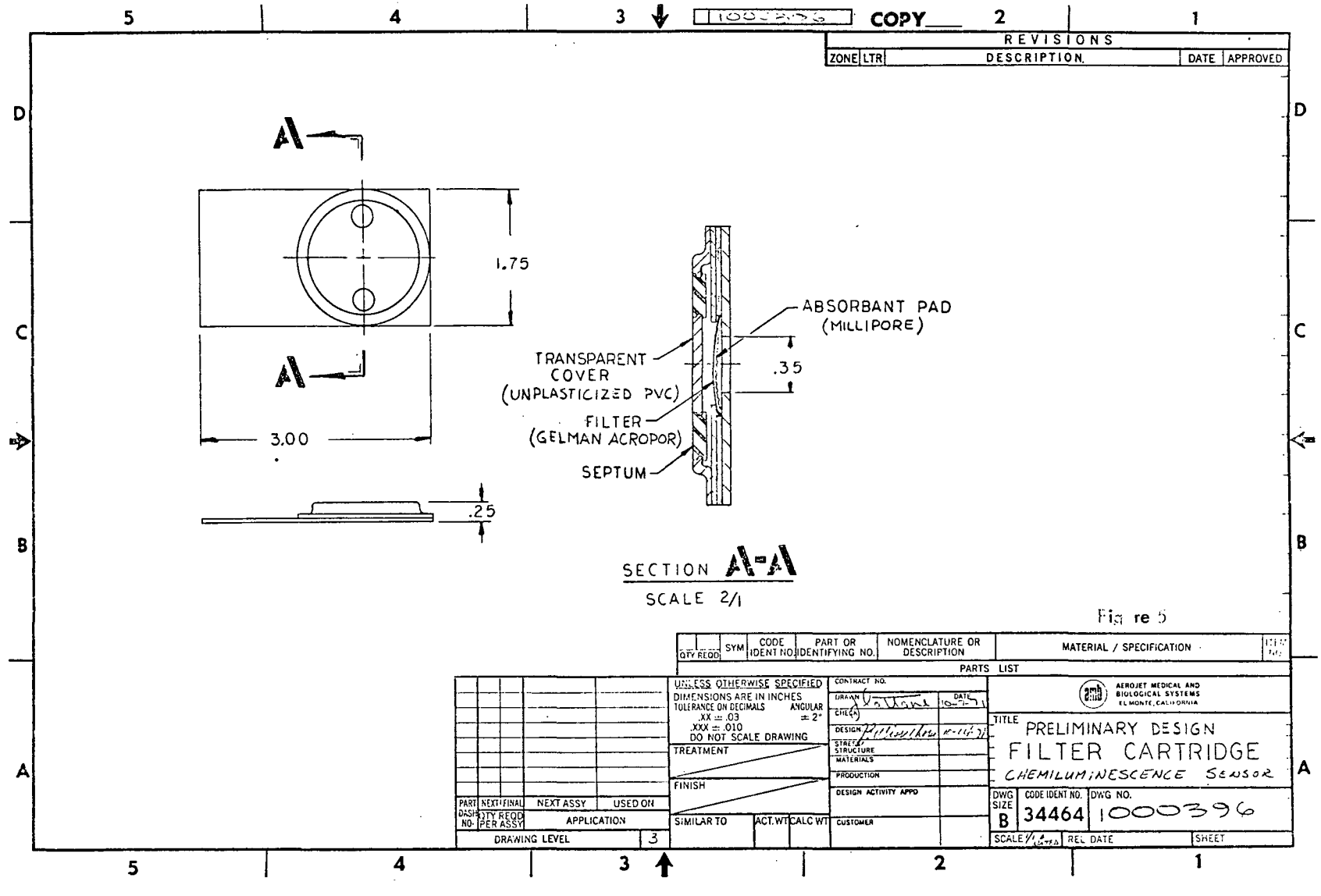
PORPHYRIN CHEMILUMINESCENCE DETECTOR

The principle of detection is based on monitoring the increase in chemiluminescence produced by the action of bacterial metal porphyrins on a luminol-hydrogen peroxide reagent. Metal porphyrins, either free or combined with proteins, are found in most living organisms. The reaction is virtually instantaneous and occurs immediately on contact of the bacteria with the aqueous reagent. The signal generated is linearly dependent on the number of bacteria present. This device responds to total number of organisms (i.e., viable + non-viable). To distinguish between viable and non-viable organisms, the signals of an incubated and unincubated sample are compared. A higher signal for the former indicates the presence of viable organisms.

Two types of systems are currently being developed for NASA, one uses a liquid sample transport, the other a modified tape sample transport. In the former, reaction between bacteria and reagent are carried out in solution, whereas in the latter, the reagent comes in contact with organisms deposited on a membrane filter (in a capsule). The latter approach is intended to minimize cross-contamination between samples by enclosing the reaction site in a capsule.

In the modified tape sample transport concept (Figures 5 and 6), individual sealed capsules, each containing a sterile membrane filter, and dispensed automatically from a cassette, are used for processing discrete water samples. Sample and processing fluids are introduced into these capsules by hypodermic needles which pierce rubber septums contained in these capsules. The liquids are pressure filtered.

Individual capsules are moved automatically from station to station on a belt. The processing sequence involves sample concentration, adding nutrient to the deposited organisms, incubation (if required), washing with 4M urea (to remove nutrient) and reaction with luminol-H_2O_2 at the last station. At the end of the processing cycle, the capsules are ejected into a container. An additional feature incorporated into the present design is the ability to detect soluble porphyrins. This is accomplished by merely reacting a portion of the unfiltered water sample directly with luminol-H_2O_2 reagent in front of the PMT.


COPY
REVISIONS
ZONE LTR DESCRIPTION DATE APPROVED
1.75
3.00
.25
ABSORBANT PAD
(MILLIPORE)
TRANSPARENT
COVER
(UNPLASTICIZED PVC)
FILTER
(GELMAN ACROPOR)
SEPTUM
.35
SECTION A-A
SCALE 2/1
QTY REQD SYM CODE IDENT NO PART OR IDENTIFYING NO. NOMENCLATURE OR DESCRIPTION MATERIAL / SPECIFICATION
PARTS LIST
UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS ANGULAR
.XX ± .03 ± 2°
.XXX ± .010
DO NOT SCALE DRAWING
TREATMENT
FINISH
CONTRACT NO.
DRAWN
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DESIGN
STRESS STRUCTURE MATERIALS
PRODUCTION
DESIGN ACTIVITY APPD
CUSTOMER
AEROJET MEDICAL AND BIOLOGICAL SYSTEMS EL MONTE, CALIFORNIA
TITLE
PRELIMINARY DESIGN
FILTER CARTRIDGE
CHEMILUMINESCENCE SENSOR
DWG SIZE B
CODE IDENT NO. 34464
DWG NO. 1000396
SCALE 1/1
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SHEET
PART DASH NO. NEXT ASSY QTY REQD PER ASSY NEXT ASSY APPLICATION USED ON
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Figure 5

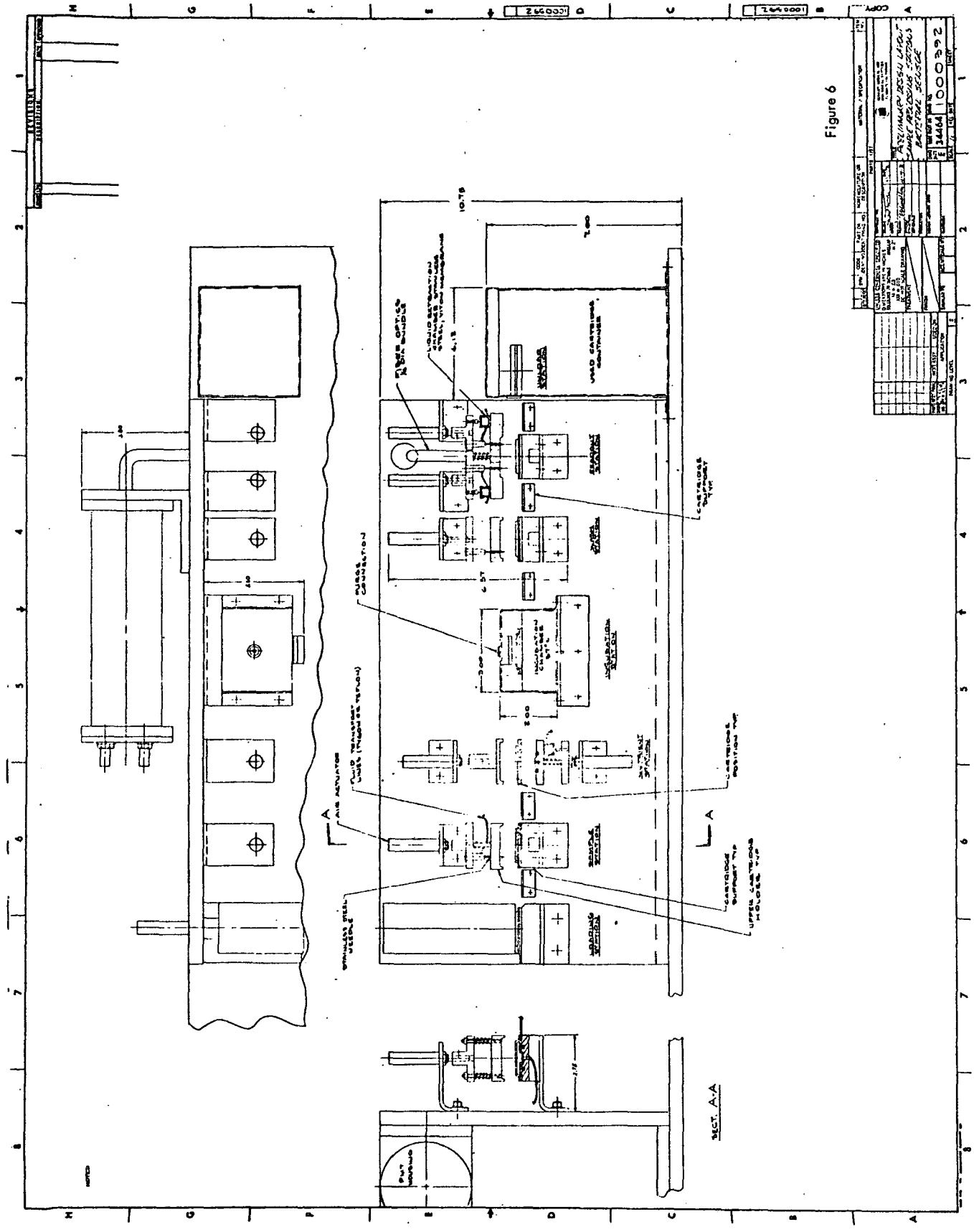

Figure 6

ATP BIOLUMINESCENCE

The firefly bioluminescence assay for adenosine triphosphate (ATP) originally prepared by McElroy* can be used for the monitoring of viable organisms. ATP, an agent found in all living cells, serves as a catalyst for energy producing reactions involved in biosynthesis and growth ot the organism.

The reaction involved in producing light is

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow{\text{Luciferase}} \text{light}$$

The ATP is supplied by the bacterial cell; luciferin and luciferase are two components which have been isolated from the firefly (luciferin has also been synthesized). Sufficient oxygen for the reaction is present in the solution. For maximum sensitivity, extraction** of the cellular ATP is required prior to reaction with the luciferin-luciferase mixture. In a typical reaction an aliquot of the bacterial extract containing the ATP is injected rapidly into a small test-tube containing the luciferin-luciferas reagent and mounted in front of a photomultiplier tube. The reaction reaches maximum light intensity in less than a second followed by an exponential decay lasting for a minute or more. The amplitude of the bioluminescent signal or the area under the curve is proportional to the amount of ATP (or number of bacteria) present. Only viable organisms contain the ATP needed to trigger the bioluminescence; when the organism dies, the ATP is rapidly consumed (disappearing in about one-half hour).

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*W.D. McElroy, Proc. Natl. Acad. Sci., U.S. 33, 342 (1949)
W.D. McElroy, Arch. Biochem. Biophys. 64, 257 (1956.

**Four methods which have been used for extracting cellular ATP include (1) boiling with water or buffer, (2) sonication, (3) acid extraction and (4) solvent extraction.

Figure 7 is a schematic representation of how this approach might be used to monitor viable cells in a regenerated water supply. After concentrating the bacteria by filtration, they are recovered by backwashing off the filter. The concentrated suspension is then passed through a sonicator (to disrupt the organism and release the ATP) and then into a cell where reaction with luciferin-luciferase occurs. The luciferin-luciferase reagent is normally stored as a lyophilized powder at dry-ice temperature (reagent is unstable at ambient) and reconstituted with Tris buffer just prior to use.

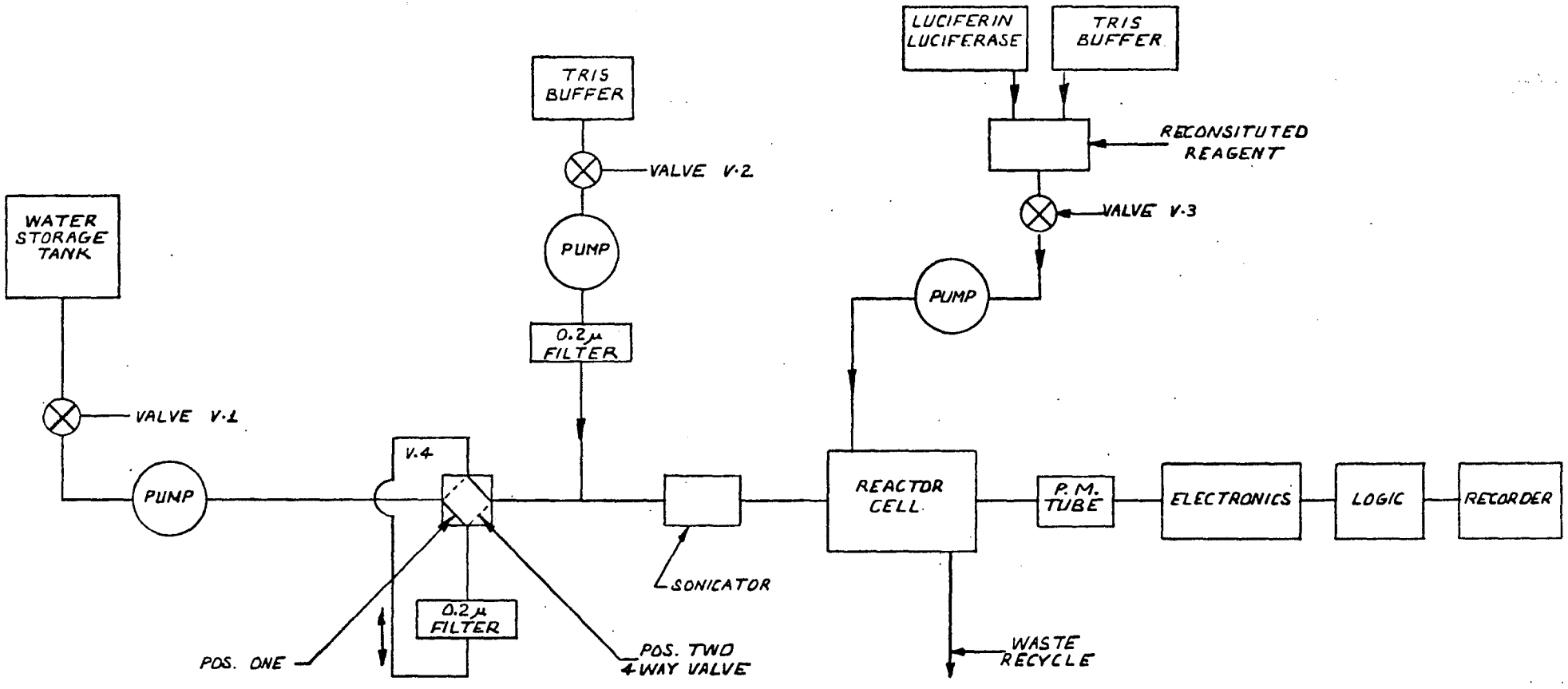


Figure 7

ATP SUB-SYSTEM
SONICATION EXTRACTION

COLONY COUNTER

In the colony counting method (shown schematically in Figures 8 and 9), the water sample is concentrated by filtering through a membrane filter contained in a capsule (dispensed automatically from a cassette), washed with nutrient at the next station and then incubated for several hours until the colony reaches a diameter of 10 to 100μ. The colonies can then be individually counted using a Vidicon readout or a flying spot scanner (Partichrome), the latter modified to pick up reflected light rather than transmitted light*. The advantage of a flying spot over a Vidicon readout is that the former can reliably count smaller colonies (i.e., 10μ instead of 100μ in diameter); thereby, cutting the required incubation time (i.e., for an unstressed E. coli to reach a colony size 10μ in diameter would require about a 3 hour incubation period compared to a 6 hour incubation for a colony 100μ in diameter).

*Prestaining of the colonies may be required with the modified Partichrome readout.

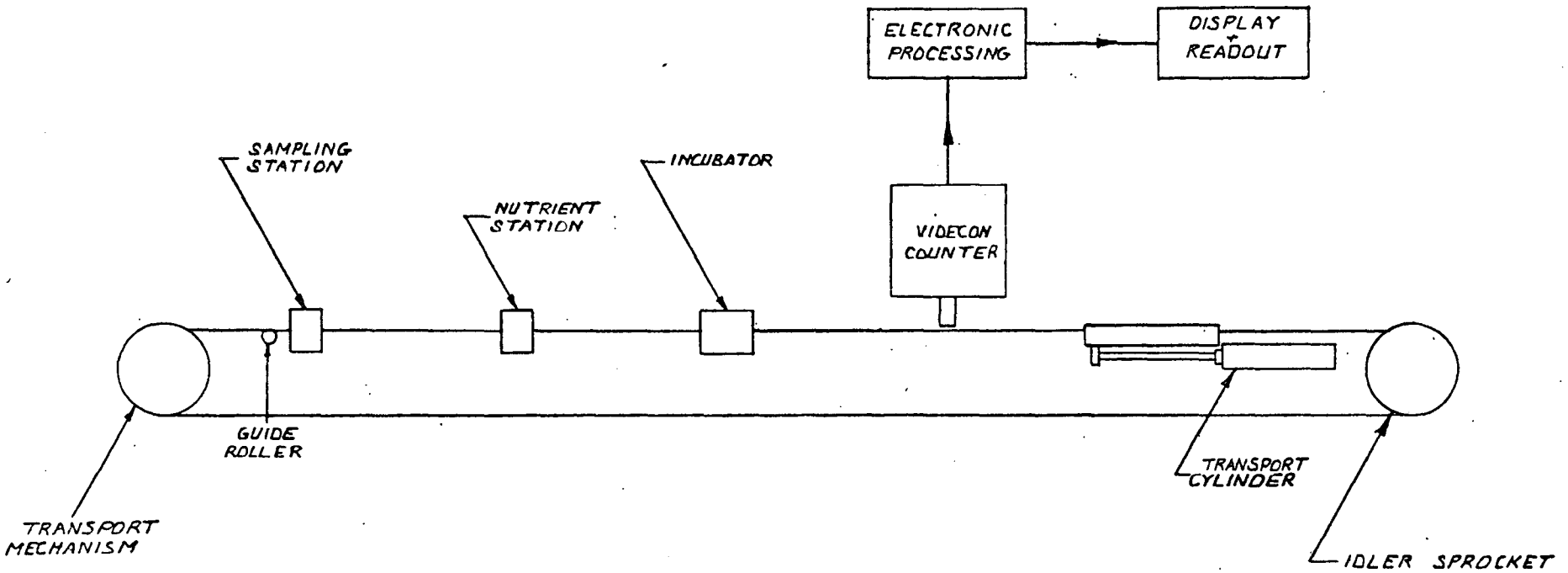


Figure 8

VIABLE COLONY COUNTING SYSTEM

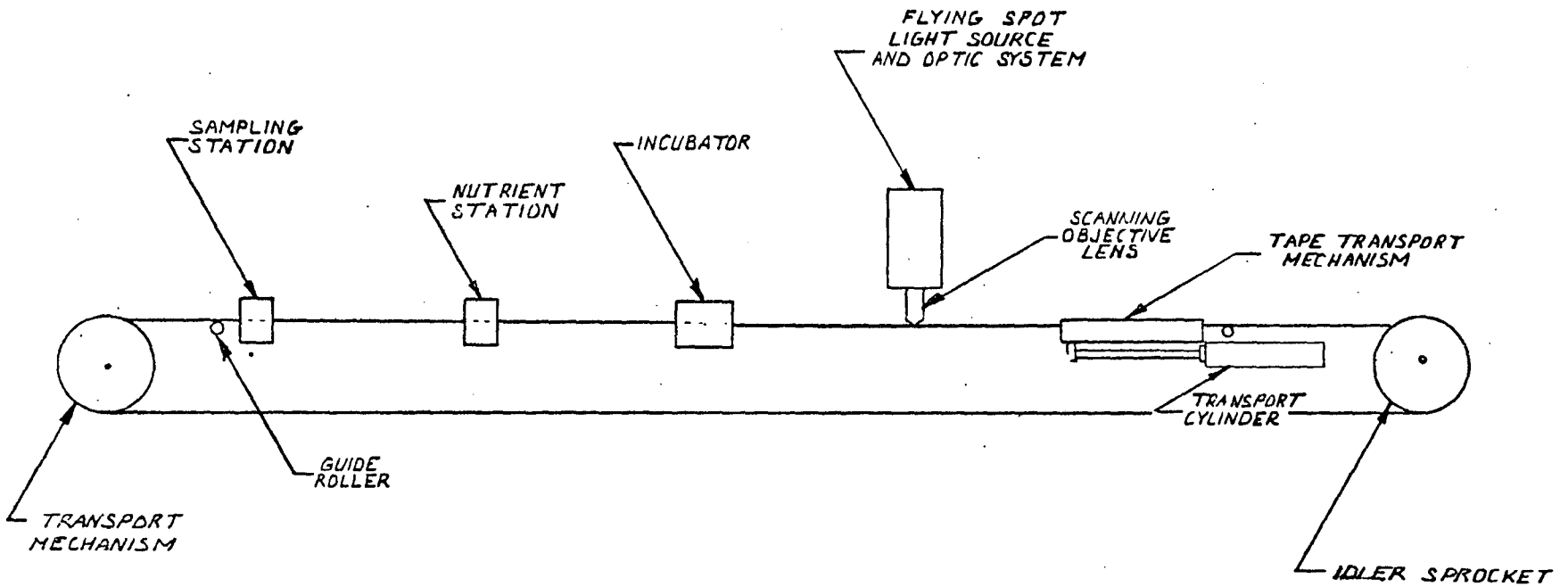


Figure 9

FLYING SPOT COLONY COUNTING SYSTEM

NEPHELOMETRIC METHOD

Nephelometry, the measurement of light scattered by particulate matter in suspension can be used for determining total cell count as well as distinguishing viable from non-viable cells. The latter can be accomplished by comparing the signals of an incubated and unincubated control.

Nephelometry is generally orders of magnitude more sensitive than turbidimetry which involves detection of particulate matter by measurement of a loss in transmitted light.

A device based on nephelometry which could be used to monitor changes in bacterial numbers (i.e., bacterial growth) is a light scattering photometer. The basic principle of operation of this instrument is shown in Figure 10. A suspension of bacteria contained in a cuvette is illuminated by a monochromater light source (such as a laser), while a collimated detector circumferentially scans the light scattered by the illuminated volume. A signal proportional to the intensity of the scattered light is recorded as a function of the angle formed between the incident and scattered beams. A comparison of this light-scattering intensity pattern with the pattern derived for an incubated bacterial suspension could be used to determine the presence of viable organisms. Bacterial growth should be reflected by a change in the scattering patterns (see Figure 11). A schematic of how this approach might be used for detection of viable organisms in a water supply is shown in Figure 12. After initial concentration of the bacterial sample on a membrane filter, the organisms are backwashed off the filter with nutrient into the light scattering cell. The intensity of the scattered light is automatically recorded as a function of scanning angle. After incubation, the scattering pattern is again determined on this same sample.

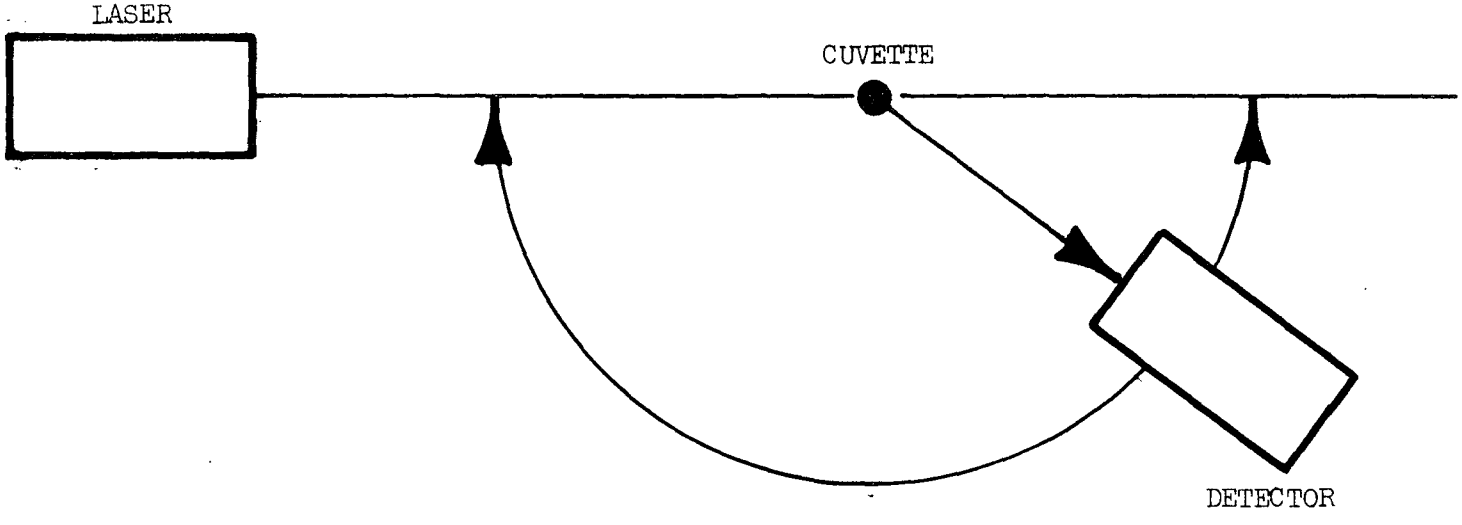
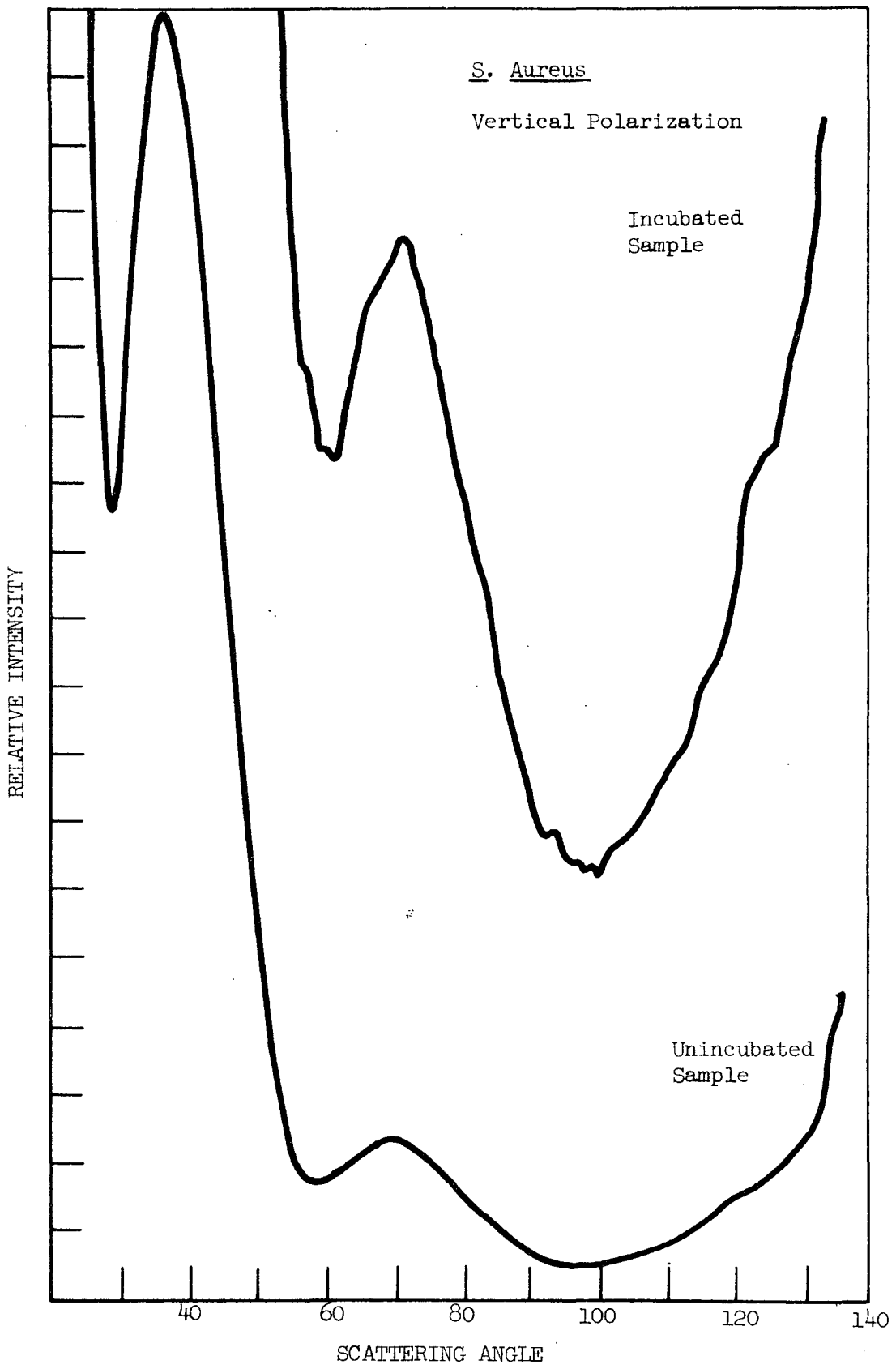


FIGURE 10 LIGHT SCATTERING PHOTOMETER

FIGURE 11

EFFECT OF INCREASE IN BACTERIAL NUMBERS ON SCATTERING PATTERN



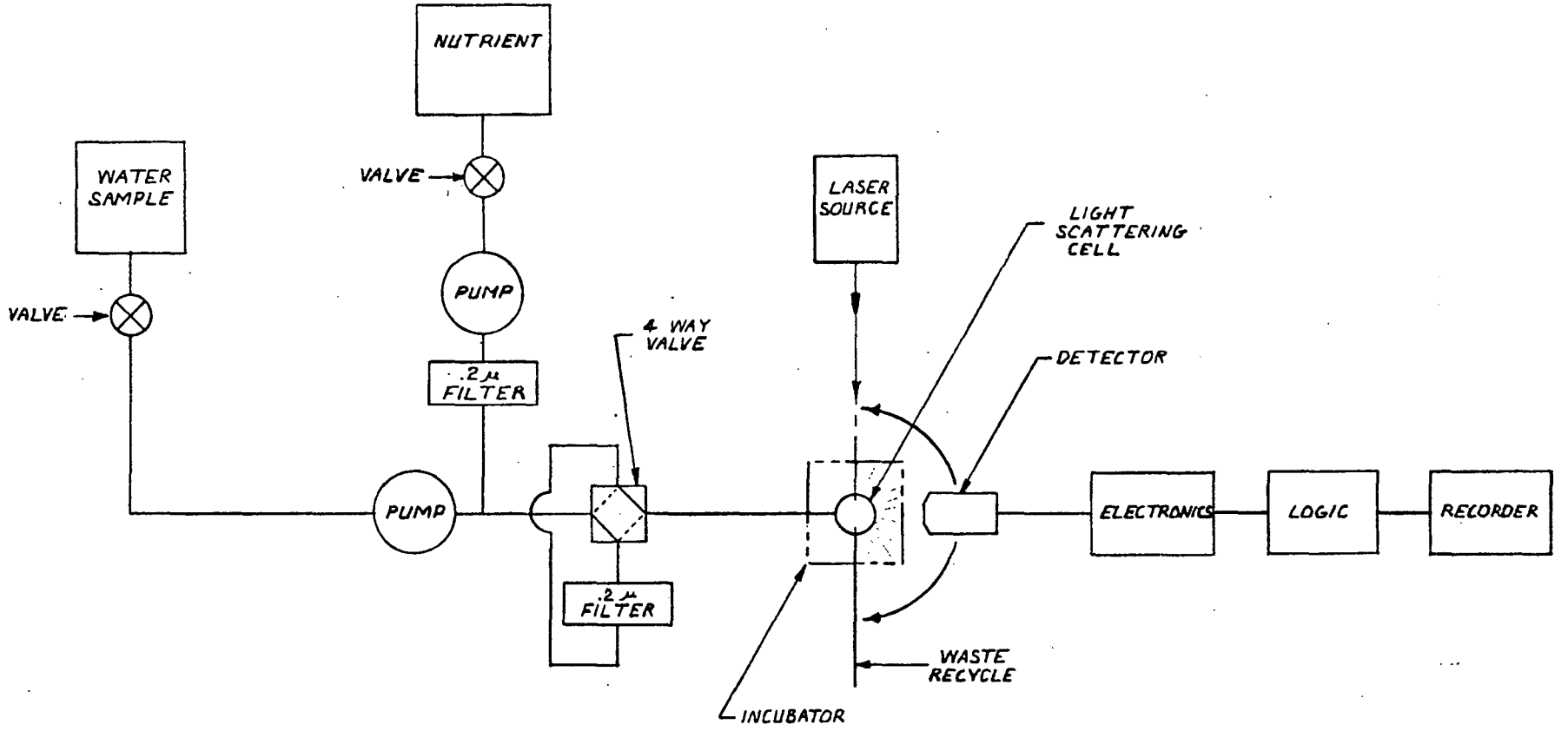


Figure 12

LIGHT SCATTERING PHOTOMETER

BEAD AGGLUTINATION

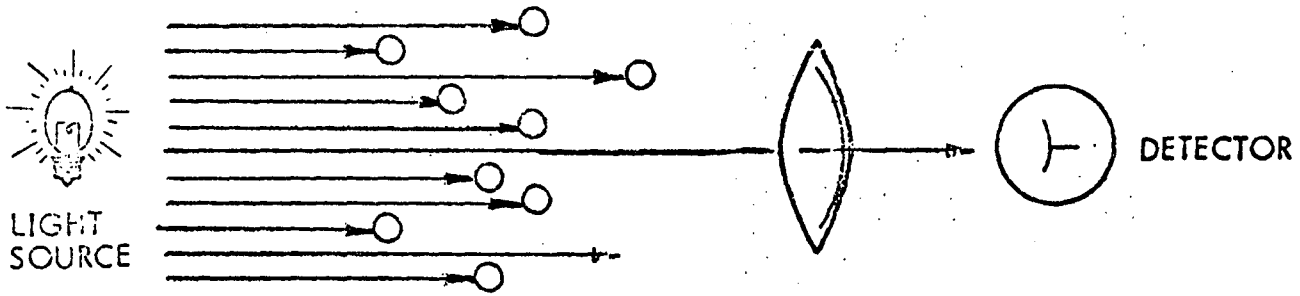
The bead agglutination or Passive Immunological Agglutination (PIA) technique was designed to detect and quantitate viruses in a spacecraft environment for both ecological and diagnostic measurements. The method is also applicable to detection of toxins, bacteria or any other antigenic material. The first step of this technique involves bonding specific antibody to polystyrene latex beads. These sensitized beads are then mixed with a virus sample. If the antibody bound to the latex bead is specific for the virus in solution, the virus then attaches to the bead. The virus is able to bind a second sensitized bead to its surface. In this manner clumps of beads are formed which are held together by the virus particles through an antigen-antibody bond. The mean clump size of these aggregates is directly proportional to the amount of viral antigen present in the sample. Size and number of clumps can be quantitated by any one of a number of particle counting methods. Two which have been successfully employed at AMB involve either a conductiometric or a photometric readout. The former (a commercially available particle sensor called a Celloscope) consists of a cylindrical aperture separating two electrode chambers filled with electrolyte. The particles being measured are suspended in the outer diameter and then drawn through the aperture, through which an electric current also passes. As each particle passes through the orifice, it changes the volume (hence resistance) of the small hemispherical volume at either end of the orifice. An electrical pulse is generated with an amplitude which is proportional to the particle volume. The sequence of particle pulses is then amplified, scaled and counted or otherwise processed electronically to yield particle count/size data.

The degree of agglutination can also be measured using a dual beam photometer developed at AMB. The effect of agglutination on the optical transmission of a bead suspension is shown in Figure 13. Individual beads in a suspension scatter and absorb light with scattering dominating. At wavelengths comparable to the bead radius or a little longer, scattering is at a maximum, and hence, the transmission is at a minimum. With greater agglutination, the beads tend to shield each other more, so that the probability of light attenuation is reduced and the transmission is increased.

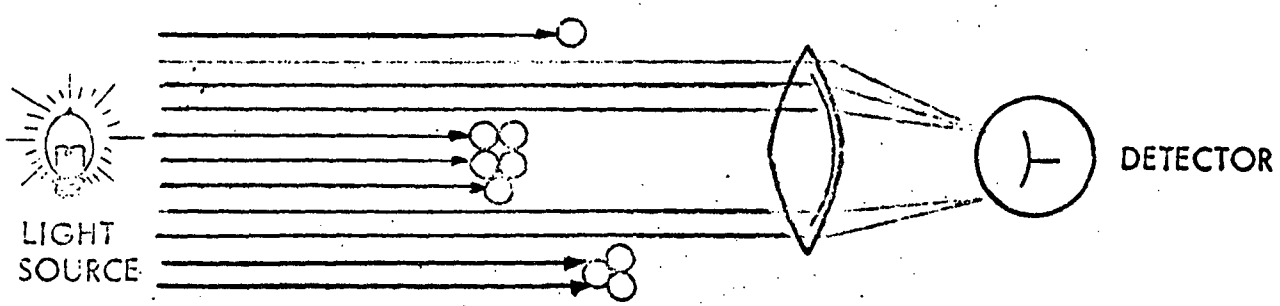
The dual beam photometer developed at AMB provides a "simultaneous" readout of a sample and a control at a particular wavelength (Figure 14). To

FIGURE 13

PHOTOMETRIC EFFECTS OF AGGLUTINATION



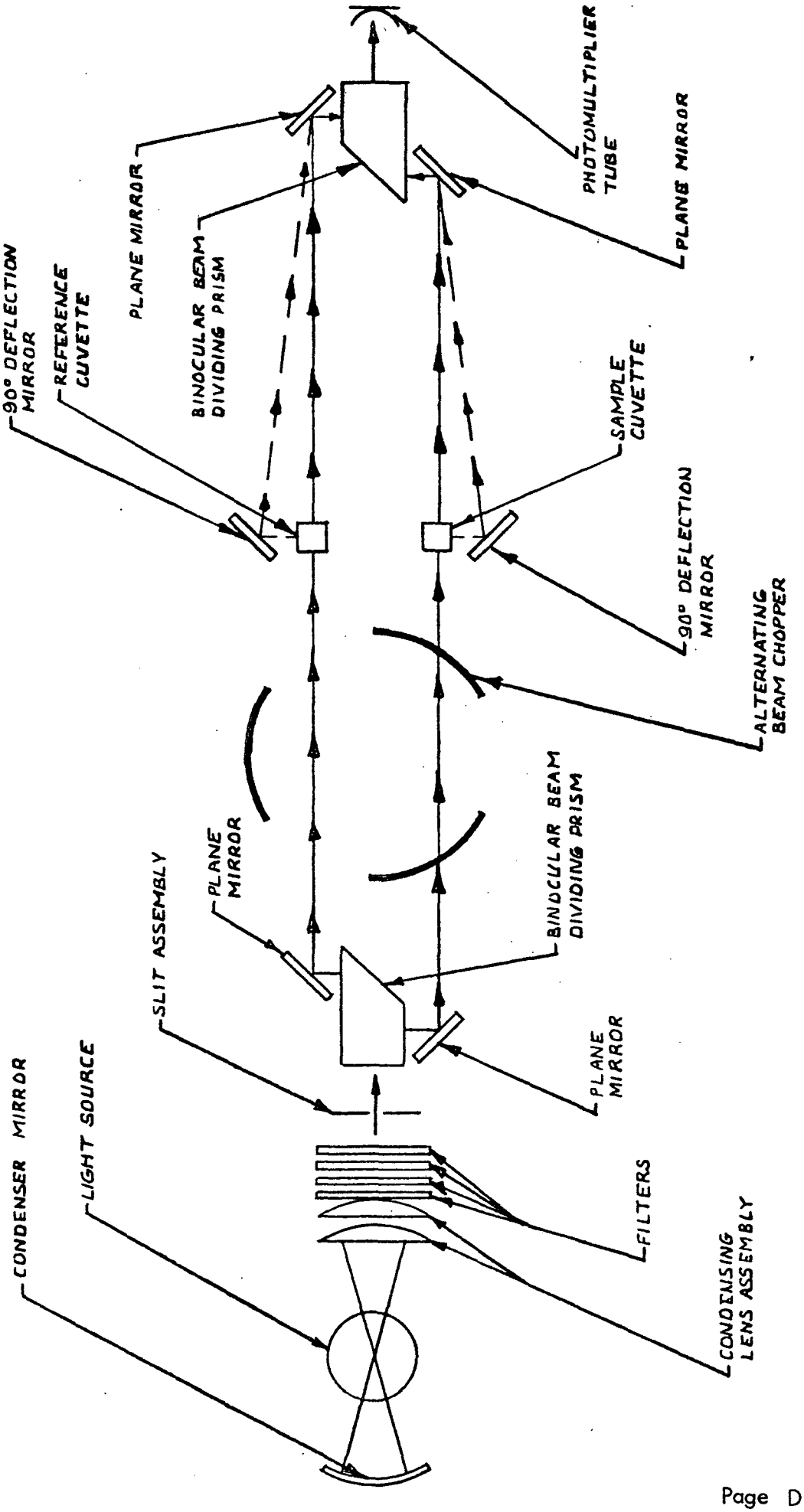
SAMPLE



SAMPLE

FIGURE 14

MEMS PHOTOMETER OPTICAL PATH



compensate for the effect of non-specific clumping which may occur under certain conditions (i.e., large changes in ionic strength of the suspending medium) beads which have been coated with gamma globulin instead of the specific viral antisera are used as a control. Detector signals received alternately from the reference and sample cuvettes by a single PMT are electrically divided and displayed on a digital readout as a ratio of sample signal-to-control signal.

A system which might be used for monitoring virus or toxins in water by the agglutination technique is shown schematically in Figure 15. Sample water is concentrated by pumping through a dual pressure dialysis module (one sample serving as a control). Dual capsules (sample and control) mounted on a single card are dispensed from a casette onto a transport mechanism. These capsules pass to the inject station where each receive about 1 ml of concentrate from the dual dialysis module. These capsules are then transported to the next station where discrete amounts of a sensitized bead suspension (one with specific antisera, the other with a gamma globulin control) are added. These capsules are incubated for one hour and then passed into the dual beam photometer where the change in light intensity between control and sample recorded.

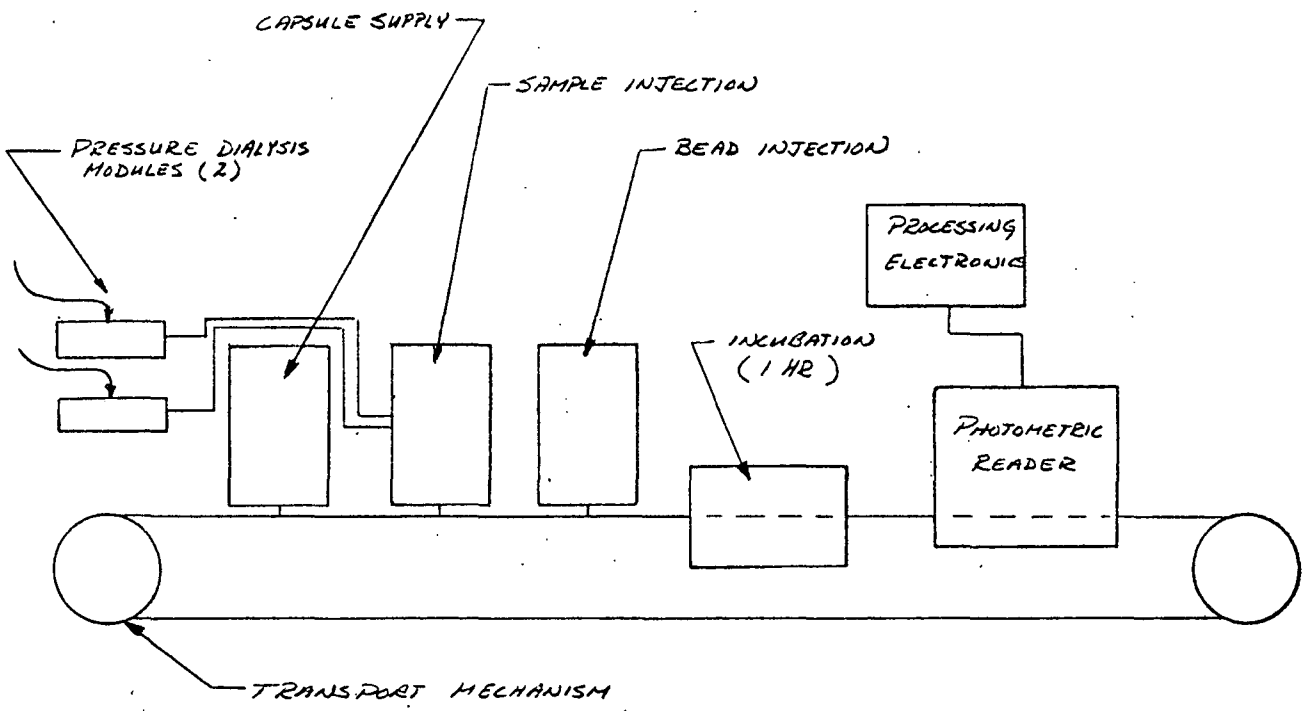


Figure 15

BEAD AGGLUTINATION COUNTING SYSTEM

Section 4

SYSTEM COMPARISON

As indicated in a previous section, it may be desirable to monitor a regenerated water supply for a number of possible contaminants; notably, viable and non-viable cells (and ability to distinguish between them), virus, toxin, fungi and lysed cells. The ability of the various systems to monitor each of these parameters is indicated in Tables 3 and 4 and critically compared in the discussion below.

1. Total Cell Count (Viable + Non-Viable Cells)

The two systems best suited for monitoring total cell count (which includes bacteria and fungi) are Porphyrin Chemiluminescence and the Partichrome.

With regard to sensitivity, the total number of (E. coli) cells required for detection are 4×10^3 to 1.2×10^4* cells for Porphyrin Chemiluminescence and 20 cells for Partichrome. If one considered concentrating the organisms contained in a 400 ml sample, then ~10-30 cells/ml and 0.05 cells/ml respectively, would be detectable by each of the methods. In terms of sensitivity, the Partichrome is at least two orders of magnitude more sensitive than the Chemiluminescence method. This is not surprising in view of the fact that in contrast to Porphyrin Chemiluminescence, the Partichrome monitors single particles. To achieve a detection probability of 95%, 20 bacterial cells would be required by the Partichrome. This assumes a fairly clean background water sample (i.d., a bacterial suspension in distilled filtered water). The nature of the background interference one is likely to encounter in reclaimed water is not known. However, some interference (magnitude not known) is to be expected from the presence of bacterial cell fragments (due to cell lysis) in the regenerated water supply. The protein specific stain used in the process will stain fragments as well as whole bacteria.

To achieve a detection threshold of 20 cells, the bacteria would have to be deposited in a narrow stripe (0.01 cm wide, 1.67 cm long) on a continuously moving porous tape that is scanned by a flying spot (150 μ scan width) provided by a mirror galvanometer (Figure 2). Minor attendant problems associated with this design are:

a. Periodic focussing (at least once in a 24 hour period) of the high magnification optics is required, (due to variations in temperature, tape tension

*Lower number is for a system utilizing a liquid sample transport. Higher number is for a system employing tape sample transport.

TABLE 3

COMPARISON OF SYSTEM FUNCTION CAPABILITIES

	Total (Viable + Non-Viable)	Viable	Toxin	Virus	Lysed Cells
Partichrome	X	X			
Porphyrin Chemiluminescence	X	X			X
ATP Bioluminescence		X			
Colony Counter		X			
Light Scattering Photometer		X			
Bead Agglutination	X		X	X	

Table 4

COMPARISON OF SYSTEM CHARACTERISTICS

	Sensitivity (Cells per Challenge)		Processing Time		Zero-G Operation	Reagent Requirements					Current** Instrument Cost	Current Instrument Weight	Current Instrument Power	Current Instrument Volume
	Total Cells	Viable Cells	Total	Viable		ml/test	Storability at Ambient	Toxicity	Other	Cost Per Test *				
1. Partichrome														
a) Flying Spot	20	N.D.+	~5 min	-	Yes	~ 0.2 ml	~ 1 yr @ ambient	None	-	< 5¢/test	~ $40,000	~60 lbs	200 watts	2.5 cu ft
b) Full-Field	$\sim 10^4$ (10 cells/ml) (400 ml vol)	N.D.+	~1/2 hr	-	Yes	~ 0.2 ml	~ 1 yr @ ambient	None	-	< 5¢/test	~ $30,000	~40 lbs	200 watts	2 cu ft
2. Porphyrin Chemiluminescence														
a) Liquid Transport	4×10^3 cells (10 E.coli/ml) (400 ml vol)	$\sim 2 \times 10^3$ (20 E.coli/ml, 100 ml vol) 2 hr incub.	1 hr	3 hrs	Yes	110 ml (total) 110 ml (viable)	3-6 months	None	-	< 5¢/test	~ $45,000	~80 lbs	1200 watts	16 cu ft
b) Tape (Capsule) Transport	1.2×10^4 cells (30 E.coli/ml) (400 ml vol)	$\sim 3 \times 10^3$ (7-8 E. coli/ml) (400 ml vol) 2 hr incub.	1/2 hr	2-1/2 hrs	Yes	67 ml (total) 67 ml (viable)	3-6 months	None	-	< 5¢/test	~ $30,000 (est)	~60 lbs (est)	800 watts (est)	12 cu ft (est)
3. ATP Bioluminescence	Not Detectable	10^3 cells/0.01 ml ($\sim 10^5$ cells/ml)	-	15 min	Yes	10-20 ml (est)	2 hrs @ ambient 6-12 months @ dry ice	None	Toxic if DMSO used Flammable if Butanol used	~50¢/test (est)	~$50,000 (est)	~50 lbs (est)	1300 watts (est)	10 cu ft (est)
4. Colony Counter (Modified Flying Spot)	-	1 cell/10 ml	-	4-5 hrs (100μ colony)	Yes	10 ml (est)	3-6 months	None	-	< 5¢/test	~ $25,000 (est)	~50 lbs (est)	1100 watts (est	6 cu ft (est)
5. Light Scattering Photometer	-	10^4 cells	-	2 hrs	Yes	20 ml (est)	3-6 months	None	-	< 5¢/test	~ $30,000 (est)	~45 lbs (est)	~ 400 watts (est)	2.5 cu ft (est)
6. Bead Agglutination***														
a) Conductimetric	0.26 HA/ml (myxovirus)	-	-	1 hr	Yes	~ 2 ml	~2 days @ ambient 6-12 months @ 5-7°C	None	-	< 10¢/test	~$20,000	~40 lbs	~ 35 watts	3 cu ft
b) Photometric	1.28 HA/ml (myxovirus)	-	-	1 hr	Yes	~ 2 ml	2 days @ ambient 6-12 months @ 5-7°C	None	-	< 10¢/test	~$30,000	~25 lbs	~ 35 watts	2.5 cu ft

* Reagent cost only; does not include cost of tape or capsule.

** Estimated cost for duplicate instrument.

*** Parameters shown are for the present manually-operated system; does not include sample concentration or automation. To include the latter two features would cost approximately an additional $10,000.

\+ N.D. - Not Determined

etc.); focus variation causes a decrease in the percentage absorption of the bacteria in the signal output.

b. Greater possibility for cross contamination of a tape sample transport.

c. Rather complex and costly instrumentation (about $40,000 per unit).

d. Nucleopore tape (0.625 in wide; $< 0.5\mu$ pore size), a polycarbonate supplied by General Electric is costly [100 ft reel ($700) is sufficient for 24 hours] and exhibits a considerable variability in flow rate. The wide variation in flow rate between different rolls of tape as well as between different locations in a particular roll has required implementation of costly acceptance test procedures on the part of the user.

Some of these problems were eliminated by modifying the deposition area and the scanning optics. Thus the organisms were deposited in a larger spot (1/4" diam.) and full-field viewing (non-flying spot) was employed, thereby simplifying the optics required considerably. Also, rather than moving continuously, the tape was indexed from station-to-station at fixed intervals. However, because of the larger viewing area, the sensitivity dropped to $\sim 10^4$ organisms, making it comparable to the sensitivities achieved with the chemiluminescence and scattering photometer system.

Although less sensitive than the flying spot design, the full-view photometer is simpler, less costly and more easily adaptable to the capsule approach (in which sample sites are isolated from one another by some enclosure).

The sensitivity range indicated earlier for the Porphyrin Chemiluminescence System is for two different types of sample transport systems developed for NASA. The greater sensitivity (i.e., 4×10^3 cells) was achieved with a liquid sample transport system in which bacteria concentrated on a filter are backwashed off and reacted with luminol-H_2O_2 in a liquid stream in front of a PMT. The lower sensitivity of 1.2×10^4 cells was achieved with a system employing a tape sample transport in which the organisms concentrated on a porous tape are

reacted with luminol-H_2O_2 directly on tape in front of a PMT. Sensitivities comparable to that achieved with the liquid sample transport system can probably be achieved by improving the optical coupling between the reaction site (i.e., surface of the tape where light is generated) and the face of the PMT. In the most recent design of the chemiluminescence approach, individual capsules, each containing a sterile filter membrane, are used to process discrete water samples. The effectiveness of this capsule design in minimizing cross contamination between samples has yet to be demonstrated. Although simpler in design, less costly to operate and possessing superior sensitivity, the liquid sample transport chemiluminescence system is probably more susceptible to cross contamination and so less desirable than the capsule approach.

One advantage of Partichrome over Porphyrin Chemiluminescence is that, whereas the former appears to possess equal sensitivity to aerobes and anaerobes alike, the latter exhibits a greatly reduced sensitivity to anaerobes (e.g., $\sim 10^6$ Cl. sporogenes required for detection by chemiluminescence).

In terms of response time, the chemiluminescence and Partichrome methods are comparable ($\sim$ 15 min). Each of these approaches are capable of zero-g operation; however, because of the varying level of complexity of the instrumentation, it appears a greater effort (i.e., cost) would be required in adapting the Partichrome and Capsule Chemiluminescence Systems than the liquid sample transport chemiluminescence system.

The reagents required for either method are generally non-toxic and could be returned to the regeneration system for re-processing.

Some of the other features of these systems are compared in Table 4.

It is to be noted that the Bead Agglutination and Scattering Photometer were not considered for obtaining total bacteria count for the following reasons:

a. Bead Agglutination: Relatively poor sensitivity ($\sim 10^5$ - 10^6 cells) anticipated.

b. Scattering Photometer: While possessing a sensitivity of $\sim$ 10^4 cells, other particulate matter could produce a false positive. The utility of this approach is in detection of viable cells (see below).

2. Viable Cell Monitoring

The systems which can be used for distinguishing viable from non-viable cells are (a) ATP Bioluminescence, (b) Colony Counting, (c) Porphyrin Chemiluminescence, (d) Partichrome and (e) Light Scattering Photometry. The problems associated with each of these methods are presented below:

a. ATP Bioluminescence

As indicated earlier, since ATP is found only in viable cells, this parameter has been used for monitoring viability.

The principle advantage of the ATP approach over the other methods is that it is more rapid ($\sim$ 15 min) and does not require the use of a nutrient. Introduction of the latter into a system is undesirable from the standpoint that it increases the possibility for bacterial buildup.

There are a number of disadvantages of this system and these are summarized below.

1) Extraction of ATP Required

For maximum sensitivity, ATP has to be extracted from the organism prior to reaction with luciferin-luciferase reagent. In the past, four extraction methods have been reported in the literature (boiling water or buffer, sonication, acid extraction, solvent extraction) each with their own disadvantage. Extraction with boiling water or buffer and sonication are probably the simplest to instrument (see Figures 7 and 16) for continuous operation but their effectiveness for completely extracting ATP without inactivating it has yet to be demonstrated. Complete disruption of micrococci within a reasonable time period would be a major problem. The power requirements for a suitable sonicator would also be quite high. Acid extraction ($HClO_4$, H_2SO_4, CCl_3COOH) requires neutralization with subsequent removal of the resulting precipitate prior to reaction with reagent; corrosion problems would also be present with this approach. Solvent extraction (ethanol, butanol, dimethylsulfoxide) aside from presenting flammability and toxicity hazards, interfere with chemiluminescence of luciferin-luciferase and so have to be removed or diluted out. Ethanol has been boiled off, dimethylsulfoxide diluted out (with dilution decreasing sensitivity proportionately) and butanol separated by liquid partitioning with octanol. The last would be particularly difficult to accomplish in a continuous system under zero-gravity unless a specially designed continuous flow centrifuge were utilized.

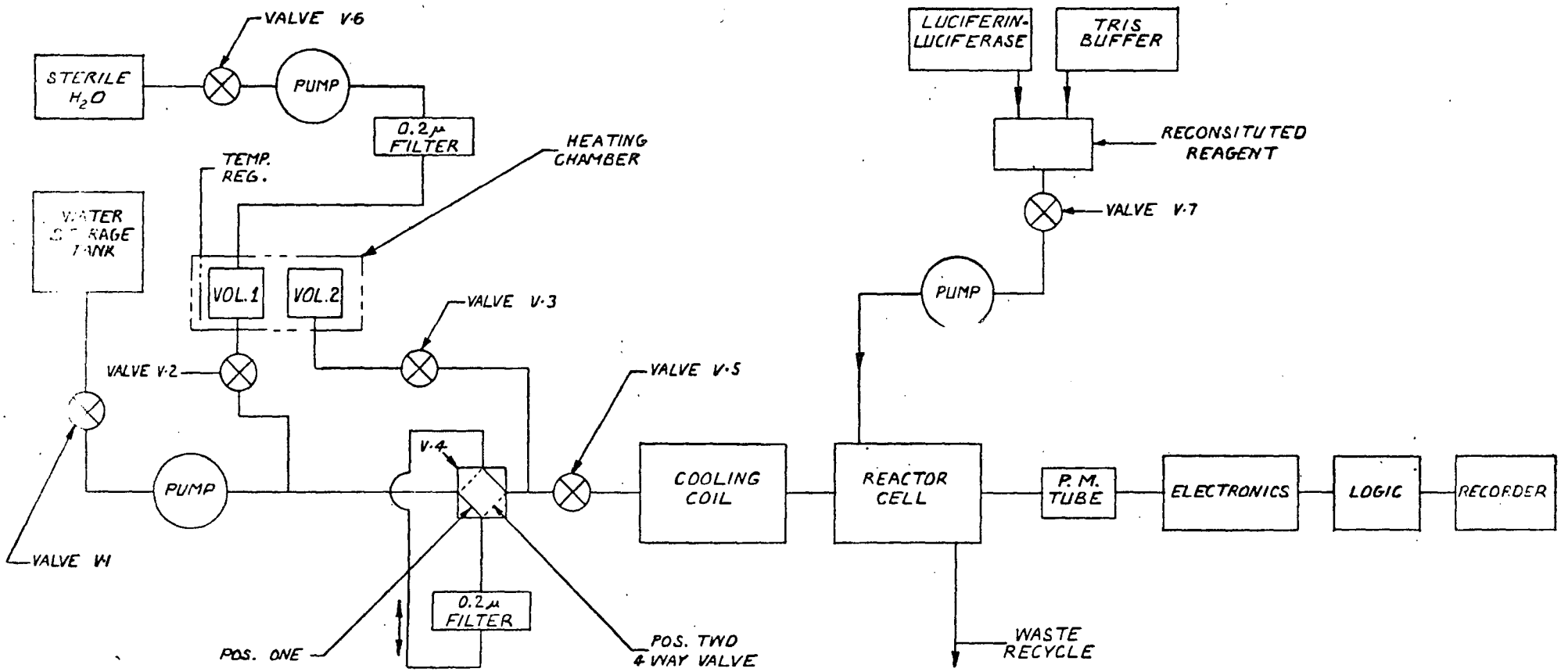


Figure 16

ATP SUB-SYSTEM
HOT WATER EXTRACTION

2) Lucerin-Luciferase Reagent Unstable at Ambient

One other inherent disadvantage of the ATP system is the availability and stability of the luciferin-luciferase reagents. Luciferin requires an elaborate synthesis procedure and has to be stored under nitrogen since it is readily oxidizable; luciferase, extractable from firefly lanterns has to be purified and stored in the frozen state. Even these purified reagents, after extended storage do not produce a clear solution on reconstitution, thereby resulting in less reproducible blanks. Automation of the reagent system itself for zero-gravity operation, presents a very difficult problem at best.

3) Sensitivity a Problem

Sensitivities for the ATP approach have only been reported for static systems, and range from 10^2 - 10^3 cells*. However, in those instances in which either DMSO or butanol are used for extraction of ATP, the 10^2 - 10^3 cells are contained in 0.01 ml volume of extract. Based on the stipulated protocols, the concentration of cells required for detection in the original sample would be 10^5 - 10^6/ml, assuming a one ml sample. While it is obvious that filtration or concentration procedures could be used to improve sensitivity, the extent to which this can be accomplished in a flow system would be critically dependent on the design employed.

4) ATP Content of Water Stressed Cells Apt to be Low

Monitoring of ATP level as a measure of viability assumes that the ATP content per bacterial cell remains constant*, regardless of species or physiological state of the growth curve. Work by Forrest** and Strange et al***, however has shown no direct relation between the ATP content and viability for certain organisms. The actual ATP level could vary as much as 10 fold in certain cases depending on the extent of aeration, salt, and nutrient concentration in the suspending medium. The maximum decrease was observed for washed cells subjected to starvation by suspension in a medium with a limited energy source.

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* G.V. Levin (Hazelton Laboratories), Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, AMRL-TR-67-71, July 1967 (Dupont).

** W.W. Forrest, J. Bact. 90, No. 4 1013 (1965).

*** R.E. Strange, et al, Nature 199, (1963).

It is to be expected that bacteria present in the storage tank of purified, or regenerated water, would be in a nutrient-limiting environment. Extended exposure to this environment should deplete the ATP content to minimal values without necessarily affecting viability. The validity of extrapolating the ATP content of freshly harvested cells to the ATP content of cells maintained in a nutrient-deficient environment is certainly open to question. Detection thresholds on the same organism could vary by as much as a log in these two extremes.

b. Colony Counting

In the two approaches described previously for colony counting, the bacterial sample was concentrated by filtration on a membrane filter contained in individual capsules. The membrane containing the deposited organisms is then wetted with liquid nutrient and incubated for several hours to reach a colony size 10 to 100 μ in diameter depending on the readout procedure employed. Where the readout system is a modified flying spot scanner*, individual colonies approximately 10 μ in diameter could be counted. With a Vidicon readout, colony sizes of the order of 100 μ would probably be required for a reasonable counting accuracy. Since the larger colony size would required longer incubation periods (i.e., 6 hours instead of 3), the modified flying spot scanner would be preferred for counting individual colonies. Whether prestaining of the colony (with a protein specific dye) would be required prior to reading with the modified Partichrome approach would have to be determined. There may be sufficient contrast between the colonies and the background for reflectance counting without staining. Whether a Vidicon or modified flying spot is employed for readout, in both cases, baseline readings would have to be taken of the unincubated controls.

The advantages of a colony counting approach for monitoring viable organisms has several distinct advantages over other

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*Adapted from the Partichrome. Typically a 1 μ flying spot is used in the original Partichrome System; in the modification being suggested, a 15 μ scanning spot is to be utilized and reflected rather than transmitted light is monitored.

approaches previously discussed (i.e., porphyrin chemiluminescence, light scattering, photometer, etc) are:

1) The excellent sensitivity ($\sim$ 0.1 cell/ml) with the smallest sample size (10 ml).

2) Method responds to viable aerobes and anaerobes alike.

3) System can be made selective for certain groups of organisms (e.g., viable coliforms) by appropriate choice of a selective nutrient. General purpose nutrients are available for growing aerobes and anaerobes alike. Although an end-to-end processing protocol for this approach has yet to be established, no insurmountable problems are anticipated in adapting it to zero-g operation. Instrumentation costs and complexity are expected to be comparable to those required for the other viable systems cited (i.e., porphyrin chemiluminescence, Partichrome or light scattering photometry).

Reagents that would be required are generally non-toxic and could be returned to the regeneration system for processing.

c. Porphyrin Chemiluminescence

The number of viable cells (E. coli) required for detection using a manually-operated tape transport system has been shown to be $\sim$ 7-8 cells/ml in a 400 ml sample*. The total sample processing time was of the order of 2-1/2 hours. Much lower sensitivites (10^3 - 10^4 cells/ml of Cl. sporogenes - 400 ml) were, however, observed for Cl. sporogenes, an anaerobe. The sample processing protocol and reagent handling problems for this approach are somewhat more involved than that required for the Colony Counting Approach.

d. Partichrome

As indicated earlier, a 1μ flying spot is normally employed in the Partichrome scanner for counting individual bacteria deposited on a porous tape. When used in this manner, maximum signal focussing becomes central and the distance between porour tape and scanning objective is generally maintained at about 0.020".

In order to permit the counting of colonies in a filter-capsule about 1/4" in depth, it would be necessary to modify the optical scanner

*AMB Contract to NASA, NAS 9-11644.

so that the flying spot is increased in size from 1μ to about 15μ in diameter. This would enable one to increase the working distance between tape and scanning objective to permit accommodation of a filter-capsule. Individual colonies 10μ in diameter could then be readily counted by the 15μ flying spot scanner.

e. Light Scattering Photometer

As indicated earlier, this can be used for detection of viability by comparing the light scattering pattern of an incubated and unincubated control (as a function of scattering angle). This device should be able to detect differences between 1×10^4 cells/ml and 2×10^4 cells/ml, thus it is anticipated that relatively short incubation intervals (i.e., $\sim$ 2 hours or less) would be required to produce a perceptible change in the scattering pattern. The type of sample processing that would be required would involve concentrating the bacterial sample to a 10^4 cells/ml initial level, the minimum detection threshold. This might be accomplished by filtering on a membrane filter and then backwashing off the filter with nutrient (Figure 12). The major drawback of this system is that it employs a liquid sample transport which increases the problem of cross-contamination between samples.

The system is fairly simple and operable under zero-g. The scattering photometer developed at AMB could be modified to automatically scan over a wide angle and generate the type of data required for this approach.

The only reagent required is liquid nutrient; being non-toxic, it can be returned to the regeneration system for re-cycling.

f. Summary

Based on the above considerations, the method of choice for viable cell monitoring would be colony counting which utilized a modified flying spot readout.

3. Detection of Virus and Toxin

The only method which can be used for monitoring virus and toxin alike is the bead agglutination method. Since this method relies on the

antigen-antibody reaction, it can be made highly specific. While this is a distinct advantage, it may be desirable to develop group antisera which would respond to more than one type of virus (i.e., group antisera for Herpes or myxovirus) rather than develop antisera for specific types which could be quite costly. Since only a limited number of bacterial toxins are of primary concern (i.e., those from Cl. botulism, Cl. welchii, Micrococcus pyrogenes, Shigella dysenteriae and Corynebacterium diphtheriae), specific antisera could be prepared for these agents.

One of the unresolved questions of monitoring viruses in regenerated water is the level of concentration that would be required for detection. Although the number of pathogenic viruses (infectious hepatitis, polio, etc.) in normal domestic sewage has been estimated* at about 10^2/ml, the number that might be present in urine or other liquid wastes is not known. In all probability, it is several orders of magnitude less. Since approximately $10^2 - 10^3$ infectious units (IFU) of myxovirus are required for detection by the bead agglutination technique, a concentration factor of $10^2 - 10^3$ would be required if the reclaimed water contained a level of one IFU/ml. Since the minimum practical working volume would be at least one ml, then a water sample of 100-1000 ml would be required for initial concentration. Although this sample size is large, the effluent from the dialysis or concentration step (see Figure 15) would be very pure (sterile) and could be returned to the potable water supply without reprocessing. Sample concentration (by dialysis) of about the same order of magnitude would be required for toxin determinations as well; here again, the water dialysate could be used without reprocessing.

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*T.R. Camp, Water and Its Impurities, Rhembold Publishing Corp., 1963, p. 88.

With regard as to which readout should be used (i.e., photometric or conductimetric), based on the results of a systems analysis summarized in Tables 5 and 6, the photometric method is preferred.

Of all the sensors, that involving bead agglutination for detection of virus and toxin is the most involved and would require the greatest effort and cost. A good share of these costs would be required for developing appropriate antisera to the various agents. Before this approach is considered for monitoring regenerated water, it would be expeditious to first define the level at which agents of interest appear in the urine so that some assessment of the concentration and sample requirements can be made. This would establish the practicability of monitoring these agents in water on a routine basis.

4. Detection of Lysed Organisms

The only instrument suitable for monitoring lysed organisms is the Porphyrin Chemiluminescence method. As indicated earlier, lysed organisms may originate from bacterial growth upstream in the regeneration cycle with subsequent lysing when they reach the pasteurization tanks at the end of the reclamation process. Readout of lysed organisms (in addition to total cells) can be carried out with the Porphyrin Chemiluminescence device shown in Figure 6. No additional reagents are required apart from that required to read total (viable + non-viable cells). In the procedure described earlier, a small aliquot ($\sim$1 ml) of the original unconcentrated water sample is reacted with the luminol-H_2O_2 reagent in an empty capsule in front of the PMT.

SUMMARY AND CONCLUSIONS

1. Apart from the chemical and physical sensors to be described later (Section 5), the biological parameters which should be monitored to establish potability of regenerated water supplies are the following:

a. Total Cell Population (viable + non-viable cells; bacteria and fungi)

Until such time as the presence of anaerobic organisms in regenerated water can be confirmed, the preferred monitoring system is Porphyrin (Capsule) Chemiluminescence. If the presence of anaerobic organisms can be demonstrated, and background problems from cell fragments is not a problem, the preferred route for total cell count would be the Flying Spot Partichrome.

TABLE 5

COMPARISON OF SYSTEM PARAMETERS

(BEAD AGGLUTINATION)

	PHOTO	COND
• REAGENT REQUIREMENTS	3.4 UNITS	1 UNIT
• PARTICLE SIZE AND DISTRIBUTION	BULK MEASUREMENT	INDIVIDUAL PARTICLE COUNT
• SAMPLING RATE	1-1 1/2 MIN/TEST	1-1 1/2 MIN/TEST
• REPRODUCIBILITY	1% (3 REPEAT RUNS)	5 - 10% (3 REPEAT RUNS)
• COMPLEXITY AND DURABILITY	OPTICS ALIGN. SIMPLE ELECTRONICS	MICRON ORIFICE MORE COMPLEX ELECTRONICS
• WEIGHT, SIZE, POWER (PROJECTED)	20 LBS .26 FT^3 35 W	15 LBS .4 FT^3 35 W
• ADAPTABILITY TO ZERO G	CUVETTE	FLOWING LIQUID
• SIGNAL PROCESSING	MINIMAL	MORE COMPLEX

TABLE 6

SYSTEMS TRADEOFF

(BEAD AGGLUTINATION)

	PHOTO	COND
• SENSITIVITY	0	0
• DOSE RESPONSE RELATIONSHIPS	0	0
• DYNAMIC RANGE	0	0
• REAGENT REQUIREMENTS	0	1
• PARTICLE SIZE AND DISTRIBUTION	0	1
• SAMPLING RATE	0	0
• REPRODUCIBILITY	1	0
• COMPLEXITY AND DURABILITY	1	0
• WEIGHT, SIZE AND POWER	0	0
• ADAPTABILITY TO ZERO GRAVITY	1	0
• SIGNAL PROCESSING	1	0
	4	2

b. Viable Cell Count

The monitoring system preferred for monitoring this parameter, is the Colony Counter with a modified flying spot for counting individual colonies in an incubated sample.

c. Virus and Toxin

The preferred system for monitoring these parameters, is the bead agglutination method with a photometric readout. In order to evaluate the practicality of monitoring these parameters on a regular basis, it is recommended that initial efforts be directed toward determining the level at which these agents appear in urine or waste water to determine the concentration and sample size that would be required.

d. Lysed Organisms

The Porphyrin (Capsule) Chemiluminescence system is recommended for monitoring this parameter in regenerated water supplies.

Section 5

CHEMICAL AND PHYSICAL SENSORS

Chemical and physical parameters which should be monitored as an indication of potability of the regenerated water supply are described below*. Suggested instrumentation suitable for monitoring these parameters have also been included.

1. Total Organic Carbon (TOC)

The TOC that is used to monitor a large number of organic compounds whether derived from human functions, cabin construction materials, reclamation system components, or materials associated with humans such as food, clothing and personal grooming accessories. Recommended limits are 100 ppm of organic carbon.

An instrument used at AMB for rapid determination of total and inorganic carbon (organic carbon obtained by differeine) is Beckman's Model 915 Total Organic Analyzer. This instrument requires only microliter sample volumes and response is within 4 minutes. Major drawbacks are the excessive weight, power and volume requirements (195 lbs, 1700 watts, and 13 cubic feet for three components).

2. Ammonia

Should be monitored as an indication of a possible serious breakthrough of biological pollution and as a source of unpleasant flavor. Recommended upper limit is < 10 ppm at pH < 7. Corning's Monovalent Cation Electrode (#476220) (with a Beckman Lazaran Reference Electrode and Orion Model 407 Filter) has been found suitable for monitoring this parameter. The suggested combination can also be used at pressure up to 5 psi and in flowing systems.

3. Hexavalent Chromium

Should be monitored because of its toxicity and the possibility of its carryover from the pretreatment chemicals used in the reclamation procedure. Maximum limit recommended in drinking water is 0.05 ppm. The wet chemical permanganate-azide method** is recommended for analysis.

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*As a point of reference, AMB has been guided by the recommendations of either the World Health Organization or the Space Science Board. (Report of the Ad Hoc Panel on Water Quality Standards for Long-Duration Manned Space Missions) Nat. Acad. of Sci, Nat. Res. Council, Sept. 1967).

**Standard Method for Examination of Water and Waste Water, American Public Health Assn., N.Y. 12th Edition 1965.

4. Nitrate and Nitrite

These should be determined because of their relationship to the processes of chemical and biochemical degradation of organic wastes. Upper toxicity limit is 10 ppm (Nitrate/Nitrite). The wet chemical brucine method* is recommended for analysis.

5. Silver Ions

Silver ions in the reclaimed water should be monitored because of the use of a silver ion generator in the post-treatment section as a microbial control. The maximum silver ion concentration allowed for potable water is given as 0.5 ppm. Either a selective-ion electrode (#39610 Beckman) or a method** based on fluorescence quenching of an eosin-phenanthroline complex can be used for monitoring silver ions.

6. Total Solids

Total solids as such are not measured, but specific conductivity is used as an indication of total ionic species and of water quality. Maximum permissible specific conductivity is 1000 μ mho/cm. Beckman's flow through conductivity cell (CEL-UD-J-2) with SOLU bridge conductivity indicator, Type RI 5 with an automatic temperature compensator are being used at AMB for making this type of measurement.

7. pH

Measuring this parameter is important since it can be used as an indication of acid carryover from the chemical pretreatment or the presence of excessive amounts of ammonia. The pH extremes recommended by the International Standards for Drinking Water (WHO) for potable water is 6.5 - 9.2. Orion's glass pH electrode (#91-01-00) with a Beckman Lazaran reference electrode have been used at AMB for making measurements in flowing streams under moderate pressure (2.5 psi).

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*Standard Methods for Examination of Water and Waste Water, American Public Health Assn., N.Y. 12th Edition 1965.

**M.T. El-Ghamry et al, Anal. Chemica Acta 47, 41 (1969).

8. Turbidity and Color

Turbidity and color are important, not only from an esthetic point of view but also as an indication of general quality deterioration, as are taste, odor and foaming. Turbidity can be measured by the use of either a visual comparator (with turbidity standards) or by a photoelectric cell arrangement. Recommended maximum - 10 Jackson units (for turbidity). Taste, odor and foaming can be evaluated subjectively.

Section 6

PRELIMINARY SKETCHES OF FLIGHT-RATED PROTOTYPES

Section 6

PRELIMINARY SKETCHES OF FLIGHT-RATED PROTOTYPES

6.1 INTRODUCTION

Based on the results of the comparative analysis presented in Section 4 of potential systems which could be used to monitor possible biological contaminants, the following systems were selected for further consideration:

a. Porphyrin (Capsule) Chemiluminescence - for monitoring total (viable + non-viable) and lysed cells.

b. Colony Counter (with modified flying spot readout) - for viable cell count.

c. Bead Agglutination - for monitoring virus and toxins.

Presented below are preliminary sketches and outline drawings of possible flight prototype configurations. In conformance with the requirements of par. 3.2.6 Exhibit A, Statement of Work for "Tape Transport Bacteria Detection System", dated November 1970, the preliminary design for the Porphyrin (Capsule) Chemiluminescence System, which is discussed first, is presented in somewhat greater detail than either of the other two systems, and consists of mechanical and electrical schematics, operating protocols, projected weight, volume and power requirements, etc. Preliminary sketches and outline drawings for advanced prototypes of the Colony Counter and Bead Agglutination Systems follow the presentation of the Porphyrin (Capsule) Chemiluminescence System.

6.2 PORPHYRIN (CAPSULE) CHEMILUMINESCENCE SYSTEM

A modified tape sample transport concept forms the basis of the proposed design. Tape is preferred to liquid sample transport since the station at which the sample is concentrated initially can be isolated more readily from the rest of the system. This feature makes it easier to maintain sterility at this station, an important consideration since the water sample is returned to the storage tank without re-purifying after passing through the sterile filter at this first station.

Instead of a moving tape, individual sealed capsules, each containing a sterile membrane filter, and dispensed automatically from a cassette, are used for processing discrete water samples. Sample and processing fluids are introduced into these capsules by hypodermic needles which pierce rubber septums contained in these

capsules. Liquids are pressure filtered (calculated pressure drop calculated to be only $\sim$ 0.5 psi/inch of needle).

Individual capsules are moved automatically from station to station on a retainer clip fastened to a belt. At the end of the processing cycle, the individual capsules are ejected from the retainer clips. The system is adaptable to zero-g operation.

The processing sequence is the same as that developed under the present contract and involves sample concentration, DBT nutrient wash, incubation, 4M Urea wash and reaction with luminol-H_2O_2 reagent at the last station. An additional feature incorporated into the present design is the ability to detect soluble porphyrins. This is accomplished by merely reacting a portion of the unfiltered water sample directly with luminol-H_2O_2 reagent. Figure 17 is a block diagram showing how this system interfaces with the regeneration water supply.

A conceptual design and potential characteristics for a flight hardware module are shown in Figure 18, and Table 7.

Reagent requirements (together with a discussion of expected shelf life) for this system are described in Appendix A.

Mechanical layout drawings, electrical and fluid flow schematics together with a description of the sample processing sequence and an engineering failure mode analysis of the proposed design are presented below in the following order:

Figure 19	Sample Processing Stations (Dwg. No. 1000392)
Figure 20	Transport Mechanism (Dwg. No. 1000393)
Figure 21	Loading Station (Dwg. No. 1000394)
Figure 22	Incubator (Dwg. No. 1000395)
Figure 23	Filter Cartridge (Dwg. No. 1000396)
Figure 24	Signal Processing Electronics (Dwg. No. 1000397)
Figure 25	Control Electronics (Dwg. No. 1000398)
Table 8	Sample Processing Description
Figure 26	Bacterial Sensor - Fluid Flow Schematic
Figure 27	Timing Sequence for Total and Viable Cell Counts
Figure 28	Timing Sequence for Soluble Signal

6.2.1 FAILURE MODE ANALYSIS

The only failure that could occur at the load station is that a cartridge is not loaded or is improperly loaded onto the transport mechanism. Position sensors

Figure 17

WATER MONITOR BLOCK DIAGRAM

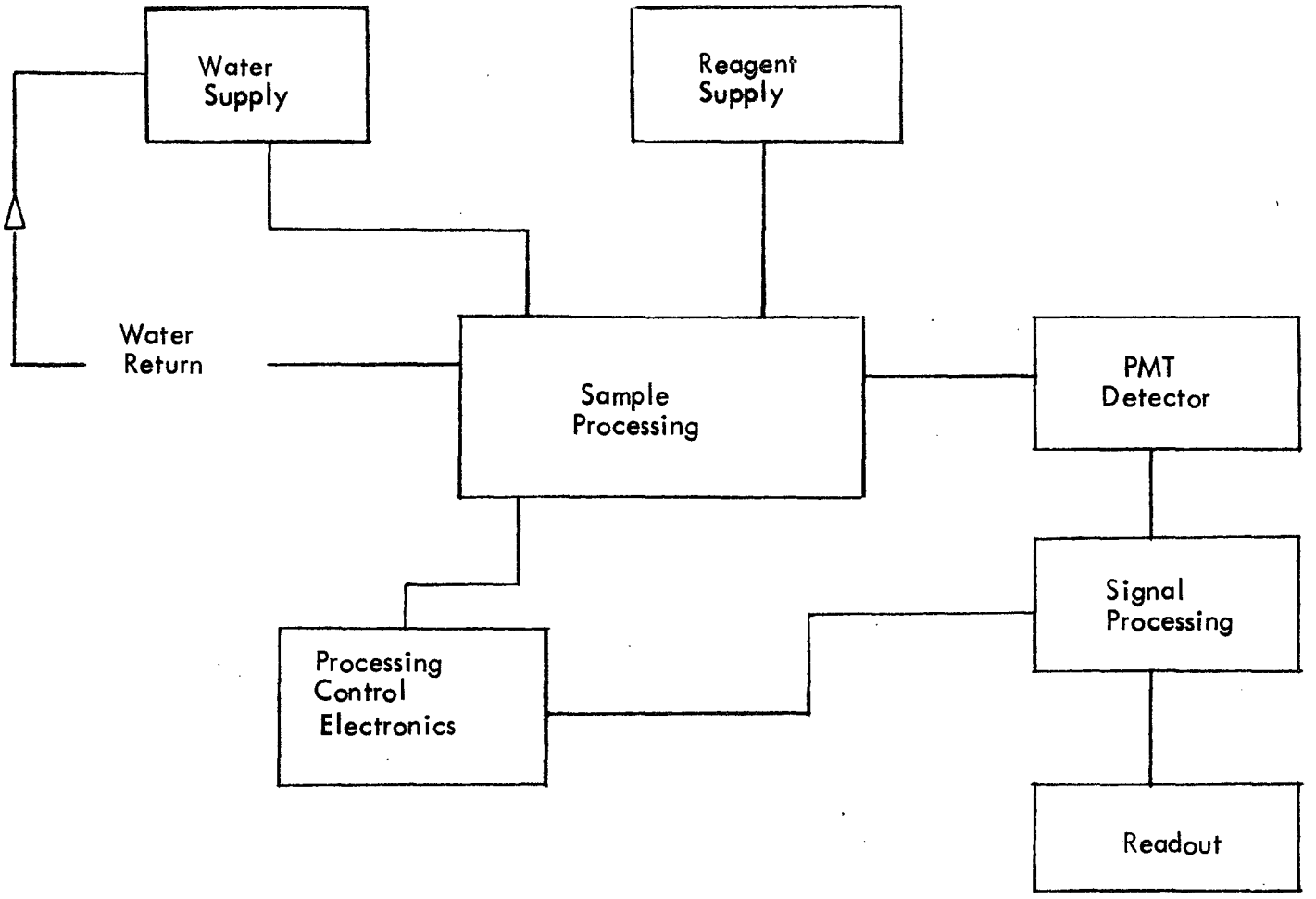


Figure 18
WATER MONITOR
FLIGHT HARDWARE CONCEPT

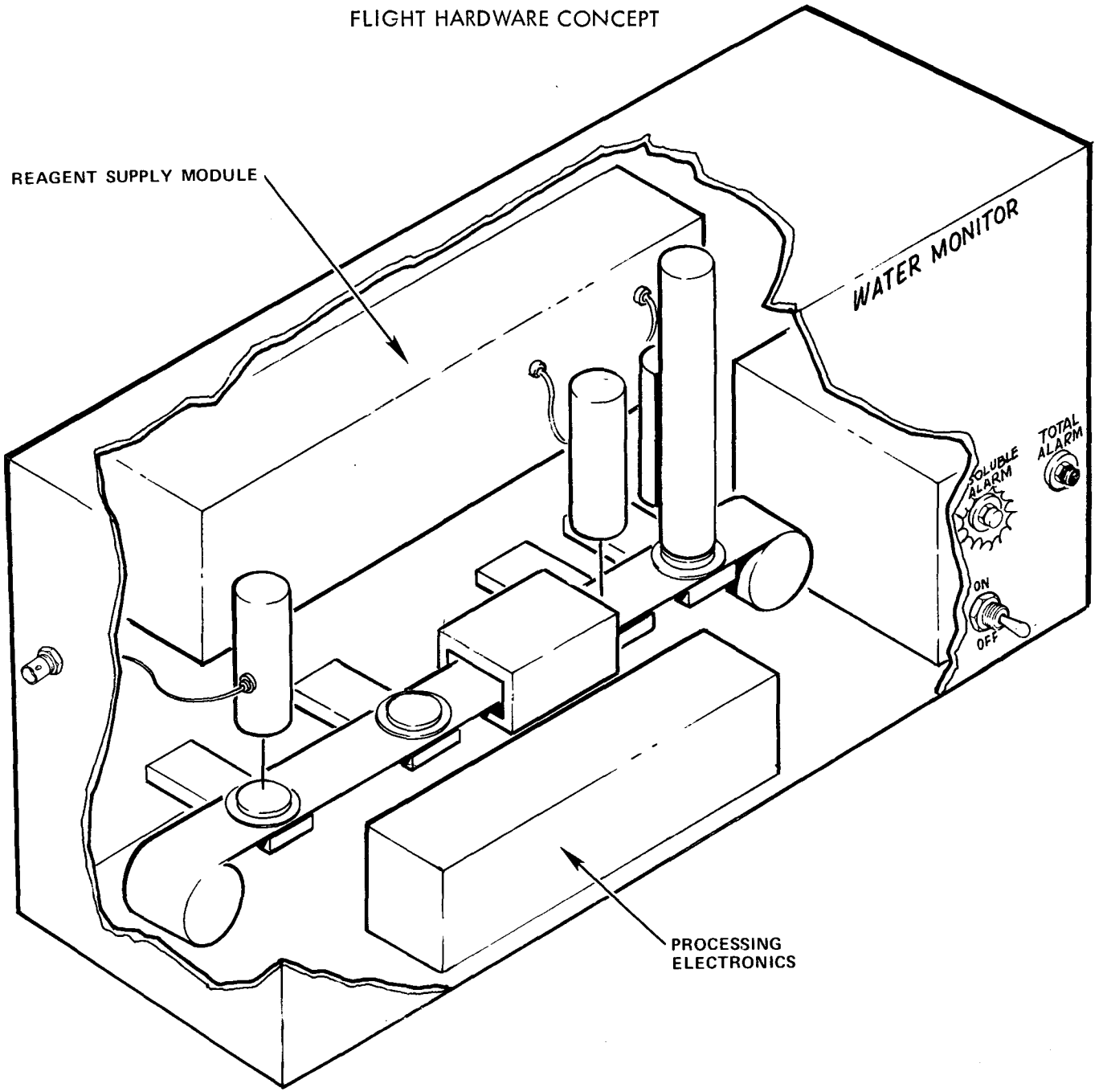


Table 7

Water Monitor Potential Characteristics

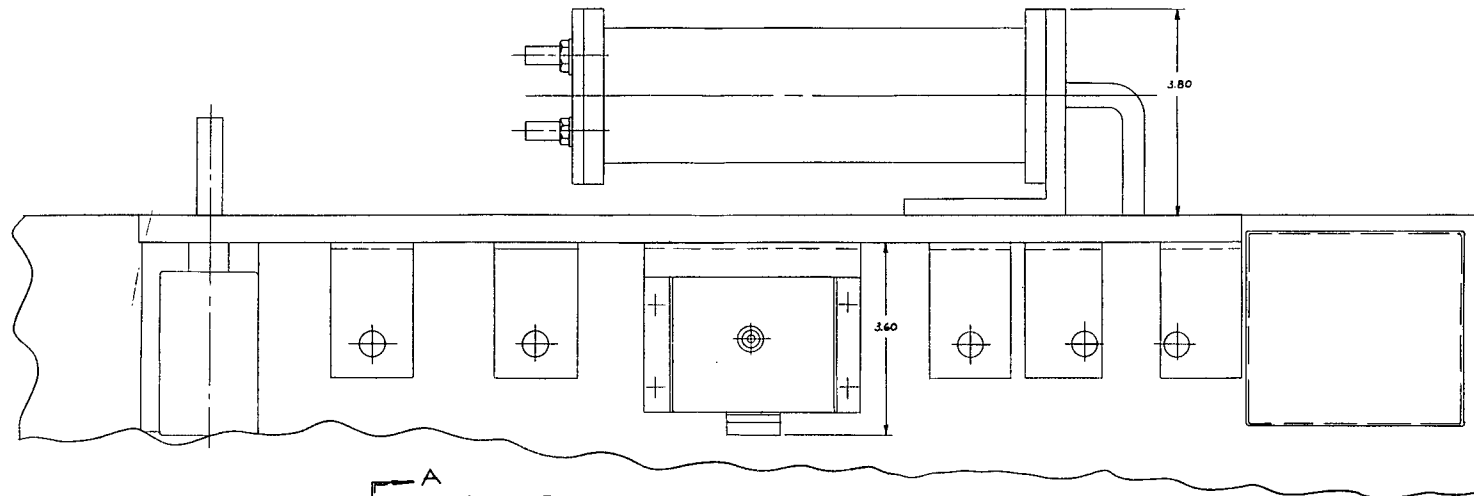
Weight	60 pounds
Space Required	34 x 11 x 12 (~3 cu ft) without reagents
Power Requirements	20 watts
Reagent Requirements	5 ml/test
Testing Rate	2 every hr (Total) 1 every 2-1/2 hr (Viable)
Sensitivity	< 10 cells/ml (Total) < 5 cells/ml (Viable)

FOLDOUT FRAME 1

FOLDOUT FRAME 2

REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

NOTES:



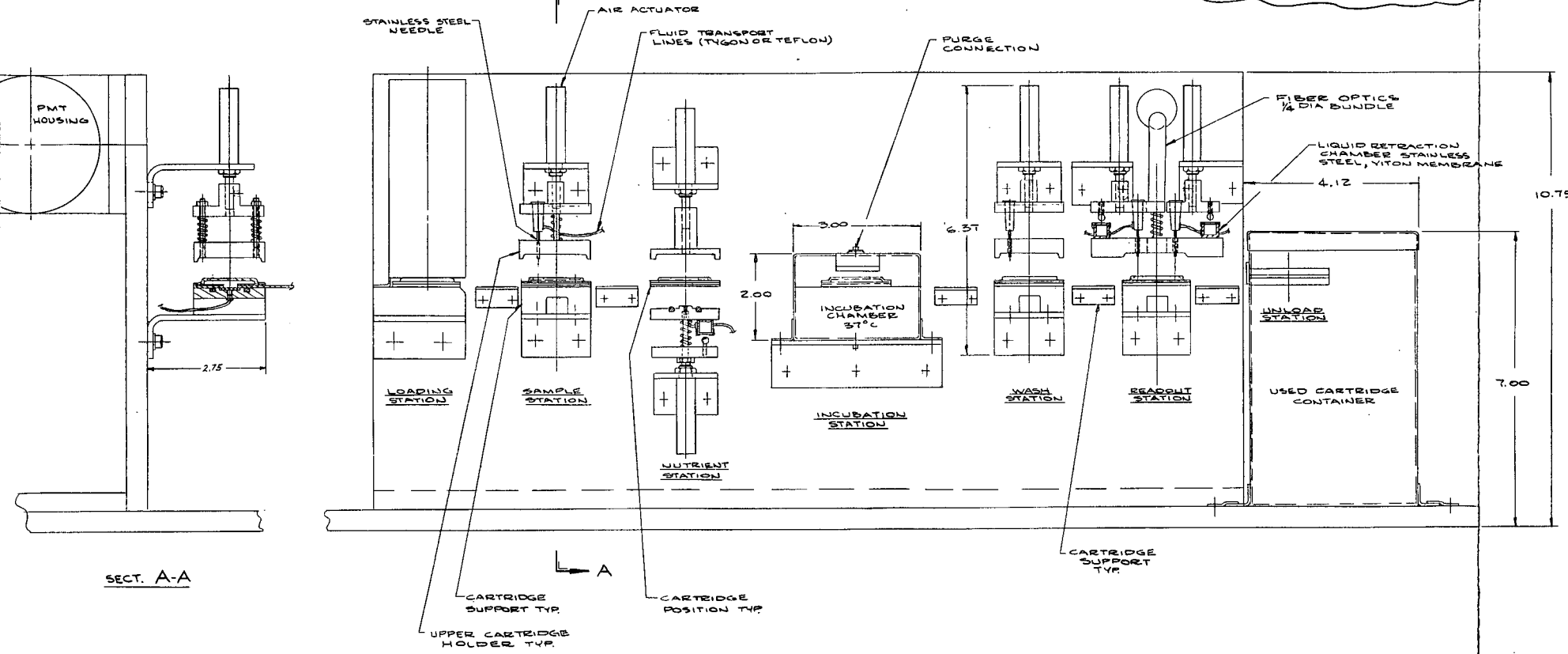


Figure 19

ITEM NO.	QTY REQD	SYM	CODE IDENT NO.	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION	MATERIAL / SPECIFICATION

PARTS LIST

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS
.XX ± .03
.XXX ± .010
DO NOT SCALE DRAWING
ANGULAR ± 2°

TREATMENT
FINISH

CONTRACT NO.
DRAWN
CHECK
DESIGN Wilmeth 10-14-71
STRESS STRUCTURES
MATERIALS
PRODUCTION
DESIGN ACTIVITY APPD
CUSTOMER

AEROJET MEDICAL AND BIOLOGICAL SYSTEMS EL MONTE, CALIFORNIA

TITLE
PRELIMINARY DESIGN LAYOUT
SAMPLE PROCESSING STATIONS
CHEMILUMINESCENCE SENSOR

DWG SIZE	CODE IDENT NO.	DWG NO.
E	34464	1000392

SCALE 1/1 | REL DATE | SHEET

PART NEXT ASSY	NEXT ASSY	USED ON
MATERIAL SPEC	APPLICATION	
NEXT ASSY		

DRAWING LEVEL 3

SIMILAR TO | ACT. WT. | CALC WT

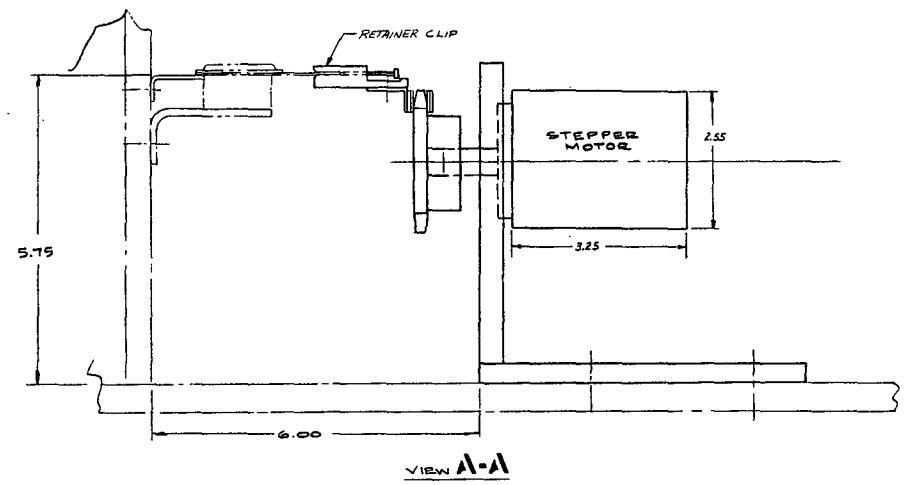
2465001 1000392 COPY

FOLDOUT FRAME 1

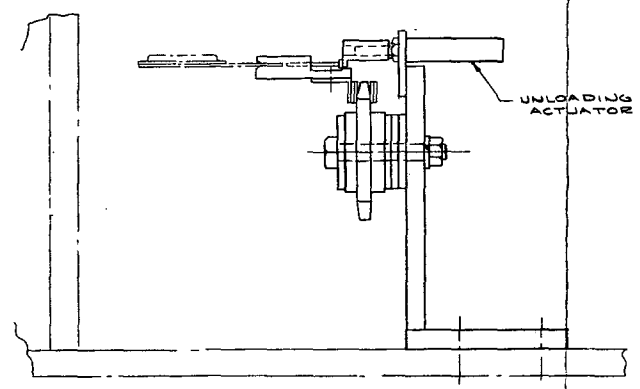
FOLDOUT FRAME 2

REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

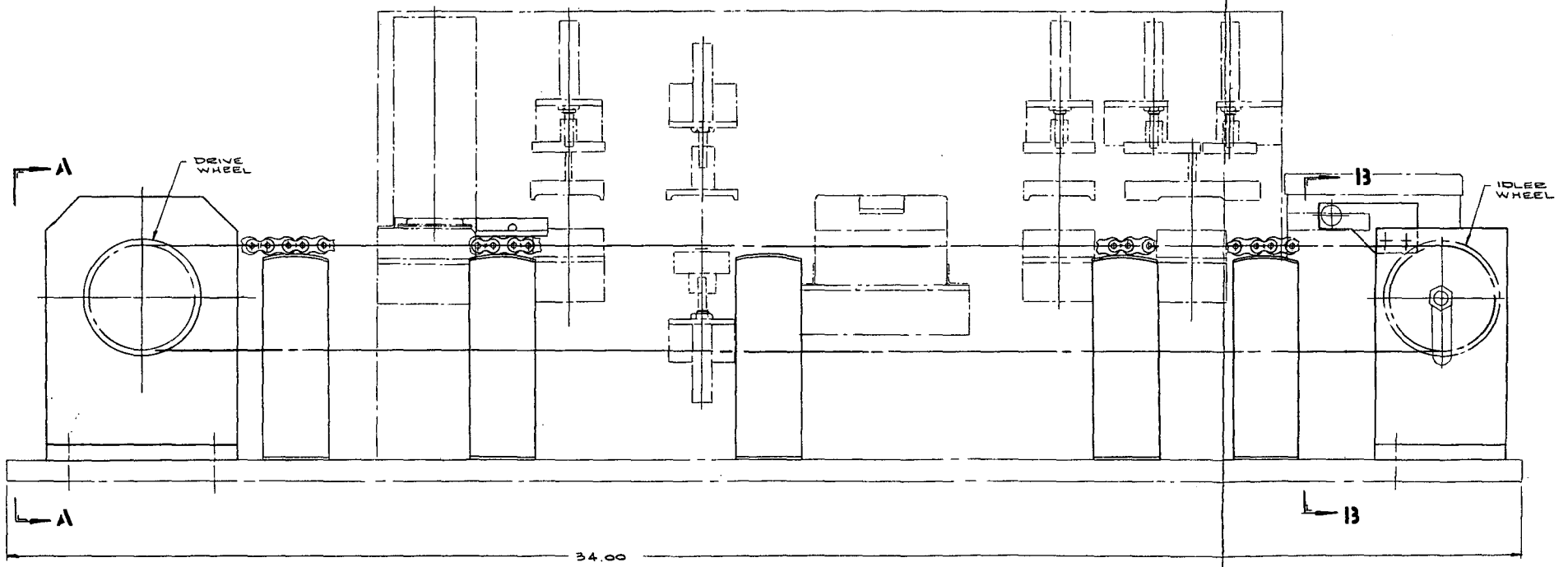
NOTES:



VIEW A-A



VIEW B-B



QTY REQD	SYM	CODE IDENT NO.	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION	MATERIAL / SPECIFICATION	ITEM NO.
PARTS LIST						

UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS
.XX ± .03
.XXX ± .010
DO NOT SCALE DRAWING

TREATMENT

FINISH

NEXT ASSY | USED ON

APPLICATION

SIMILAR TO | ACT. WT. | CALC WT

CUSTOMER

DRAWING LEVEL 3

CONTRACT NO.

DRAWN | DATE 10-4-71

CHECKED

ENGINEERING APPROVAL

MANUFACTURING APPROVAL

PRODUCTION

DESIGN ACTIVITY APPD

ACRANET BIOMEDICAL AND BIOLOGICAL SYSTEMS EL MONTE, CALIFORNIA

TITLE
PRELIMINARY DESIGN LAYOUT
TRANSPORT MECHANISM
CHEMILUMINESCENCE SENSOR

DWG SIZE	CODE IDENT NO.	DWG NO.
E	34464	1000393

SCALE 1/1 | REL DATE | SHEET 1 OF 1

1000393 001

1000393 002

COPY

Figure 20

FOLDOUT FRAME 1

FOLDOUT FRAME 2

REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

NOTES:

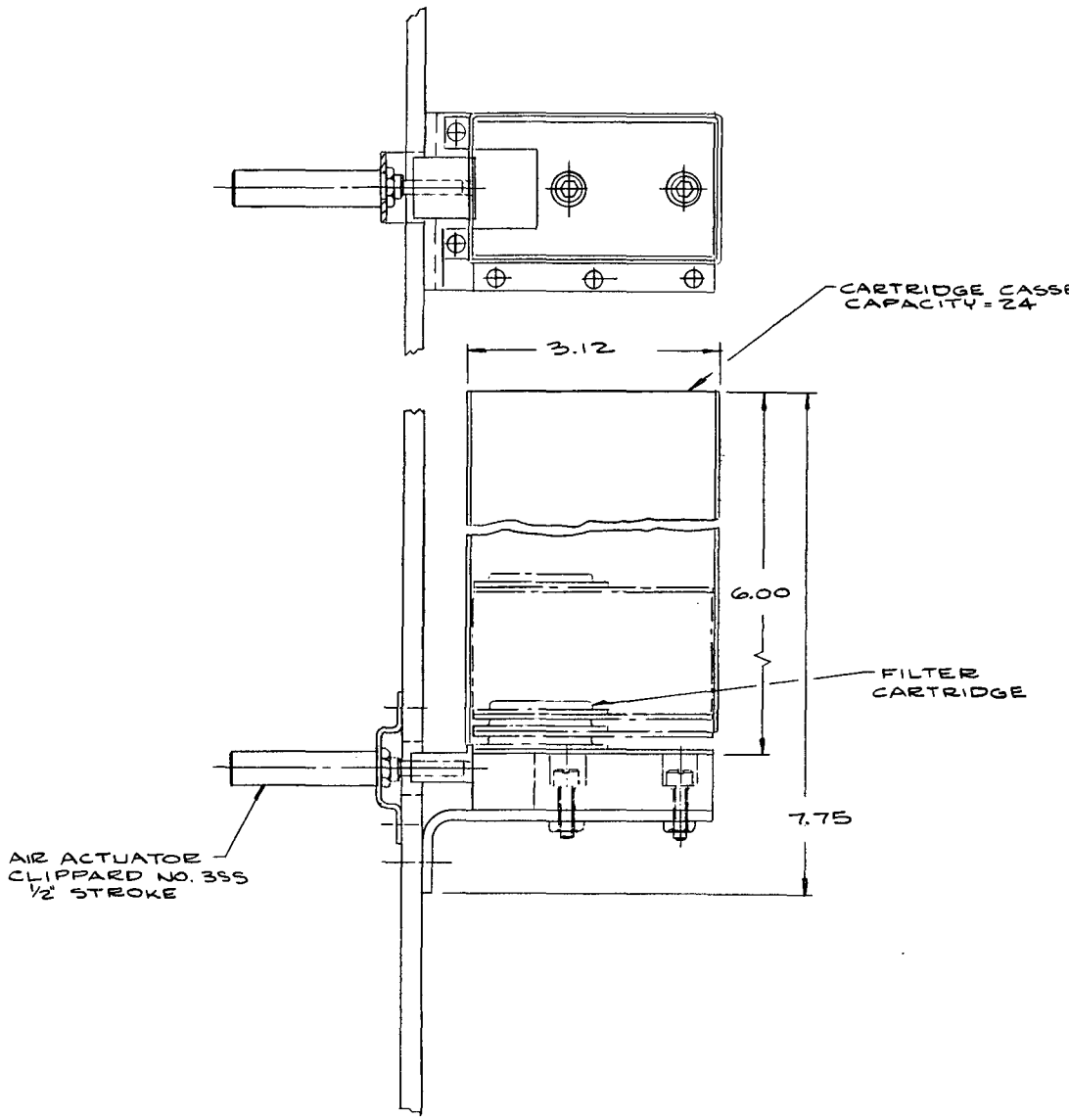


Figure 21

QTY REQD	SYM	CODE IDENT NO.	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION	MATERIAL / SPECIFICATION	ITEM NO.

PARTS LIST

PART DASH NO.	NEXT ASSY QTY REQD PER ASSY	FINAL	NEXT ASSY APPLICATION	USED ON

DRAWING LEVEL 3

UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS ANGULAR
.XX ± .03 ± 2°
.XXX ± .010
DO NOT SCALE DRAWING

TREATMENT

FINISH

SIMILAR TO | ACT. WT | CALC WT

CONTRACT NO.		
DRAWN		DATE 9-28-71
CHECK		
DESIGN	J. Winterhalter	10-14-71
STRESS/ STRUCTURE		
MATERIALS		
PRODUCTION		
DESIGN ACTIVITY APPD		
CUSTOMER		

AEROJET MEDICAL AND BIOLOGICAL SYSTEMS
EL MONTE, CALIFORNIA

TITLE PRELIMINARY DESIGN
LAYOUT, LOADING STATION
CHEMILUMINESCENCE SENSOR

DWG SIZE	CODE IDENT NO.	DWG NO.
D	34464	1000394

SCALE 1/1 | REL DATE | SHEET 1 OF 1

1000394

COPY

70-009 (7-70) 7/70 DIETERICH-POST CLEARPRINT 1020

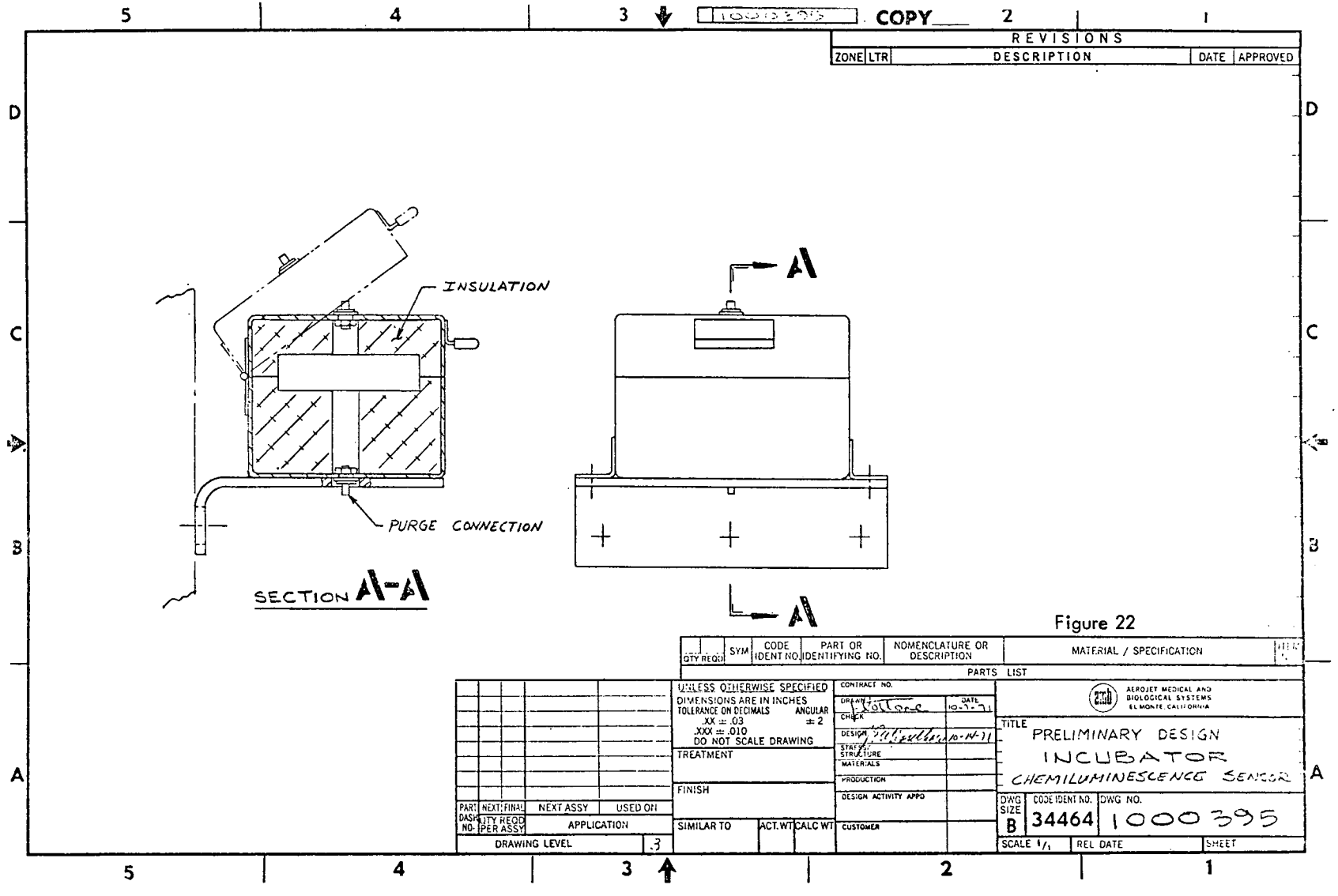

INSULATION
PURGE CONNECTION
SECTION A-A
A
A
COPY
REVISIONS
ZONE LTR
DESCRIPTION
DATE
APPROVED
PARTS LIST
QTY REQD
SYM
CODE IDENT NO.
PART OR IDENTIFYING NO.
NOMENCLATURE OR DESCRIPTION
MATERIAL / SPECIFICATION
UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS
ANGULAR
.XX ± .03
± 2
.XXX ± .010
DO NOT SCALE DRAWING
TREATMENT
FINISH
CONTRACT NO.
DRAWN
CHECK
DESIGN
STRESS STRUCTURE
MATERIALS
PRODUCTION
DESIGN ACTIVITY APPD
CUSTOMER
DATE
10-7-71
10-14-71
AEROJET MEDICAL AND BIOLOGICAL SYSTEMS
EL MONTE, CALIFORNIA
TITLE
PRELIMINARY DESIGN
INCUBATOR
CHEMILUMINESCENCE SENSOR
DWG SIZE
B
CODE IDENT NO.
34464
DWG NO.
1000395
SCALE 1/1
REL DATE
SHEET
PART DASH NO.
NEXT ASSY
USED ON
QTY REQD PER ASSY
APPLICATION
DRAWING LEVEL
3
SIMILAR TO
ACT. WT.
CALC WT
5
4
3
2
1

Figure 22

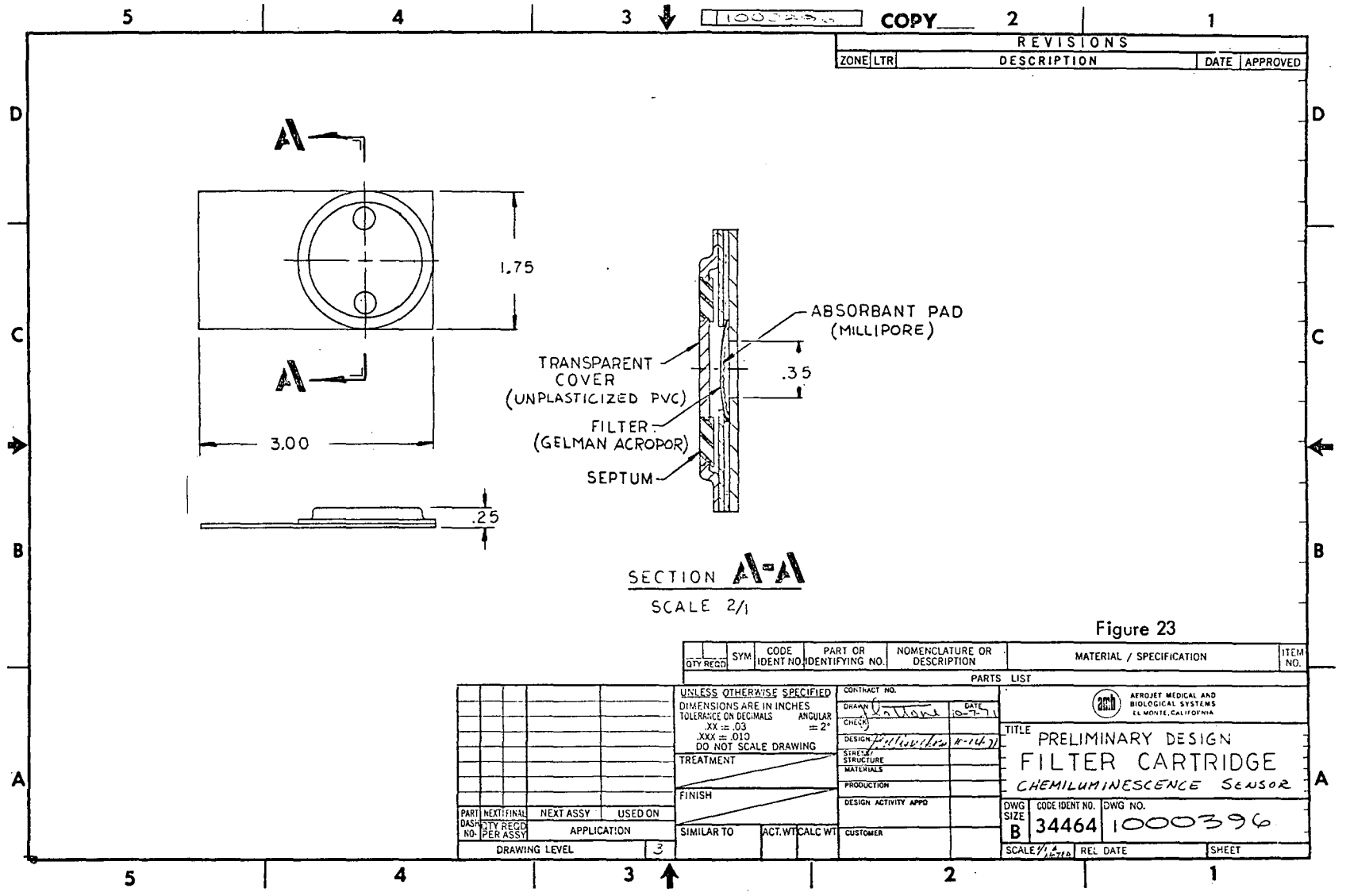

COPY
REVISIONS
ZONE LTR DESCRIPTION DATE APPROVED
1.75
3.00
.25
ABSORBANT PAD
(MILLIPORE)
TRANSPARENT
COVER
(UNPLASTICIZED PVC)
FILTER
(GELMAN ACROPOR)
SEPTUM
.35
SECTION A-A
SCALE 2/1
QTY REQD SYM CODE IDENT NO. PART OR IDENTIFYING NO. NOMENCLATURE OR DESCRIPTION MATERIAL / SPECIFICATION ITEM NO.
PARTS LIST
UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS ANGULAR
.XX ± .03 ± 2°
.XXX ± .010
DO NOT SCALE DRAWING
TREATMENT
FINISH
CONTRACT NO.
DRAWN
CHECK
DESIGN
STRESS/STRUCTURE
MATERIALS
PRODUCTION
DESIGN ACTIVITY APPD
CUSTOMER
DATE
AEROJET MEDICAL AND BIOLOGICAL SYSTEMS
EL MONTE, CALIFORNIA
TITLE
PRELIMINARY DESIGN
FILTER CARTRIDGE
CHEMILUMINESCENCE SENSOR
PART DASH NO. NEXT ASSY QTY REQD PER ASSY FINAL NEXT ASSY APPLICATION USED ON
SIMILAR TO ACT. WT CALC WT
DRAWING LEVEL 3
DWG SIZE B
CODE IDENT NO. 34464
DWG NO. 1000396
SCALE 1/1 LISTED
REL DATE
SHEET

Figure 23

FOLDOUT FRAME 1

FOLDOUT FRAME 2

REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

NOTES:

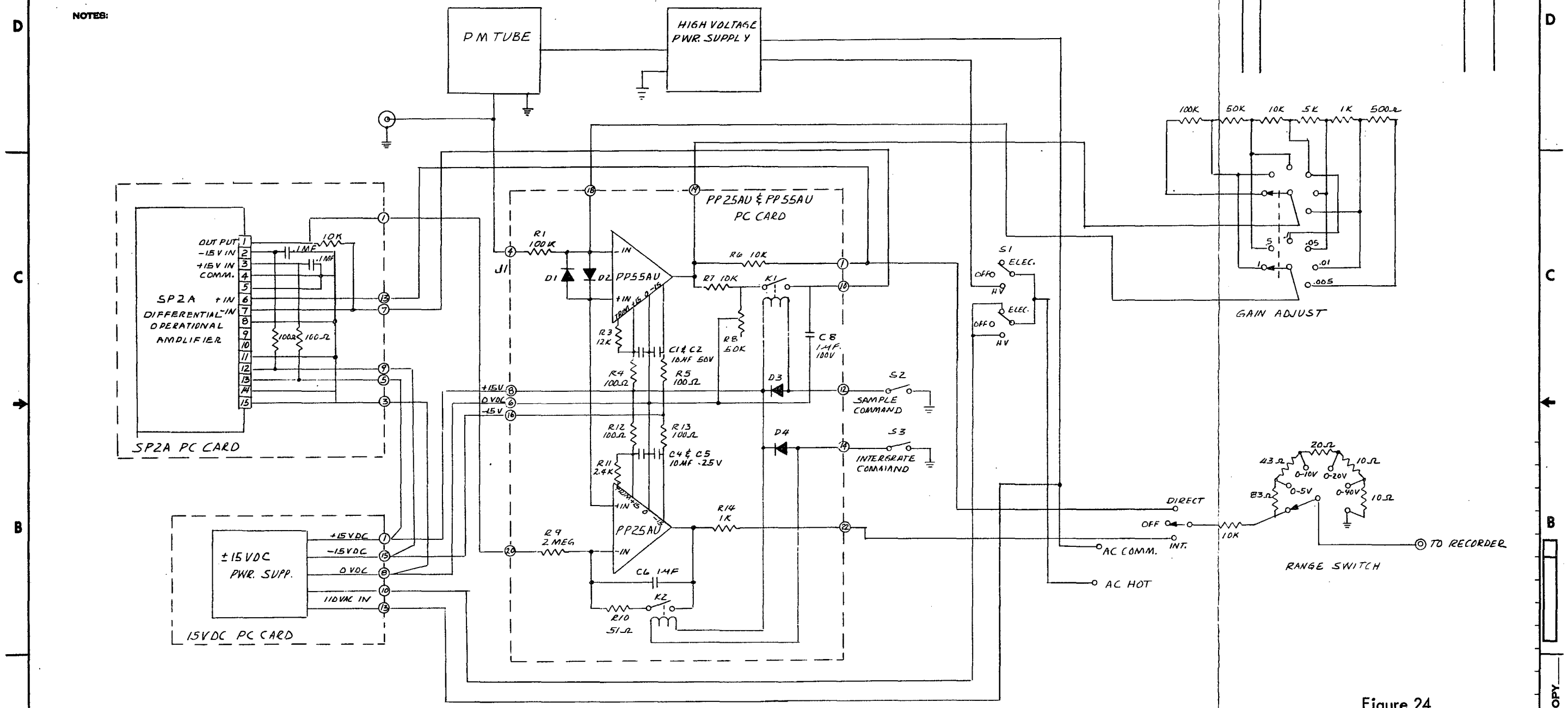


Figure 24

QTY REQD	SYM	CODE IDENT NO.	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION	MATERIAL / SPECIFICATION	ITEM NO.

PARTS LIST

PART DASH NO.	NEXT ASSY	FINAL	NEXT ASSY	USED ON			
	QTY REQD PER ASSY		APPLICATION				
	DRAWING LEVEL		3				

UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS ANGULAR
.XX ± .03 ± 2°
.XXX ± .010
DO NOT SCALE DRAWING

TREATMENT

FINISH

SIMILAR TO | ACT. WT | CALC WT

CONTRACT NO.

DRAWN	A. ARAGON	10-6-71
CHECK		
DESIGN	L. Aragon	10-6-71
STRESS/ STRUCTURE		
MATERIALS		
PRODUCTION		
DESIGN ACTIVITY APPD		
CUSTOMER		

AEROJET MEDICAL AND BIOLOGICAL SYSTEMS
EL MONTE, CALIFORNIA

TITLE
SIGNAL PROCESSING ELECTRONICS
CHEMILUMINESCENCE SENSOR

DWG SIZE	CODE IDENT NO.	DWG NO.
D	34464	1000397

SCALE — | REL DATE | SHEET

70-029 (7-70) 7/70 DIETERICH-POST CLEARPRINT 1020

COPY

FOLDOUT FRAME 1

FOLDOUT FRAME 2

REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

NOTES:

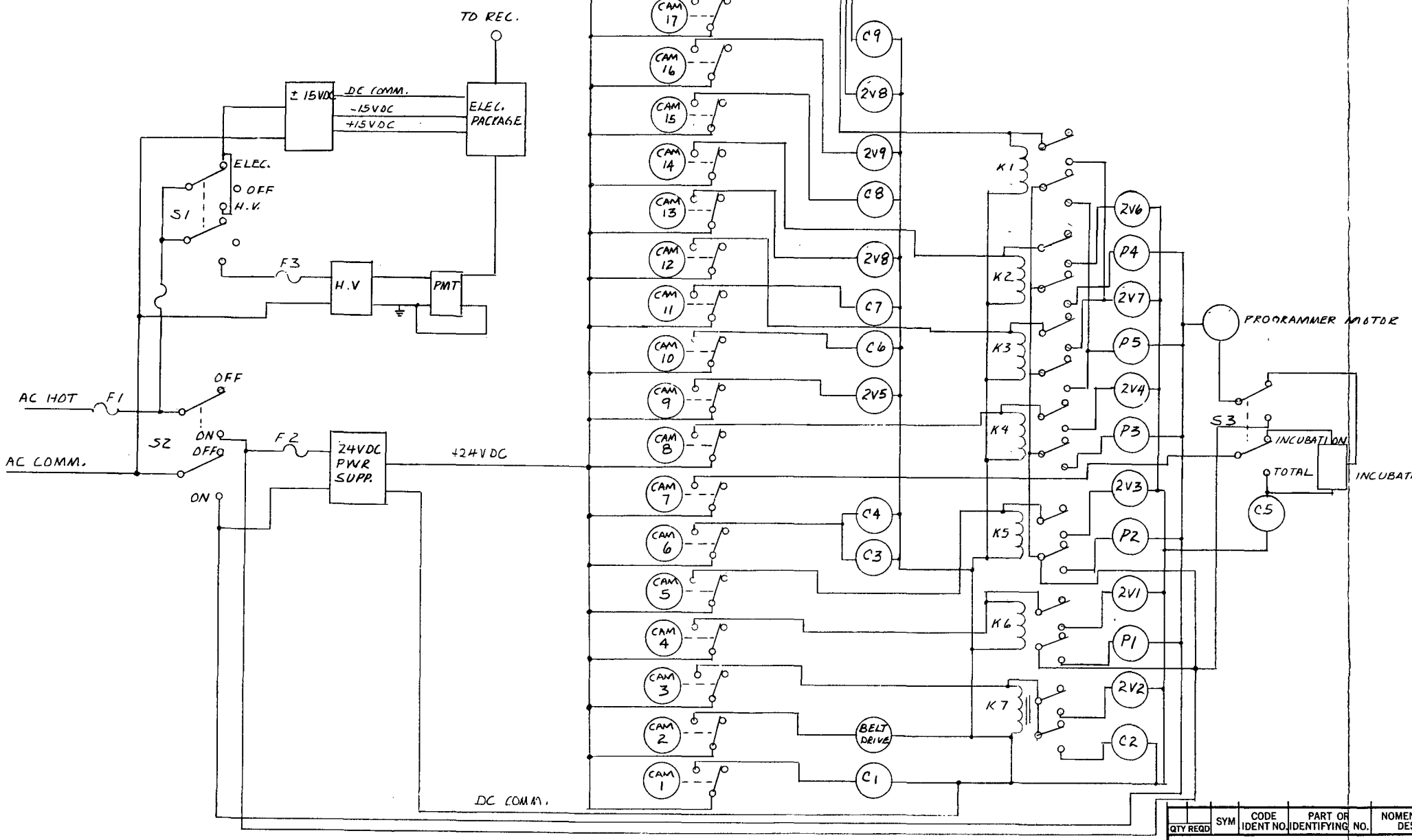


Figure 25

QTY REQD	SYM	CODE IDENT NO.	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION	MATERIAL / SPECIFICATION	ITEM NO.

PARTS LIST

PART DASH NO.	NEXT ASSY QTY REQD PER ASSY	FINAL	NEXT ASSY	USED ON
			APPLICATION	
DRAWING LEVEL		3		

UNLESS OTHERWISE SPECIFIED
DIMENSIONS ARE IN INCHES
TOLERANCE ON DECIMALS ANGULAR
XX ± .03 ± 2°
XXX ± .010
DO NOT SCALE DRAWING

TREATMENT

FINISH

SIMILAR TO | ACT. WT | CALC WT

CONTRACT NO.

		DATE
DRAWN	L C McAuley	10-6-71
CHECK		
DESIGN	C ARAGON	10-6-71
STRESS/ STRUCTURE		
MATERIALS		
PRODUCTION		
DESIGN ACTIVITY APPD		
CUSTOMER		

AEROJET MEDICAL AND BIOLOGICAL SYSTEMS
EL MONTE, CALIFORNIA

TITLE
CONTROL ELECTRONICS
CHEMILUMINESCENCE SENSOR

DWG SIZE	CODE IDENT NO.	DWG NO.
D	34464	1000398

SCALE — | REL DATE | SHEET

70-009 (7-70) 7/70 DIETERICH-POST CLEARPRINT 1020

COPY

Table 8

SAMPLE PROCESSING DESCRIPTION
(Refer to Dwg. No. 1000392)

Station 1 - Load Cartridge

a. Activate air cylinder to slide cartridge out of cassette and into retainer clip mounted to belt.

b. Advance cartridge to Station 2.

Station 2 - Collect Sample

The drive mechanism will preposition the cartridge over the filter support screen.

a. Air cylinder is activated.

1) First the cartridge holder comes down and makes contact with the flange around the rim of the cartridge, holding the cartridge in place, making a water tight seal on the underside of the cartridge and effectively enclosing the cartridge to protect against bursting the cartridge during pressure filtration.

2) As the cylinder comes down further, the needle penetrates the septum.

b. At this time, pressure filtration of the liquid sample will proceed. (Filtering of 400 ml at a rate of 40 ml/min). A support under the cartridge has a stainless steel screen or porous plug to support the filter during pressure filtration.

c. At the end of pressure filtration, the sample flow is stopped and air is passed through the cartridge to remove some of the sample water.

d. The cylinder is then deactivated to cause withdrawal of the needle.

e. The cartridge is then indexed to Station 3.

Station 3 - Nutrient

a. The upper and lower cylinders are activated at the same time to come together around the cartridge. The O-ring on the lower support make a seal to prevent leakage of nutrient.

b. Nutrient is applied through the bottom of the filter cartridge. The nutrient will displace some of the air inside the cartridge.

c. The lower support has a "liquid retraction chamber". The membrane on the chamber is pressed inward by a spherical ball. Membrane deflection reduces the chamber volume so that at the time the lower support is withdrawn, excess nutrient will be drawn back into the support reducing the probability or free liquid floating around inside the instrument under zero gravity.

d. At this time the cartridge is passed to the incubator station.

Station 4 - Incubation

a. As the cartridge approaches the incubator, the incubator will open and accept the cartridge.

b. When the cartridge has stopped in the incubator, the incubator will close around the cartridge to make a vapor seal.

c. Incubation then takes place. Inlet and outlet connections are provided for purging the incubator with an atmosphere other than air (CO_2 or N_2) if desired.

d. At the end of incubation the incubator will open and the cartridge will pass to the urea station.

Station 5 - Urea Wash

a. The operation at this station is similar to that at Station 2. A "liquid retraction chamber" is used on the top and bottom supports at this station.

b. At the end of the wash, air is passed through the cartrdige. place during which the pressure inside the cartridge is allowed to decay.

c. At the end of the pressure decay, the cylinder is deactivated to cause withdrawal of the needle.

d. The cartridge is then passed to the readout station.

Station 6 - Readout

a. The lefthand and righthand cylinders are activated causing insertion of the needles.

b. Premix flow to the cartridge is now started. The fluid is withdrawn through the righthand needle.

c. After the Premix is stopped, a H_2O wash is started to cleanout the withdrawal needle and the support screen.

d. After the H_2O is shut off, the pressure in the cartridge is allowed to decay and then both needles are withdrawn.

e. The cartridge then passes to Station 7.

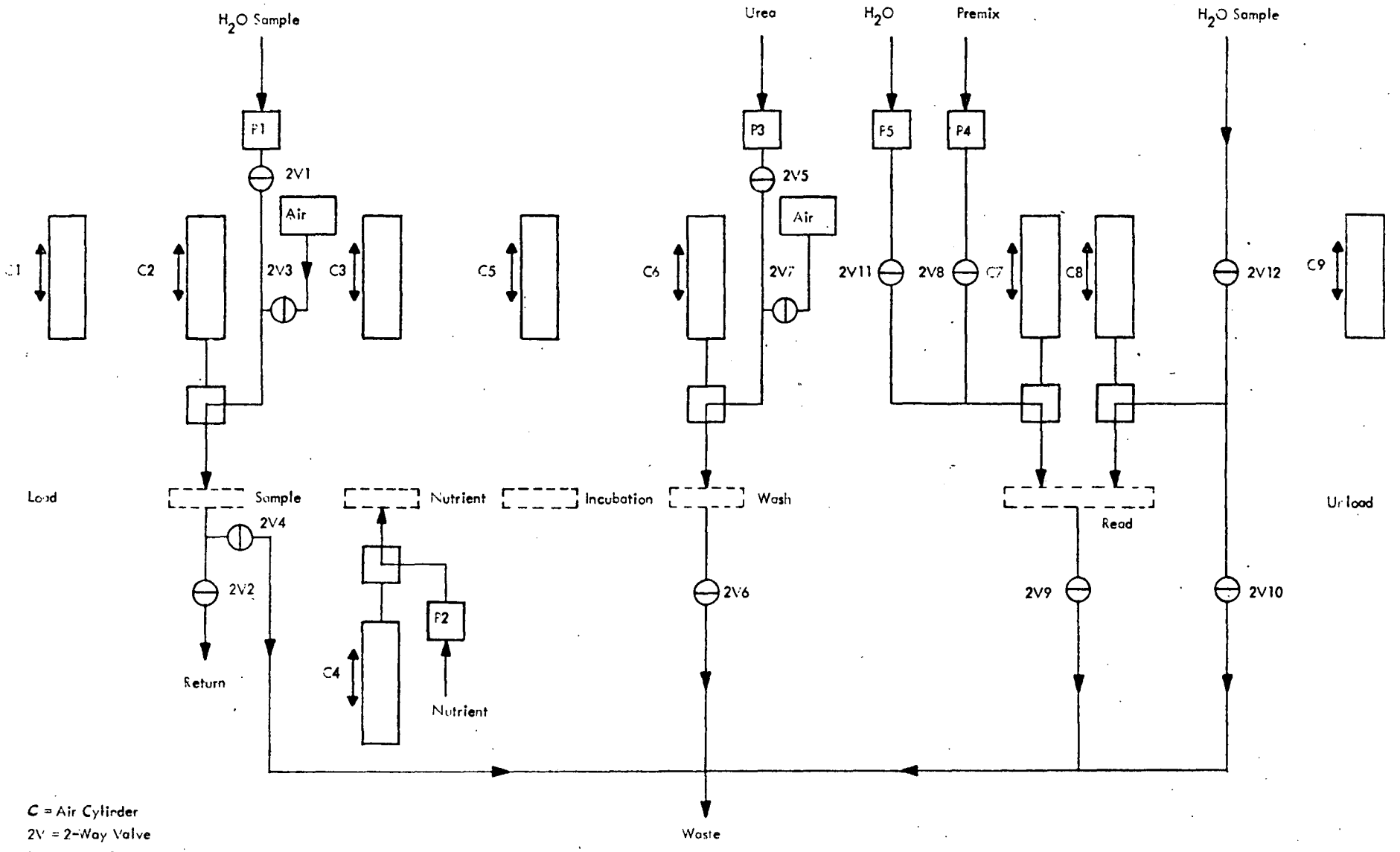
f. For reading soluble signals, an empty filter cartridge is loaded on the transport mechanism and indexed directly to the readout station where a steady flow of Premix and sample H_2O enter the cartridge through the two needles. The solution is withdrawn through the filter membrane.

Station 7 - Cartridge Ejection

At this point a cylinder is activated which ejects the cartridge from the retainer clip.

Figure 26

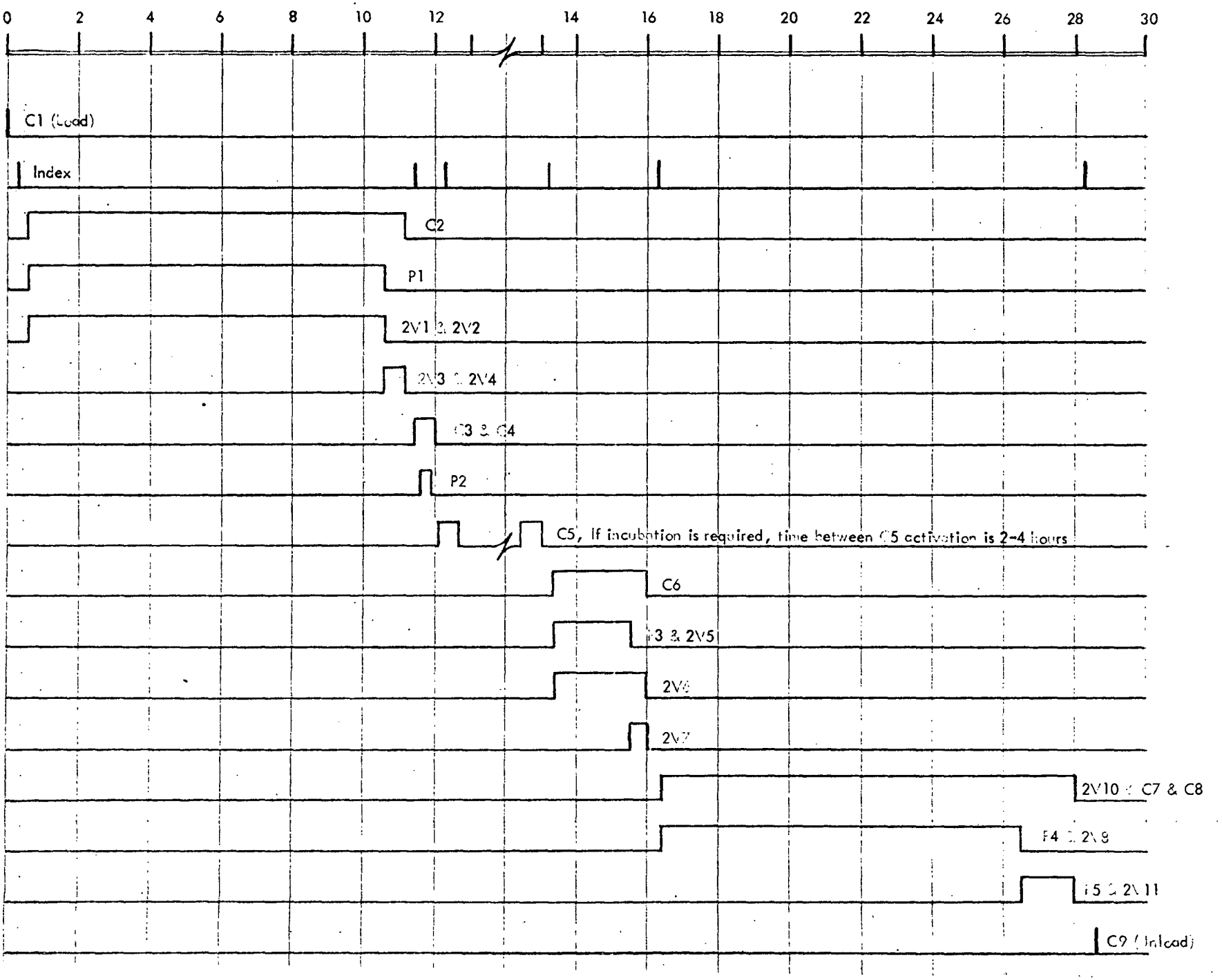
CHEMILUMINESCENCE SENSOR - FLUID FLOW SCHEMATIC



C = Air Cylinder
2V = 2-Way Valve
P = Reagent Pump

Figure 27

TIMING SEQUENCE FOR TOTAL AND VIABLE CELL COUNTS



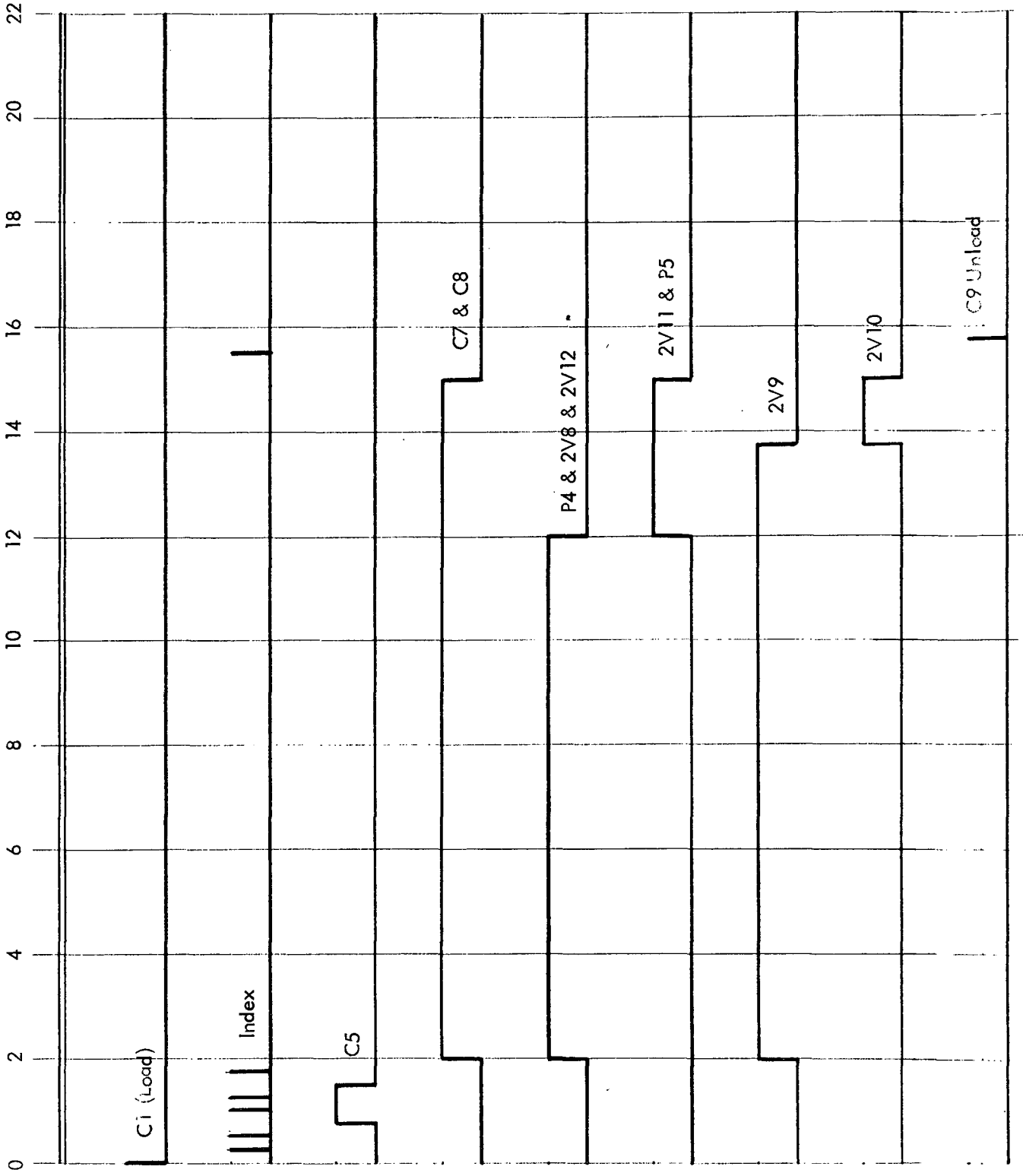


Figure 28
TIMING SEQUENCE FOR SOLUBLE SIGNAL

at subsequent processing stations would sense whether or not failure at the load station occurred.

If a cartridge were not properly loaded on the transport mechanism, several failures could occur at subsequent processing stations. The needle might hit the plastic cartridge and could break, the cartridge might not be properly supported and sample water may not pass through the cartridge but would wet other parts of the system. This type of failure could be prevented using position sensors to determine that the cartridge has been properly positioned at the load station. These conditions are typical of all stations employing flow of fluid. If the actuators failed at stations employing liquid transfer, then the fluid would not be confined to the capsules and fluid lines. This sort of failure is prevented by monitoring the actuator position before pumping any fluids. Cartridge position sensors are desirable at each processing station to insure that a cartridge did not "hang up" at some previous station.

With respect to the problem of cross-contamination between stations, this has been greatly minimized by virtue not only of the capsule and transport design but in the procedure selected for processing the individual water samples.

The possibility of transfering organisms from Station 1 (sample concentration, Figure 19) to Station 2 (nutrient addition) is practically nil since introduction of the water sample into the capsule at Station 1 is through a rubber septum in the upper face of the capsule and nutrient addition is through the bottom of the capsule at Station 2. Should the nutrient source become contaminated by bacteria, the fact that the nutrient is passed through an in-line filter as well as a membrane filter in the underside of the capsule itself precludes contaminating the capsule from this source.

Should the outside of the septum become contaminated at Station 1 and bacterial transfer occur from septum to needle at Station 4 (Urea Waste Station), bacterial growth would be inhibited due to the bacteriostatic action of the 4M Urea. Any trace amount of bacteria on the surface of the needle which might be introduced into the capsule would be insignificant, at this stage of the processing.

The transport mechanism (see Figure 20) used for moving the capsule from station to station also can be ruled out as a source of cross-contamination since it is designed to grasp the edge of the capsule (see Figure 20) and thus avoid direct contact with any of the processing stations (including the incubator).

If septum contamination did occur, flushing with 4M Urea followed by a sterile water rinse may be all that may be required for decontamination. For more extensive contamination dismantling of the affected parts and washing more thoroughly with a bactericide may be required. An alternate approach would be blanketing the component in an ethylene oxide atmosphere overnight and flushing thoroughly with sterile air.

6.3 VIABLE COLONY COUNTER SYSTEM DESCRIPTION (Figure 29)

A capsule* is loaded onto the transport mechanism and is then transferred to the sample station. At the sample station, a needle penetrates a rubber septum in the capsule and the water sample is concentrated by filtering through a membrane filter inside the capsule. The needle is then withdrawn and the capsule is transported to the next station where nutrient is applied to the sample. The capsule is then passed to an incubator where colonies are allowed to grow to a size of about 10 microns. The incubated sample is then transported to the read station where it is positioned in view of a flying spot scanner. Colonies are then counted and the total is displayed. As a final step, the capsule is unloaded from the transport mechanism.

---

*See Figure 23 for capsule design. Load and transport mechanisms for this system are the same as for Porphyrin (Capsule) Chemiluminescence System.

FOLDOUT FRAME 1

FOLDOUT FRAME 2

REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

NOTES:

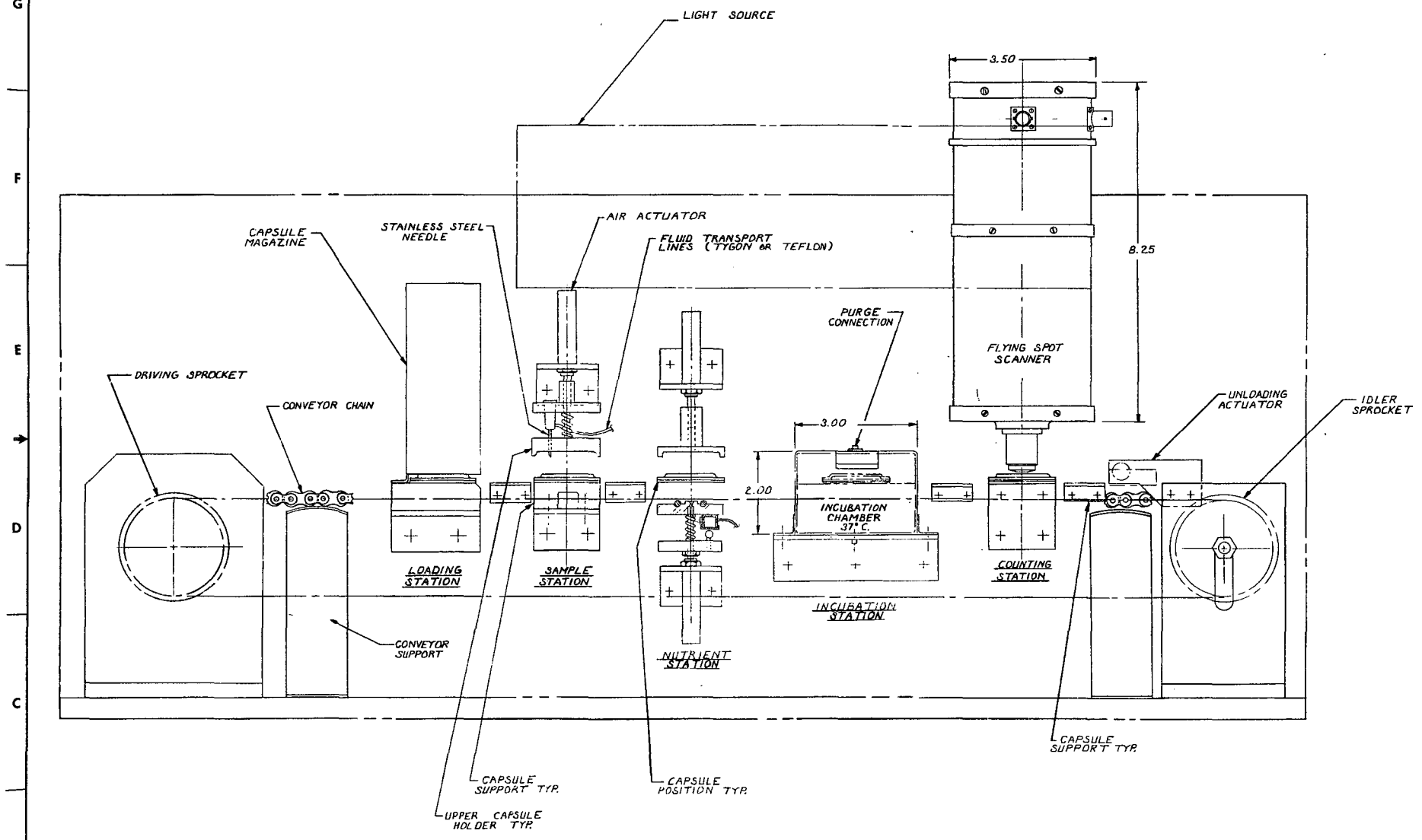


Figure 29

QTY REQD	SYM	CODE IDENT NO.	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION	MATERIAL / SPECIFICATION	ITEM NO.
PARTS LIST						

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES. TOLERANCE ON DECIMALS .XX ± .03 .XXX ± .010 DO NOT SCALE DRAWING. ANGULAR ± 2°

TREATMENT

FINISH

MATERIAL TO

NEXT ASSY | USED ON | APPLICATION | DRAWING LEVEL 3

CONTRACT NO.

DRAWN D. MULLIGAN 6-7-72

CHECK

ENGINEER

MATERIALS

PRODUCTION

DESIGN ACTIVITY APPD

CUSTOMER

AEROJET MEDICAL AND BIOLOGICAL SYSTEMS EL MONTE, CALIFORNIA

TITLE: PRELIMINARY DESIGN LAYOUT VIABLE COLONY COUNTING SYSTEM

DWG SIZE E | CODE IDENT NO. 34464 | DWG NO. 1000412

SCALE FULL | REL DATE | SHEET 1 OF 1

1000412

COPY

6.4 BEAD AGGLUTINATION SYSTEM DESCRIPTION (Figure 30)

Sample water enters the system and is pumped through a dual pressure dialysis module. There are separate dialysis cells for the sample and control solutions. The concentrate flow is adjusted so that a concentration ratio of approximately 200:1 is achieved. The filtrate may be retumed to the storage tank or may be dumped for reprocessing. Dual capsules (sample and control) on a single card are dispensed from a cassette and prepositioned at the inject station by a transport mechanism. After about 1 ml of concentrate is formed at each dialysis cell, the concentrate is injected into the capsule.

The capsules are then transported to the next station where specified amounts of sample and control sensitized beads are injected (proportions of beads and H_2O concentrate are approximately 1:1). The capsules are then passed to the incubator where the solutions are allowed to react for one hour. The capsules are then transported to the photometric reader.

At the reader a light beam is split into two equal beams (balanced electronically). These beams are modulated (chopped), passed through the two capsules, recombined and sent to a single detector. Beam modulation is necessary so that the single detector and the electronics can distinguish between the sample signal and control signal. The signal is proportional to the amount of light transmission through the photometer cell. The processing electronics displays the ratio of sample-to-control signal. Agglutination causes an increase in light transmission through the bead suspension. The photometric reader senses the difference in light transmission and hence, agglutination.

FOLDOUT FRAME 1

FOLDOUT FRAME 2

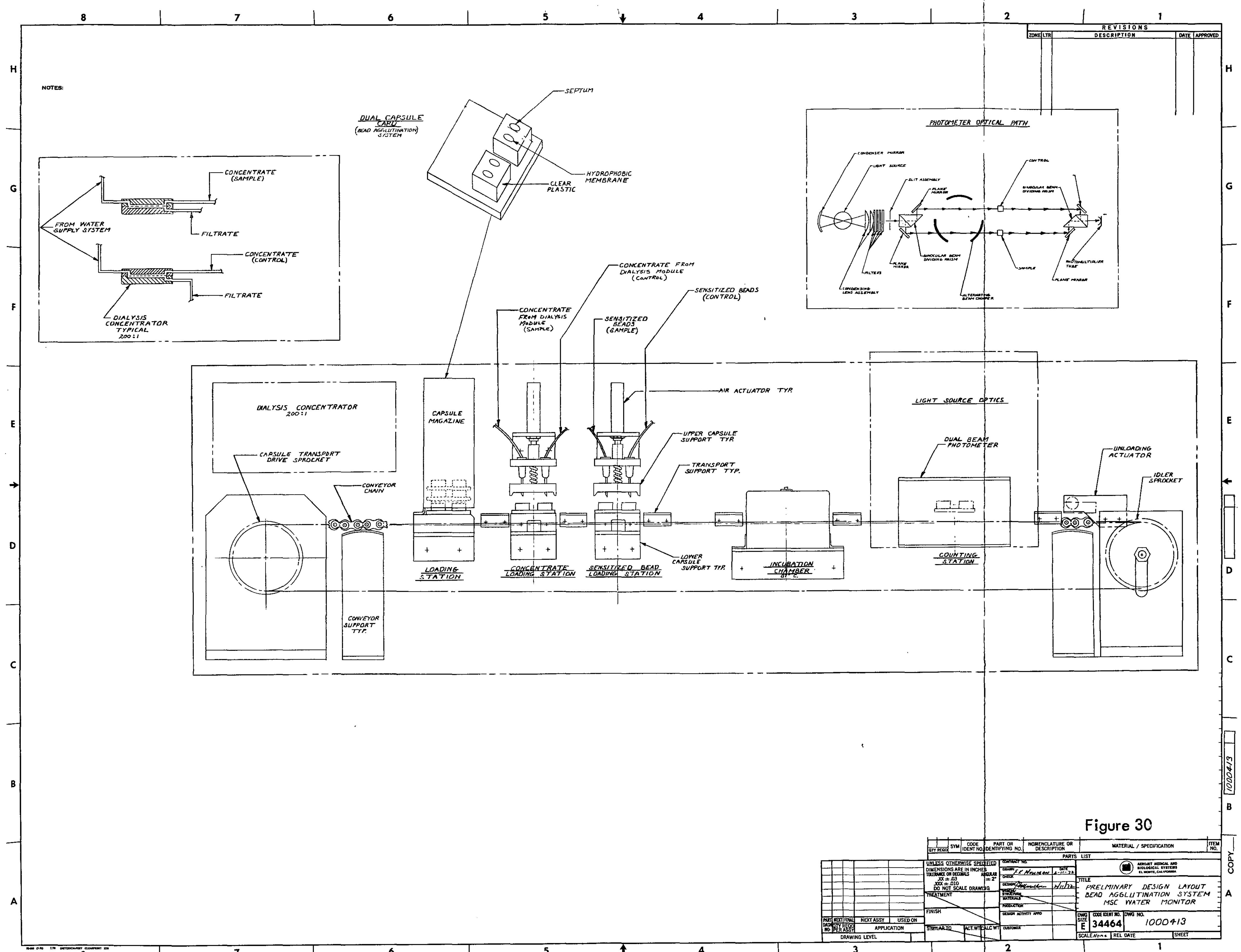


Figure 30

Section 7

PROGRAM PROJECTIONS

Section 7

PROGRAM PROJECTIONS

Detailed program projections are presented below for the three systems (i.e., Porphyrin (Capsule) Chemiluminescence, Colony Counter and Bead Agglutination) selected by NASA for further consideration. Program schedules, tasks/phases, manpower and material cost estimates required for the development of each of the systems to a flight-rated status have been included.

The material is presented in the following order:

1. Task/Phase Description - Program Schedules
   - Porphyrin (Capsule) Chemiluminescence
   - Colony Counter
   - Bead Agglutination
2. Program Cost & Manpower Estimates

PORPHYRIN (CAPSULE) CHEMILUMINESCENCE

(For Total and Lysed Cells)

PHASE 1
(8 Months Duration)

OBJECTIVES

Design, fabricate and test a fully automated chemiluminescence prototype based on the "Capsule" approach which is capable of monitoring total, viable and lysed cells. Major tasks to be performed (Figure 31) include:

1. Optimize Capsule Design

Prime consideration will be given to minimize cross contamination between stations; design will be supported by backup laboratory studies.

2. Optimize Conditions for Bacterial Growth

Includes investigation of various parameters (nutrient, relative humidity, ambient atmosphere, temperature, etc.) required for growing the aerobes.

3. Design, Fabricate and Checkout of Automated System

This prototype will be fully automated and except for the reagent feed system, will be operable under zero-g.

4. Evaluate Instrument and Finalize Design

The sensitivity and reliability of detection for viable and non-viable cycles will be established for the two test organisms. At the conclusion of the test series, design of the instrument will be upgraded for optimum performance. System will then be delivered to MSC.

Phase 2
(16 Months Duration)

OBJECTIVES

Modify the system for zero-g operation and field test a fully automated system. Specific tasks (Figure 32) to include:

1. Modification of Reagent Supply Subsystem for Zero-g Operation

This would include a design effort as well as a laboratory support study to select materials of construction (i.e., bladder materials suitable for space use possessing the desired flexibility and chemical compatibility).

Figure 31

PORPHYRIN (CAPSULE) CHEMILUMINESCENCE

PROGRAM SCHEDULE-PHASE 1

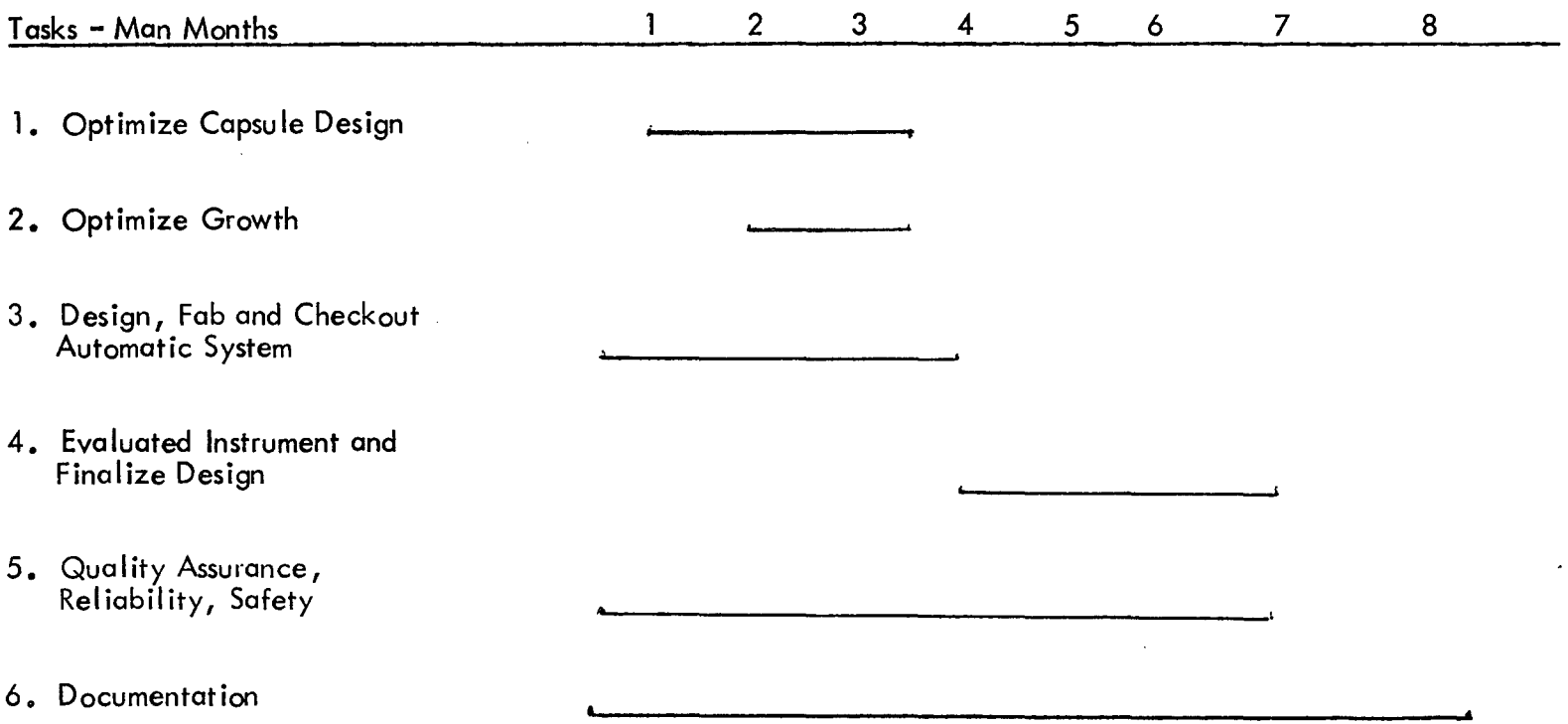
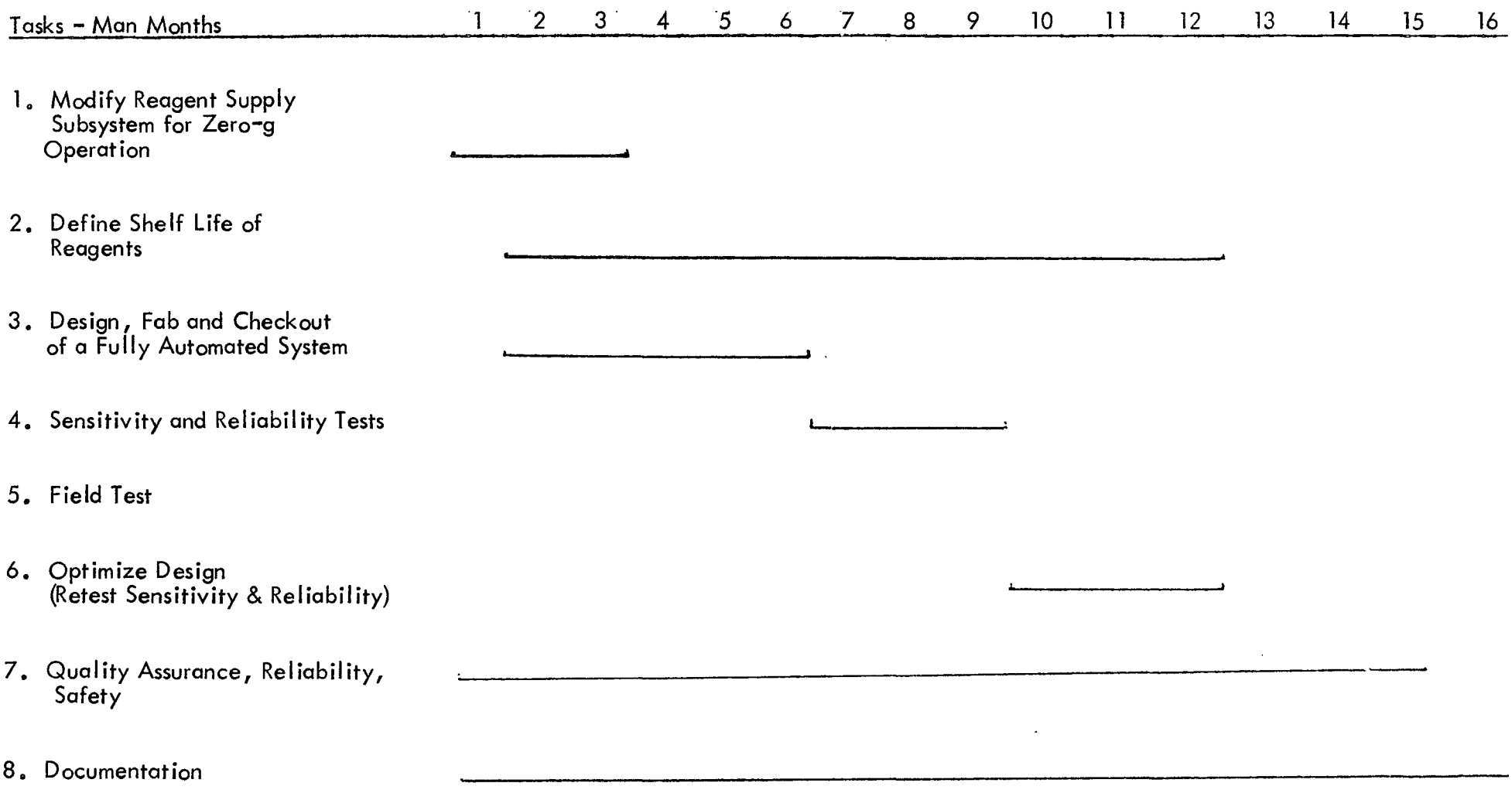


Figure 32

PORPHYRIN (CAPSULE) CHEMILUMINESCENCE

PROGRAM SCHEDULE-PHASE 2



2. Define Reagent Shelf Life

Including component compatibility.

3. Design, Fabrication and Checkout of a Fully Automated System

This prototype would be capable of zero-g operation.

4. Sensitivity and Reliability Tests

Detection limits delineated for total and viable cycles as well as for soluble porphyrins (E. coli test organisms).

5. Field Test

This system would be interfaced with a water regeneration system and field tested for 3 months.

6. Optimize Design

Based on the results of the field studies, the system will be modified to optimize performance. Sensitivity and reliability will be redetermined on a modified unit.

Phase 3
(16 Months Duration)

OBJECTIVES

Field testing of a miniature and modularized flight-rated prototype. Referring to Figure 33, major tasks are to include:

1. Design, Fabrication and Checkout of a Miniaturized and Modularized Flight-Rated Prototype

Design will incorporate explosion-proof features and materials of construction suitable for space application.

2. Sensitivity and Reliability Evaluation

Sensitivity limits and reliability of detection for total, viable and soluble cycles will be established.

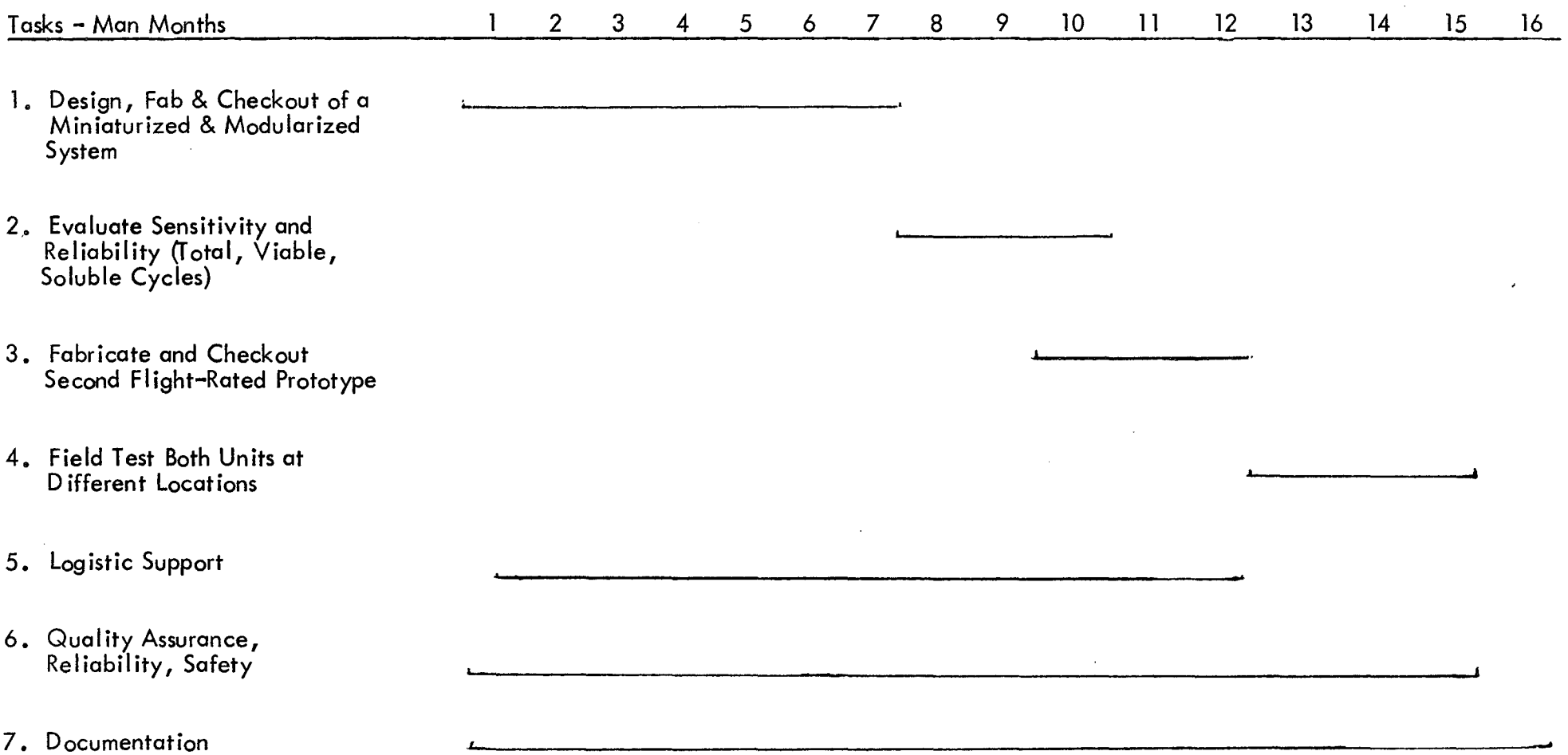
3. Fabricate and Checkout Additional Flight-Rated Prototype

Incorporating any improvements suggested from the sensitivity and reliability evaluation; (upgrade both units).

Figure 33

PORPHYRIN (CAPSULE) CHEMILUMINESCENCE

PROGRAM SCHEDULE-PHASE 3



4. Field Test

Both units are to be interfaced with different water regeneration systems and evaluated over a 3-month period.

5. Logistic Support

Reagent and component replacement package to be designed and assembled. Shelf life of reagents and maintenance schedule for critical components to be defined.

Phase 4
(17 Months Duration)

OBJECTIVES

Qualification and acceptance testing of a flight-rated unit. Major tasks are to include (Figure 34) the following:

1. Design, Fabricate and Checkout of a Flight-Rated Prototype

Based on results of field testing in Phase 3, a more advanced model will be designed and fabricated.

2. Sensitivity and Reliability

Sensitivity and reliability of this flight-rated prototype will be evaluated (for total viable and soluble cycle) using E. coli as the test organism.

3. Qualification Testing

Sensitivity and reliability of the system will be confirmed. The system will be subjected to vibration, shock and other tests required to achieve a flight-rated status.

4. Fabrication of a Flight-Rated Unit

A second unit will be fabricated and checked out for use in acceptance testing prior to delivery to MSC.

5. Acceptance Testing

Flight-rated unit will be subjected to tests for sensitivity and reliability using E. coli as the test organism.

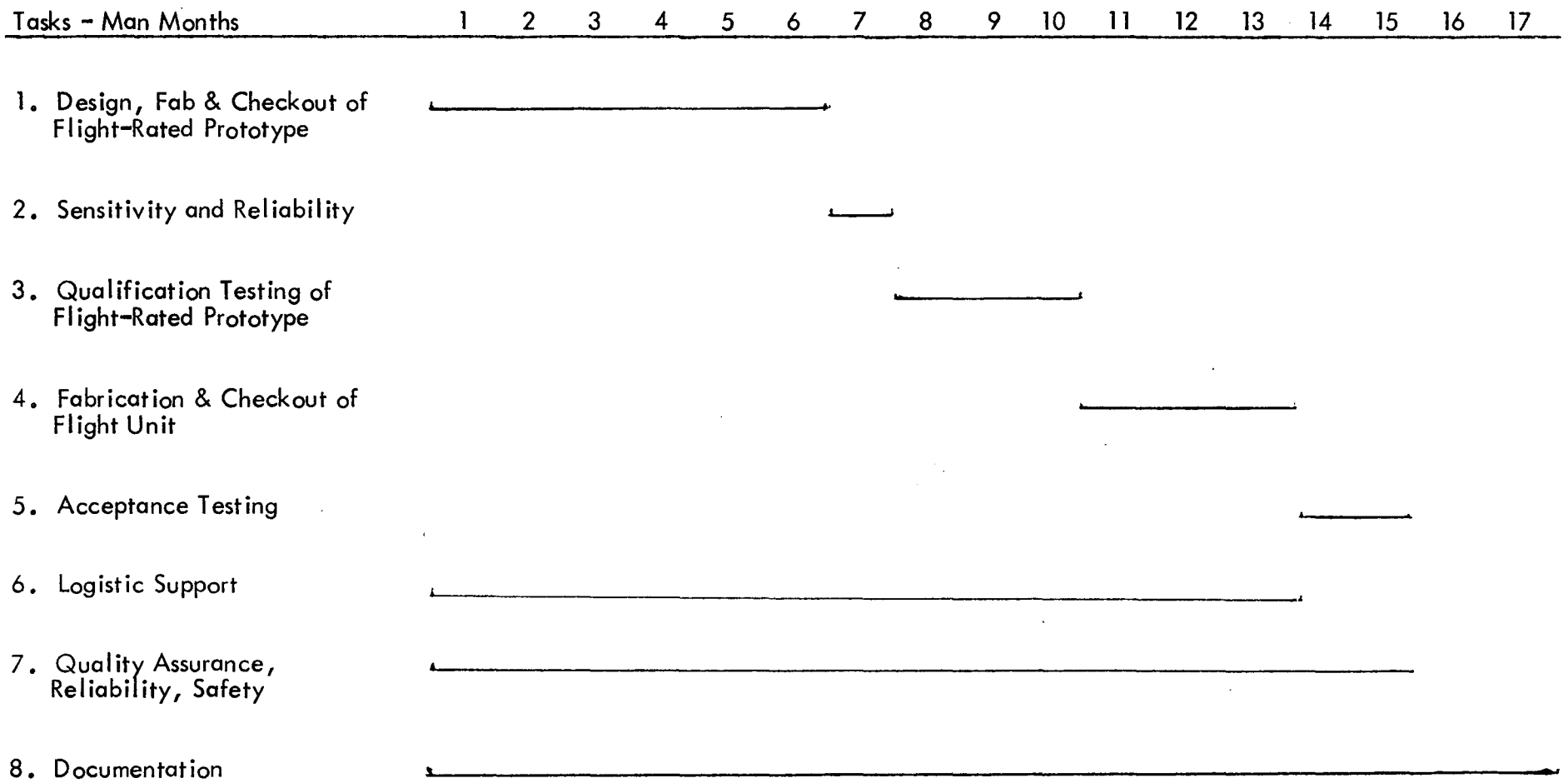
6. Logistic Support

Reagents and technical support required for the operation and maintenance of the unit will be provided and subjected to qualification and acceptance testing.

Figure 34

PORPHYRIN (CAPSULE) CHEMILUMINESCENCE

PROGRAM SCHEDULE-PHASE 4



COLONY COUNTER
(For Viable Cells)

PHASE 1
(8 Months Duration)

OBJECTIVES

To demonstrate the feasibility of the colony counting method for monitoring of viable organisms in a regenerated water supply. A manually-operated (capsule) transport system (with modified flying spot readout) is to be utilized to optimize process variables. Major tasks (Figure 35) are to include the following:

1. Optimize Capsule Design

To minimize cross contamination between samples, sterile filter-capsules dispensed from a cassette will be utilized for processing individual water samples. Optimum capsule design will be established in conjunction with backup laboratory studies. Capsule materials and sterilization technique selected will be checked for their effect on bacterial growth.

2. Protocol Development

The optimum procedure for growing the test organisms in the capsule and one which is compatible with a flying spot readout will be developed.

3. Transport Mechanism

The optimum design for dispensing and transporting the capsules from station to station will be arrived at through backup laboratory studies.

4. Design, Fabrication and Checkout of Laboratory Prototype

An integrated manually-operated laboratory breadboard containing all the stations required for processing the sample will be designed, fabricated and checked out.

5. Sensitivity and Reliability Studies

The detection threshold for viable E. coli will be established using the laboratory breadboard.

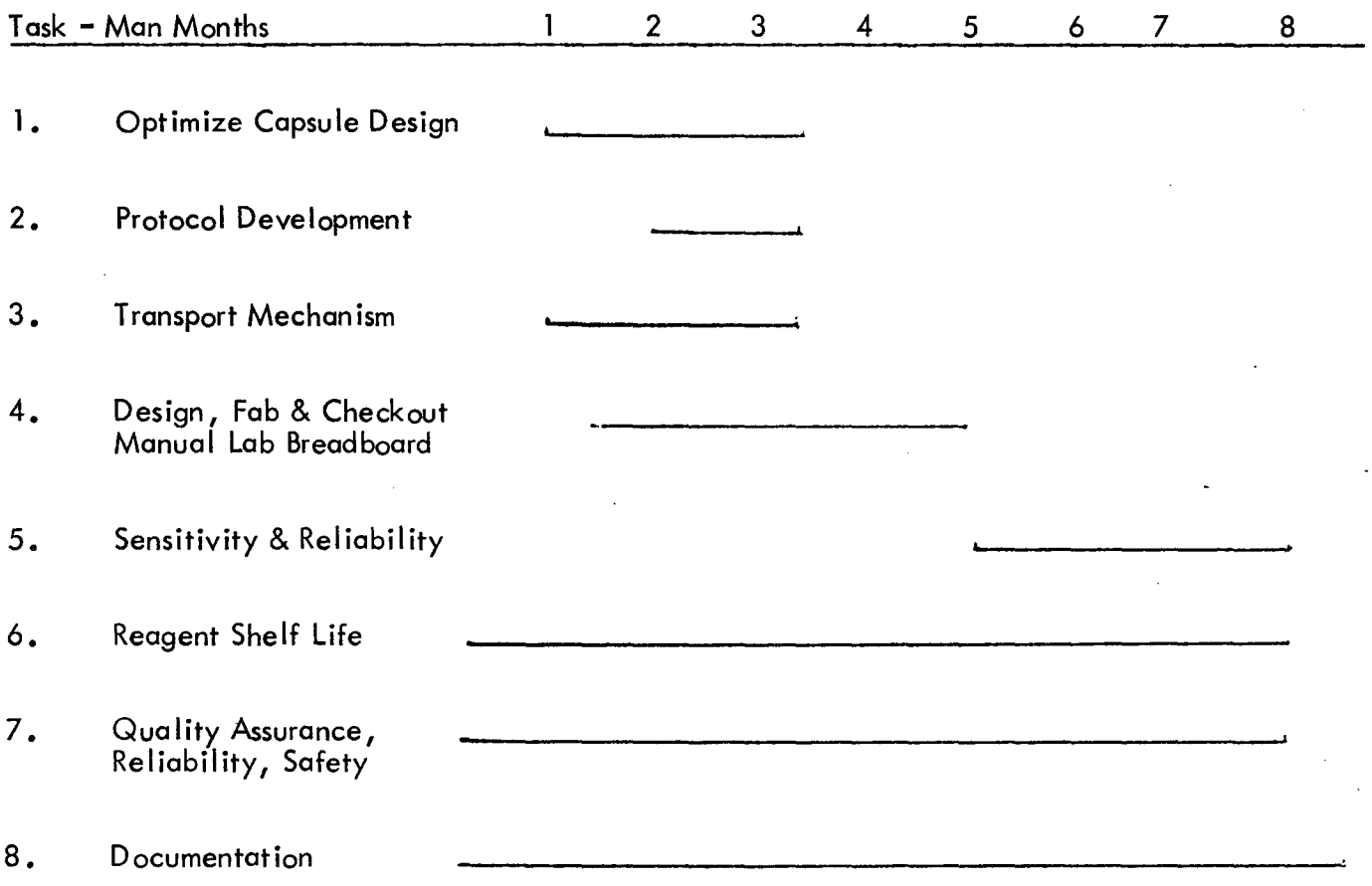
6. Reagent Shelf Life Studies

The compatibility and shelf life of required reagents and supplies will be determined.

Figure 35

COLONY COUNTER

PROGRAM SCHEDULE-PHASE 1



PHASE 2
(13 Months Duration)

OBJECTIVES

To design, fabricate and field test a fully-automated laboratory prototype. Major tasks to be performed (see Figure 36) are as follows:

1. Design, Fabrication and Checkout of an Automated Prototype

The laboratory prototype developed under Phase 1 will be modified as required to permit integration into a fully automated prototype system capable of operation in a zero-g environment.

2. Evaluation of Sensitivity and Reliability

The sensitivity and reliability of detection of the automated system toward viable E. coli will be established.

3. Field Test

This system will be field tested (3-month period) with a water regeneration system to establish interface problems and system reliability.

PHASE 3
(16 Months Duration)

OBJECTIVES

To field test a miniaturized and modularized flight-rated prototype. Referring to Figure 37, major tasks are to include:

1. Design, Fabrication and Checkout of a Miniaturized and Modularized Flight-Rated Prototype

Design will incorporate explosion-proof features and materials of construction suitable for space application.

2. Sensitivity and Reliability Studies

Sensitivity limits and reliability of detection toward viable E. coli will be established.

3. Fabrication and Checkout of an Additional Flight-Rated Prototype

4. Field Test

Both units are to be field tested with different water-regeneration systems over a 3-month period.

Figure 36

COLONY COUNTER

PROGRAM SCHEDULE - PHASE 2

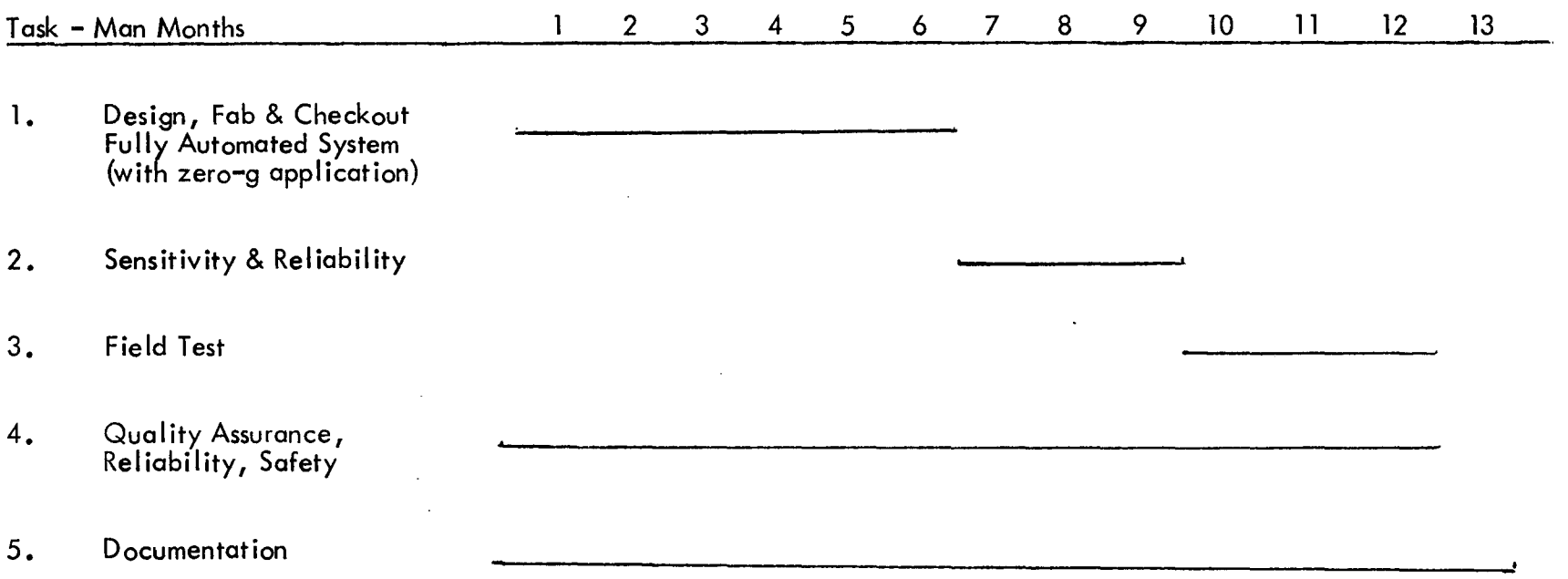
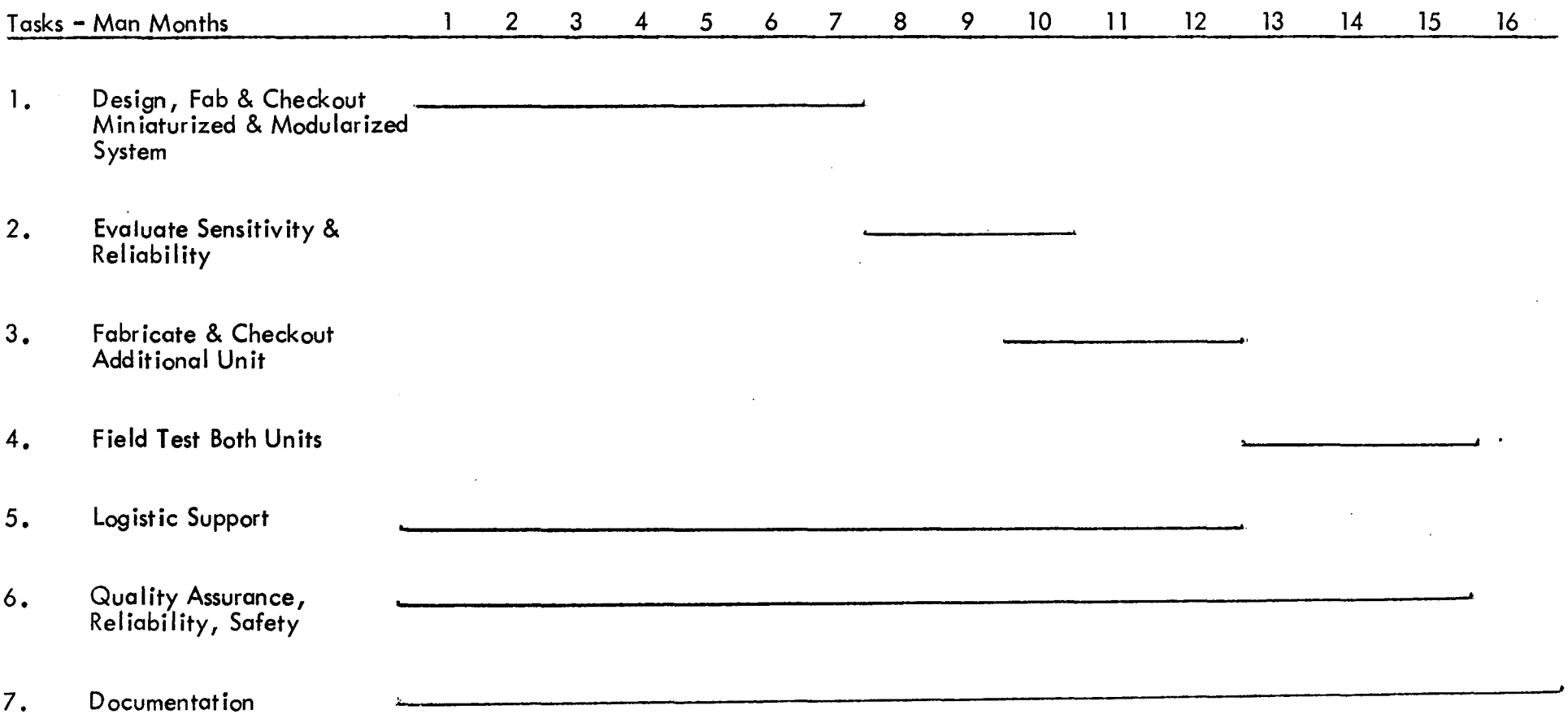


Figure 37

COLONY COUNTER

PROGRAM SCHEDULE - PHASE 3



5. Logistic Support

Reagent and component replacement package to be designed and assembled. Shelf life of reagents and maintenance schedule for critical components are to be defined.

PHASE 4
(19 Months Duration)

OBJECTIVES

Qualification and acceptance testing of a flight-rated unit. Major tasks are to include (Figure 38) the following:

1. Design, Fabrication and Checkout of a Flight-Rated Prototype

Based on the results of field testing in Phase 3, a more advanced model of the colony counter will be designed and fabricated.

2. Sensitivity and Reliability

The sensitivity and reliability of detection for viable E. coli will be determined.

3. Qualification Testing

The sensitivity and detection reliability of the flight-rated prototype will be confirmed. System will be subjected to vibration, shock and other tests required to achieve a flight-rated status.

4. Fabrication of a Flight-Rated Unit

A second unit will be fabricated and checked out for use in acceptance testing prior to delivery to MSC.

5. Acceptance Testing

Flight-rated unit will be subjected to tests for sensitivity and reliability using E. coli as a test organism.

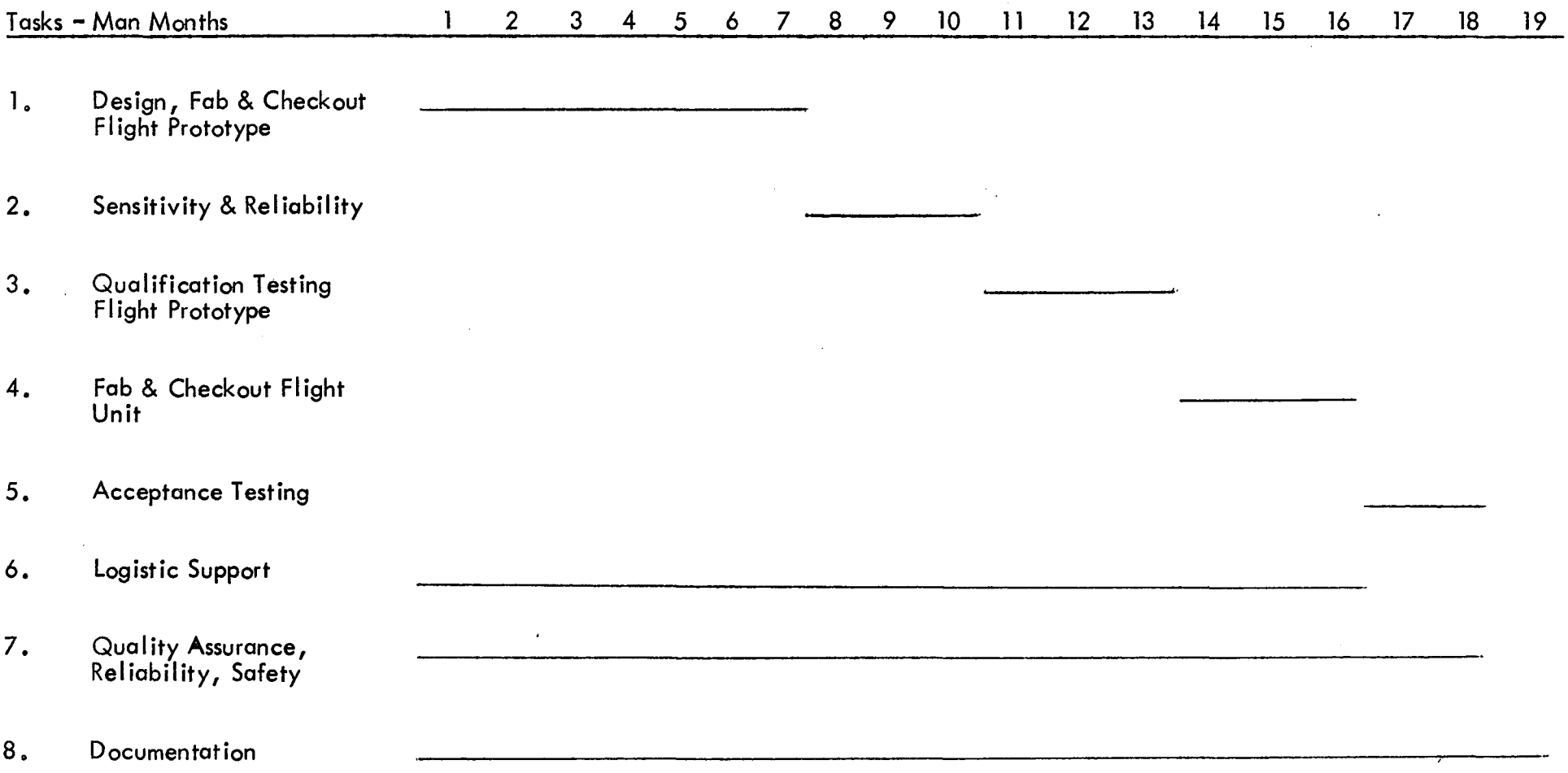
6. Logistic Support

Reagents and technical support required for operation and maintenance of the unit will be provided and subjected to qualification and acceptance testing.

Figure 38

COLONY COUNTER

PROGRAM SCHEDULE - PHASE 4



BEAD AGGLUTINATION METHOD

(For Virus and Toxin)

PHASE 1
(15 Months Duration)

OBJECTIVES

To fabricate and test a manual laboratory prototype to demonstrate feasibility against a representative virus and bacterial toxin. Specific tasks to be performed are as follows: (Refer to Figure 39).

1. Definition of Virus and Toxin Concentration in Liquid Wastes

This information would help in defining:

a. the extent of concentration that would be needed for detection,

b. the type of concentration approach and

c. the practicality of monitoring for these agents on a regular basis. Conventional assay techniques would be used for establishing the concentration of agents of interest in wine and other liquid wastes (i.e., wash water, condensate, etc.).

2. Evaluation of Concentration Methods

The most attractive system for continuous automatic operation appears to be pressure dialysis and should be examined first. Backup methods include liquid partition, filtration, continuous (Sharples) centrifugation and chemical concentration techniques.

3. Dual Capsule Design

Sterile disposable dual capsules, mounted on a single card, will be utilized for reaction and readout of the concentrated sample and control. The optimum capsule design will be established in conjunction with backup laboratory studies. Capsule materials and sterilization techniques selected will be checked to insure freedom of interference with reaction or photometric readout (through the capsule).

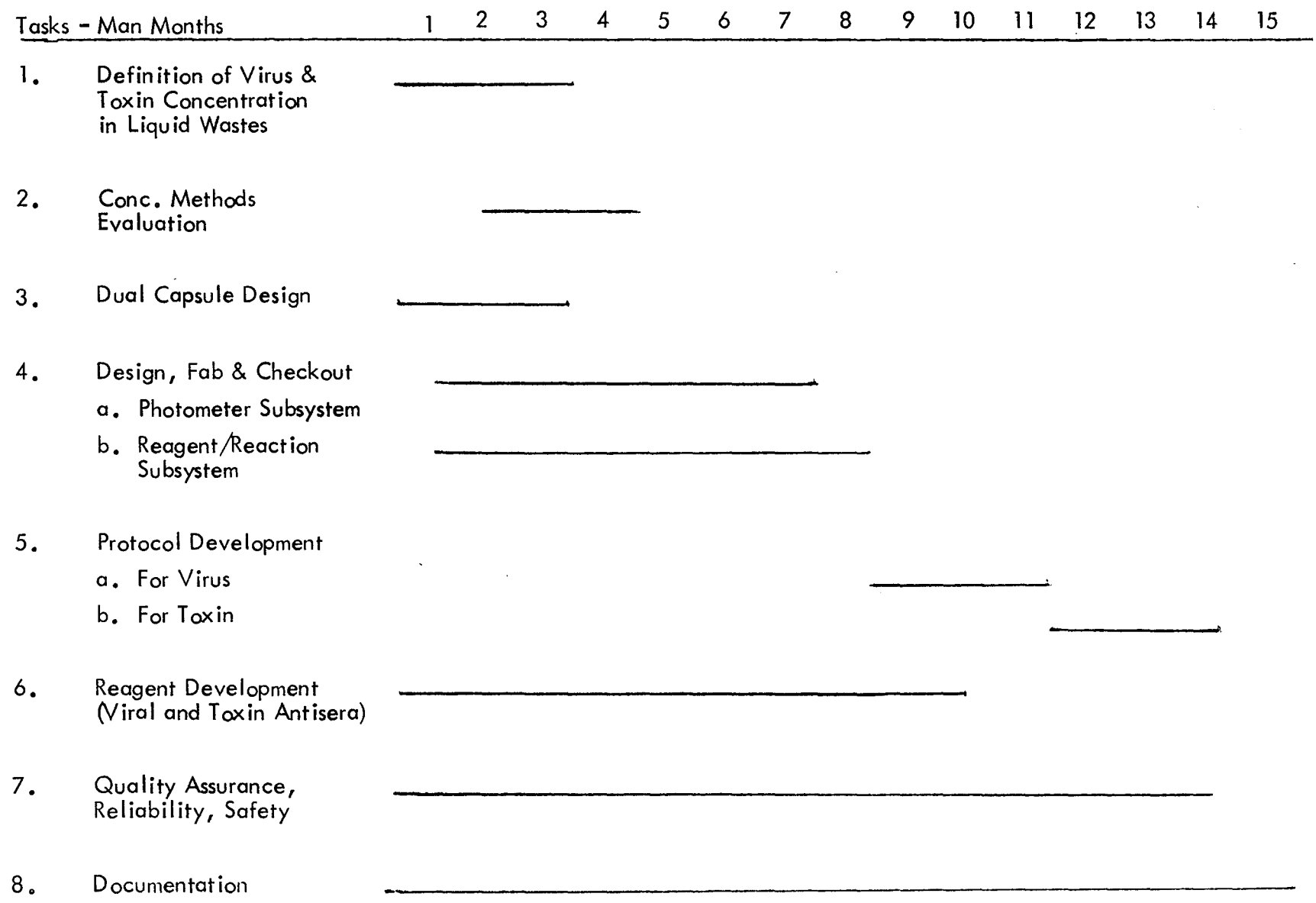
4. Design, Fabrication and Checkout of a Manually-Operated Laboratory Prototype

For maximum flexibility in protocol development, a manually-operated laboratory prototype in which the sample is transported manually between each of the subsystems (i.e., sample concentration and treatment, reaction and readout) would be fabricated based on the principles outlined previously. The photometric readout subsystem and the reagent/reaction subsystem are fabricated and checked as subsystems before integration.

Figure 39

BEAD AGGLUTINATION

PROGRAM SCHEDULE - PHASE 1



5. Protocol Development

This would involve developing appropriate processing protocols for detection of a representative virus (i.e., adenovirus type) and a bacterial toxin (i.e., Staph. enterotoxin) in a regenerated water supply.

6. Reagent Development

This would entail developing appropriate antisera (for an adenovirus and Staph enterotoxin) for coating of the polystyrene beads. Specifically this would involve isolating and characterizing the desired antigens, preparing a vaccine, immunization, isolation and fractionation of the antibody and preparation of the sensitized reagents.

PHASE 2
(12 Months Duration)

OBJECTIVES

Adapt process to zero-g operation and demonstrate feasibility with a fully automated prototype system.

Major tasks to be performed (see Figure 40) are as follows:

1. Design, Fabrication and Checkout of an Automated Prototype

The subsystems and procedures developed under Phase 1 will be modified as required to permit integration into a fully automated prototype system capable of operation in a zero-g environment.

2. Evaluation of Sensitivity and Reliability

An automated laboratory prototype will be utilized to establish the sensitivity and reliability of detection toward a representative virus and bacterial toxin.

3. Reagent Preparation

Entails preparing antisera (for myxovirus and Staph. enterotoxin) for sensitizing polystyrene beads.

PHASE 3
(12 Months Duration)

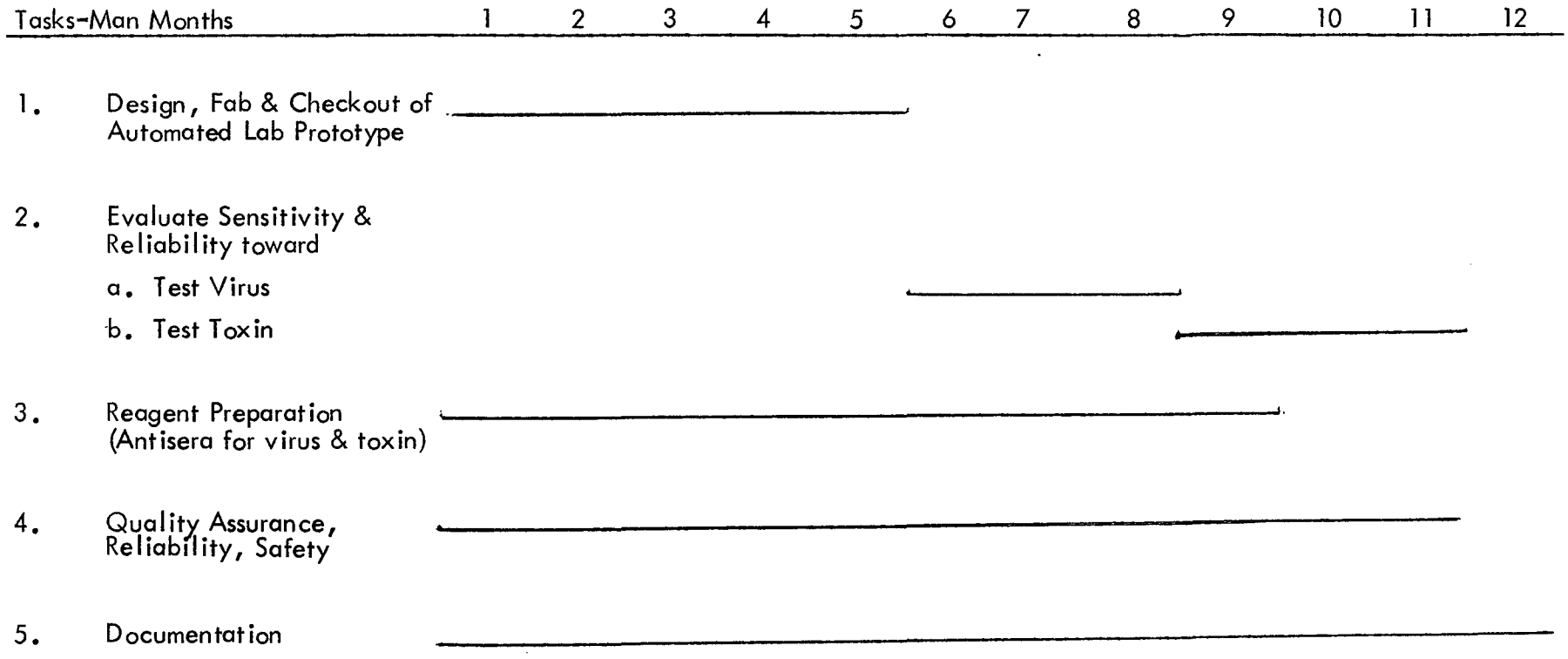
OBJECTIVES

Automated unit developed under Phase 2 will be field tested with various

Figure 40

BEAD AGGLUTINATION

PROGRAM SCHEDULE - PHASE 2



water regeneration systems. Specific tasks to be performed are the following (see Figure 41).

1. Checkout System

Automated system developed under Phase 2 will be tested to establish anticipated interface problems with different water regeneration systems. Necessary logistic support for field evaluation will be established.

2. Field Test

Actual hookup of water monitor with regeneration system will be made and tests conducted over a 3 months interval.

3. Rework System

System will be modified in light of field data to provide more optimal performance.

4. Field Test

Reworked unit will be field tested again either with the same or a different water regeneration system.

5. Optimize Design

Design for the unit will be finalized incorporating improvements suggested by field trials. The sensitivity and reliability of the final unit will be established; maintenance schedules for system components will be defined.

PHASE 4
(18 Months Duration)

OBJECTIVES

Design, fabricate and field test a miniaturized and modularized flight-rated prototype. Antisera developed for several agents (viruses and toxins) of interest. Referring to Figure 42 , the various tasks to be performed are as follows:

1. Design, Fabrication and Checkout of Miniaturized and Modularized Flight-Rated Prototype

Design will also incorporate explosion-proof features and materials of construction compatible with space application.

2. Sensitivity & Reliability Evaluation

Detection limits of instrument will be established against 3 group viruses and 3 toxins of interest.

Figure 41

BEAD AGGLUTINATION

PROGRAM SCHEDULE - PHASE 3

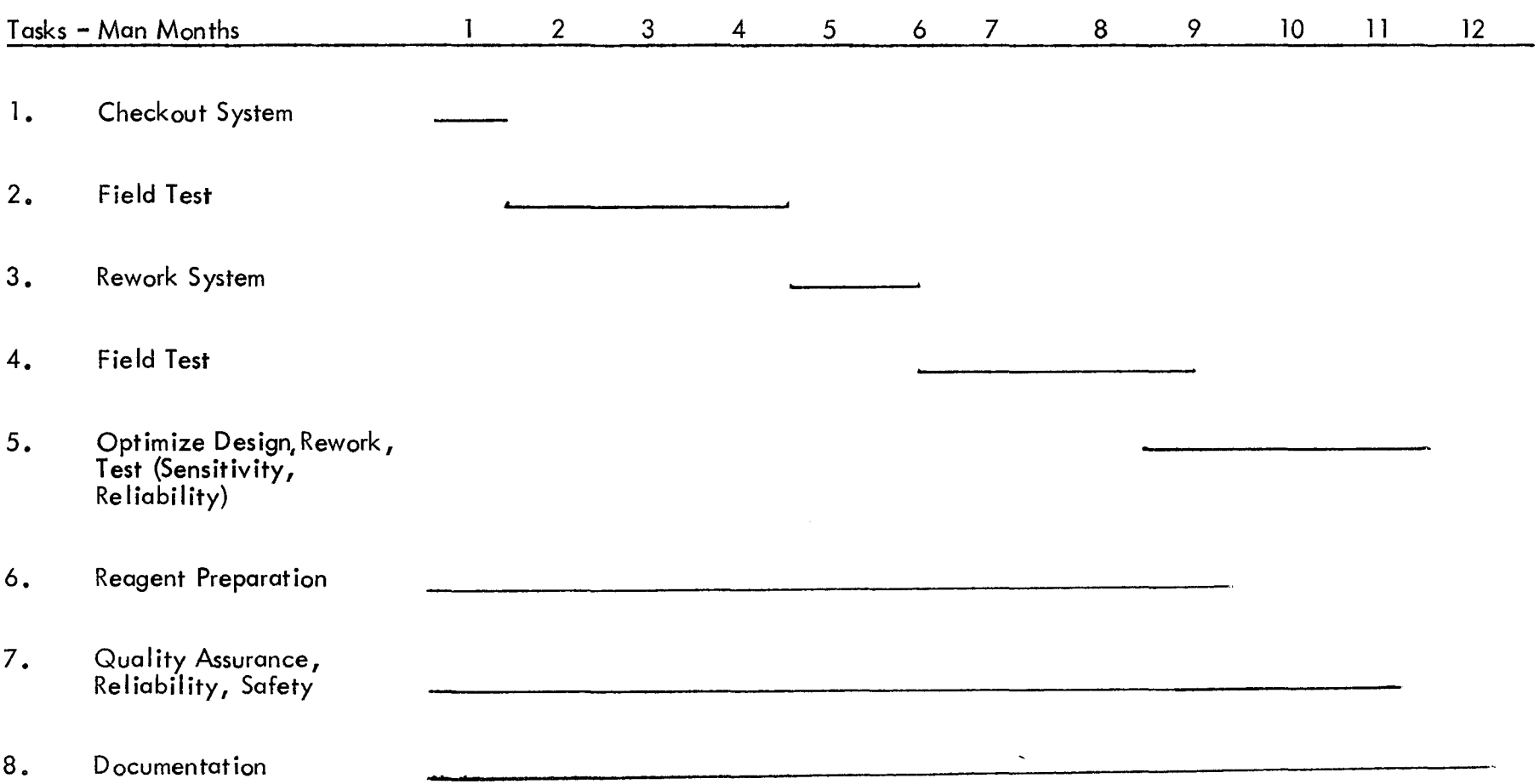


Figure 42

BEAD AGGLUTINATION

PROGRAM SCHEDULE - PHASE 4

Tasks - Man Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Design, Fab & Checkout Miniaturized & Modularized System	———	———	———	———	———	———	———	———										
2. Evaluate Sensitivity & Reliability Against Number of Virus & Toxin									———	———	———	———						
3. Build Additional Unit of Flight-Rated Prototype											———	———	———	———				
4. Field Test Both Units at Different Locations															———	———	———	
5. Reagent Preparation	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———		
6. Quality Assurance, Reliability, Safety	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———		
7. Documentation	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———

3. Fabrication of Additional Flight-Rated Prototype

Fabrication of additional flight-rated prototype incorporating any improvement suggested from the sensitivity evaluation (first unit will also be up-graded).

4. Field Test

Both units to be field tested at different locations interfacing with difference water-regeneration systems.

4. Reagent Preparation

Group antisera will be prepared for viruses of interest (i.e., myxovirus, adenovirus, infectious hepatitis). Specific antisera will also be prepared for exotoxins produced by Clostridium botulinum, Micrococcus pyogenes, (Staphylococcus) and Shigella dysenteriae.

Phase 5
(18 Months Duration)

OBJECTIVES

Qualification and acceptance testing of a flight-rated unit. Specific tasks (see Figure 43) include:

1. Design, Fabrication and Checkout of a Flight-Rated Prototype

Based on results of field testing in Phase 4, hardware design will be upgraded for optimum performance. Flight-rated prototype will be fabricated and checked out prior to submission for qualification testing.

2. Sensitivity & Reliability

Sensitivity and reliability against a single virus and toxin will be determined.

3. Qualification Testing

Sensitivity and reliability of the unit against agents of interest will be confirmed. System will also be subjected to vibration, shock and other tests required to achieve a flight-rated status.

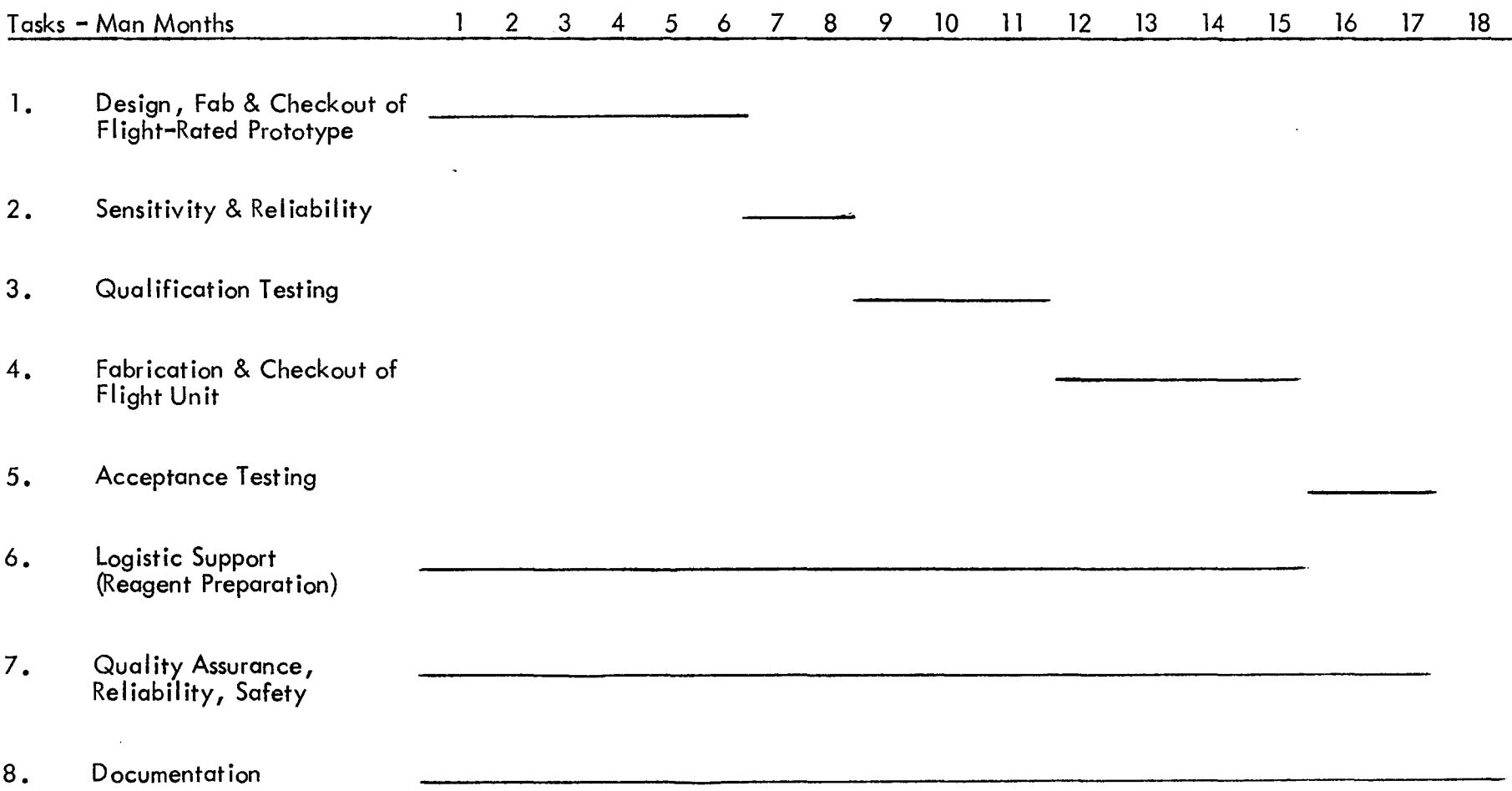
4. Fabrication of Flight-Rated Unit

An additional unit will be fabricated for use in acceptance testing prior to delivery to MSC.

Figure 43

BEAD AGGLUTINATION

PROGRAM SCHEDULE - PHASE 5



5. Acceptance Testing

Flight-rated unit will be subjected to tests and sensitivity and reliability using agents of interest.

6. Logistic Support

Reagents and technical support required for operation and maintenance of the unit will be provided and subject to qualification and acceptance testing.

Table 9

PROGRAM COST & MANPOWER ESTIMATES

	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
1. Porphyrin (Capsule) Chemiluminescence					
Duration, Months	8	16	16	18	-
Total Manhours	2800	3650	4200	23,500	-
Material Costs, $	18,000	20,000	59,000	88,000	-
Outside Testing, $	-	-	-	65,000	-
Total Price, $	79,000	93,000	168,000	716,000	-
2. Colony Counter					
Duration, Months	8	13	16	19	-
Total Manhours	2900	4100	5300	25,500	-
Material Costs, $	18,500	25,000	55,000	82,500	-
Outside Testing, $	-	-	-	65,000	-
Total Price, $	82,000	118,000	190,000	752,000	-
3. Bead Agglutination					
Duration, Months	15	12	12	18	18
Total Manhours	7350	6400	5314	13,200	28,500
Material Costs, $	45,000	58,500	18,500	135,000	135,000
Outside Testing, $	-	-	-	-	65,000
Total Price, $	195,000	188,000	135,000	426,000	862,000

*Includes Reagent Costs