Research Report

PLANETARY QUARANTINE

SC-RR-71 0681
September 1971

A COMPUTERIZED PROGRAM FOR STATISTICAL TREATMENT OF BIOLOGICAL DATA

A. L. Roark
Planetary Quarantine Systems Studies Division
M. C. Reynolds
Planetary Quarantine Applied Science Division

SANDIA LABORATORIES

OPERATED FOR THE UNITED STATES ATOMIC ENERGY COMMISSION BY SANDIA CORPORATION | ALBUQUERQUE, NEW MEXICO; LIVERMORE, CALIFORNIA

Issued by Sandia Corporation,
a prime contractor to the United States Atomic Energy Commission

---

NOTICE

SC-RR-71 0681

A COMPUTERIZED PROGRAM FOR STATISTICAL TREATMENT OF BIOLOGICAL DATA

A. L. Roark
Planetary Quarantine Systems Studies Division

M. C. Reynolds
Planetary Quarantine Applied Science Division
Sandia Laboratories, Albuquerque, New Mexico 87115

Completed - September 1971

Abstract

Biologists frequently conduct experiments which measure the patterns of inactivation of bacterial populations after exposure to a lethal environment. This document discusses a computer program which calculates many of the quantities that have proven to be useful in the analysis of such experimental data.

This work was conducted under Contract No. W-12,853, Planetary Programs, Office of Space Science and Applications, NASA Headquarters, Washington, D. C.

Contents

	Page
Introduction	5
Determination of Survivors	5
Statistical Methods	7
The Program	15
References	22

Figures

	Page
1. Sample Assay and Dilution Procedure	6
2. Program Flow Chart	16
3. Input Format	17
4. Output Format	18
5. Example of Input Data	19
6. Example of Output Data	20
7. Graphical Representation of Program Output	21

A COMPUTERIZED PROGRAM FOR STATISTICAL TREATMENT OF BIOLOGICAL DATA

Introduction

In the programs now underway in the Planetary Quarantine Department, it is frequently necessary to compare subtle changes in the destruction pattern of microorganisms. The use of standard pour plate techniques[1,2] for microbial assay during experimentation in some cases yields hundreds of data bits (plate counts). These must be reduced in a way that these successive samples taken during process application represent the destruction rate of microorganisms as a consequence of the process. This destruction rate is best described by a survivor curve since it relates the number of surviving organisms at any time to the sterilization process. The survivor curve is usually a y-axis plot of the logarithm of the number of organisms surviving the sterilization treatment versus the equivalent process time on the x-axis. This process time versus log of survivors or logarithmic model seems to be the most practical representation of data since essentially all thermoradiation and most heat and radiation sterilization has exhibited the logarithmic order of destruction. Consequently, the comparison of treatments can be made on the basis of the slope of the survivor curve or the D-value determined from the slope.

Based on this rationale, a computerized program has been developed to handle the statistical aspects of the data reduction. With plate counts of each successive sampling periods as an input, the program computes the mean value of the replicate plate counts, the variance, standard deviation, upper and lower .95 confidence intervals and the coefficient of variation for each sampling interval. Based on the coefficient of variation values for a sampling period, the dilution or data set exhibiting the best values are selected for each period. These best sets are then used in computing the survivor curve based on a least square fit of the logarithmic model.

Determination of Survivors

At any specific sampling period the procedure for assay is as follows: Four replicate samples are generally used for each sampling period. Aluminum foils or 0.020" thick square planchets are used as a substrate for the test organisms. After exposure to the sterilization treatment the substrate material is placed in a beaker with 10 ml sterile water and insonated for two minutes to suspend the organisms. From this base suspension, measured amounts of the inoculum are transferred to

petri dishes or additional dilution blanks[3] as required to result in plate counts between 30 and 300 colonies per plate. Within this range, the counts can be accurate, and the possibility of interference of the growth of an organism with that of another is minimized.

The determination of viable population from the resultant plate count is made as follows:

> Using the arrangement of dilution*, Figure 1, the inoculum from each of the four replicate samples for a single time period is plated in duplicate. Consequently, there are eight plates for each sampling period at a single level of dilution. Sometimes as many as three dilutions are plated out with the best set of data used as the surviving population at that sampling period.

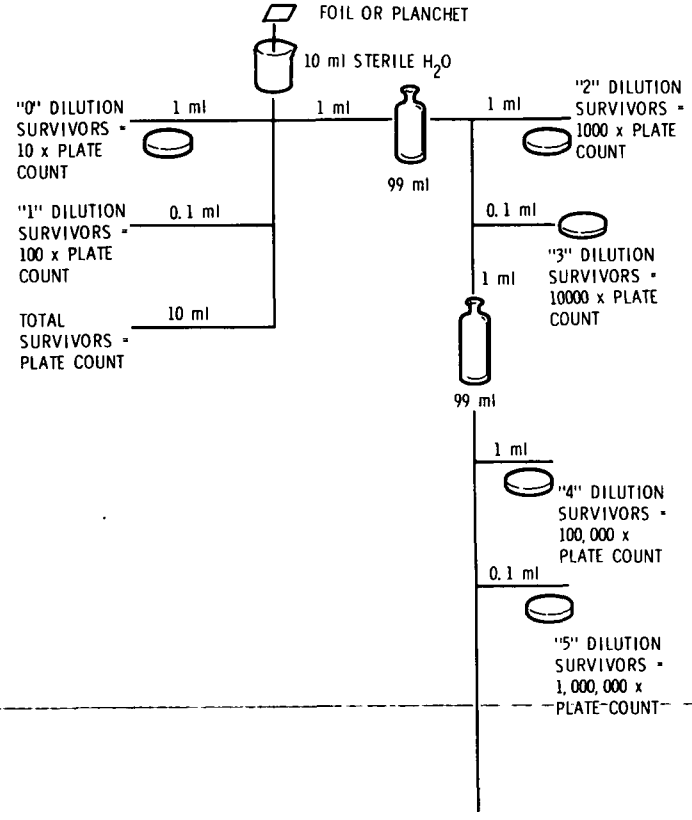

Figure 1. Sample Assay and Dilution Procedure

*For consistency in the input data, the total survivor plate counts will be assigned on order of dilution of "-1".

Statistical Methods

If we consider a single microorganism of a given type, we see that its loss of viability in a lethal environment is a random event. This fact has been explained in terms of natural variations between microorganisms brought about, in part, by their past history and by the hypothesis that loss of viability is due to the occurrence of chemical reactions[4]. In modeling the inactivation of microorganisms, researchers have usually attempted to derive expressions for the probability of single spore survival as a function of time of exposure to a given environment.

As we have pointed out earlier, instead of looking at the inactivation of a single spore, an experimenter considers the number of survivors in a given population as a function of time. We shall let the random variable $N(t)$ be the number of survivors at time t and let $p(t)$ represent the probability of single spore survival at time t. The model we shall assume defines the conditional probability as

$$\text{Prob.}\{N(t) = k | N(0) = N_o\} = \binom{N_o}{k} [p(t)]^k [1 - p(t)]^{N_o - k} . \tag{1}$$

Using the definition of conditional probabilities we have

$$\text{Prob.}\{N(t) = k\} = \sum_{N_o = 0}^{\infty} \text{Prob.}\{N(0) = N_o\} \ \text{Prob.}\{N(t) = k | N(0) = N_o\} . \tag{2}$$

Combining (1) and (2) yields

$$\text{Prob.}\{N(t) = k\} = \sum_{N_o = 0}^{\infty} \binom{N_o}{k} [p(t)]^k [1 - p(t)]^{N_o - k} \ \text{Prob.}\{N(0) = N_o\} . \tag{3}$$

We are usually interested in the expected value of the number of survivors as a function of time. Using the expression (3) it can be shown that

$$E(N(t)) = E(N(0))\, p(t) . \tag{4}$$

This is the basic expression for our model. In particular, the most widely used expression for the probability of single spore survival is provided by what is known

as the log model. Using this model we would have

$$p(t) = 10^{-t/D}$$

and thus (4) becomes

$$E(N(t)) = E(N(0))\ 10^{-t/D}. \tag{4'}$$

In this model, D is assumed to be a fixed parameter for a microorganism belonging to a homogeneous population.

Let us return for a moment to the experimental method used and consider the quantities we wish to compute for each dilution and each time period. Let us define

$x_{ij}(t_\ell)$ = number of colonies on plate i of dilution j at sampling period ℓ,

$\ell = 1, \ldots, M$

$j = 1, \ldots, K_\ell$

$i = 1, \ldots, N_{j\ell}$

where

M = number of sampling periods

K_ℓ = number of dilutions at sampling period ℓ

$N_{j\ell}$ = number of plates of dilution j for sampling period ℓ

and

t_ℓ = time of sampling period ℓ (in any units desired).

The mean of the plate counts for a particular dilution and sampling period is

$$\bar{x}_j(t_\ell) = \frac{1}{N_{j\ell}} \sum_{i=1}^{N_{j\ell}} x_{ij}(t_\ell)$$

while the variance of the distribution of plate counts can be approximated by the sample variance which is given by

$$s_j^2(t_\ell) = \frac{\sum_{i=1}^{N_{j\ell}} (\bar{x}_j(t_\ell) - x_{ij}(t_\ell))^2}{N_{j\ell} - 1}$$

$$= \frac{\sum_{i=1}^{N_{j\ell}} x_{ij}(t_\ell)^2 - N_{j\ell}\,\bar{x}_j(t_\ell)}{N_{j\ell} - 1} \quad . \tag{5}$$

Similarly, the standard deviation is approximated by the sample standard deviation which is given by

$$s_j(t_\ell) = \sqrt{s_j^2(t_\ell)} \quad . \tag{6}$$

To be more precise, let $\sigma_j^2(t_\ell)$ be the variance in the plate counts (this includes natural variation as well as any errors). The sample variance is a random variable which depends on the counts of the replicate plates. It can be shown that

$$\sigma_j^2(t_\ell) = E(s_j^2(t_\ell)) \quad .$$

Another desirable quantity for each dilution at each time interval is the confidence interval for the mean. This confidence interval is given for a particular time period by the expression

$$[L_j(t_\ell),\ U_j(t_\ell)] = \left[\bar{x}_j(t_\ell) - \frac{k s_j(t_\ell)}{\sqrt{N_{j\ell}}},\ \bar{x}_j(t_\ell) + \frac{k s_j(t_\ell)}{\sqrt{N_{j\ell}}}\right] \quad . \tag{7}$$

It is well known that as the number of samples, $N_{j\ell}$, becomes larger the parameter k for the α confidence limit should be chosen as the 100 $\alpha/2$ percentage point of the normal distribution. Thus, for the .95 confidence, k = 1.96 if $N_{j\ell}$ is large. Unfortunately, the number of plates of a given dilution at a given sampling time is usually small. In this case, the 100 $\alpha/2$ percentage point of the Student's t-distribution with $N_{j\ell} - 1$ degrees of freedom is more appropriate for k. Thus we can approximate k to sufficient accuracy by

$$k = \chi \left[1 + \frac{\chi^2 + 1}{4(N_{j\ell} - 1)} + \frac{(\chi^2 + 3)(5\chi^2 + 1)}{96(N_{j\ell} - 1)^2} \right] , \qquad (8)$$

where χ is the 100 $\alpha/2$ percentage point of the normal distribution. For our .95 confidence interval we let $\chi = 1.96$ in (8) to get our k for (7).

A good measure of the amount of spread in a particular set of data has been found to be the relative standard deviation[5]. This is more commonly known as the coefficient of variation. For each sampling period and each dilution it is defined to be

$$C_j(t_\ell) = \frac{s_j(t_\ell)}{\bar{x}_j(t_\ell)} .$$

In calculating the fit to the data of our "straight line" model, we wish to use the dilution at each time period which has the "tightest" data. We shall use the coefficient of variation as an index of the spread. Therefore, we let

$$X(t_\ell) = \bar{x}_J(t_\ell)$$

and

$$\sigma^2(t_\ell) = \sigma_J^2(t_\ell)$$

where J is chosen to minimize $C_j(t_\ell)$ for $j = 1, \ldots, K_\ell$. Let the order of this dilution (as defined in Figure 1) be d_ℓ.

We are now prepared to again consider the problem of applying our model to the data. Let

$$Y(t_\ell) = X(t_\ell) \times 10^{d_\ell + 1} . \qquad (9)$$

Then $Y(t_\ell)$ is an estimate of $E(N(t_\ell))$. In our model we wish to use $Y(t_\ell)$, $\ell = 1, \ldots, M$ to determine E(N(0)) and D as accurately as possible and to obtain some measurements of the statistical variations. Taking the natural log on both sides of (4') we obtain

$$\log E(N(t)) = \log (E(N(0)) + \gamma t , \qquad (10)$$

where

$$\gamma = \frac{2.303}{D} \quad . \tag{11}$$

With this model in mind, let us consider the equation

$$y(t) = \alpha + \beta t + \epsilon \tag{12}$$

where ϵ is a random variable representing the variation of the measured values about the line $\alpha + \beta t$.

Comparing (10) and (12) we see that we are assuming that

$$\alpha = \log (E(N(0))$$

or

$$E(N(0)) = e^{\alpha} \tag{13}$$

and

$$\beta = \frac{2.303}{D} = \gamma \quad . \tag{14}$$

The random variable ϵ in (12) represents the variation of the mean of the plate counts from the log model. This is assumed to be independent of time. This is consistent with assuming that the distribution of the variation in plate counts from the log model is independent of time. Let

$$y_{\ell} = \log Y(t_{\ell}) \quad .$$

Then y_{ℓ} is a sampled value of the random variable $\log E(N(t_{\ell}))$. Let us assume that ϵ is normally distributed and that

$$E(\epsilon) = 0.$$

For later convenience, let the variance of the distribution of ϵ be represented by σ_{ϵ}^2.

We are now prepared to calculate α and β. The following definitions will prove valuable:

1. $$\bar{t} = \frac{\sum_{\ell=1}^{M} t_\ell}{M} \tag{15}$$

2. $$\bar{y} = \frac{\sum_{\ell=1}^{M} y_\ell}{M} \tag{16}$$

3. $$b = \frac{\sum_{\ell=1}^{M} (t_\ell - \bar{t})(y_\ell - \bar{y})}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2} = \frac{\sum_{\ell=1}^{M} (t_\ell - \bar{t})\, y_\ell}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2} \tag{17}$$

4. $$a = \bar{y} - b\bar{t} \tag{18}$$

The quantities a and b depend on the samples used. The Gauss-Markoff theorem[6] tells us that a and b coincide with the maximum likelihood estimates of α and β and that they are unbiased, i.e.,

$$E(a) = \alpha$$

and

$$E(b) = \beta .$$

Letting

$$Z(t) = a + bt$$

and defining the standard error of estimate, $S_{y|t}$, by

$$S^2_{y|t} = \frac{\sum_{\ell=1}^{M} (Z(t_\ell) - y_\ell)^2}{M - 2}, \tag{19}$$

the Gauss-Markoff theorem also tells us that $S^2_{y|t}$ is an unbiased estimate of σ^2_ϵ, i.e.,[7]

$$E(S^2_{y|t}) = \sigma^2_\epsilon .$$

In addition to the Gauss-Markoff theorem, our assumptions on ϵ imply that a and b are also the minimum variance unbiased estimates of α and β respectively from among the class of all linear estimates. The standard deviation in b, which is given by

$$\sigma_b = \sqrt{\sigma_\epsilon^2 \Big/ \sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}$$

can be approximated by the standard error in the slope,

$$S_b = \frac{S_{y|t}}{\sqrt{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}} \quad .$$

Similarly, the standard deviation of the distribution of a,

$$\sigma_a = \sigma_\epsilon \sqrt{\frac{1}{M} + \frac{\bar{t}}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}}$$

can be approximated by the standard error in a*,

$$S_a = S_{y|t} \sqrt{\frac{1}{M} + \frac{\bar{t}}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}} \quad ,$$

where $S_{y|t}$ is given by (19).

It is also desirable in many applications to have a measure of how closely the variation in the log of the means of plate counts can be explained on the basis of only the variation of time in the lethal environment. The correlation coefficient, r, is defined by

*The quantity S_a shall be called the standard error in the estimated intercept.

$$r = \frac{\sum_{\ell=1}^{M} (t_\ell - \bar{t})(y_\ell - \bar{y})}{\sqrt{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2 \sum_{\ell=1}^{M} (y_\ell - \bar{y})^2}} = \frac{\sum_{\ell=1}^{M} t_\ell y_\ell}{\sqrt{\sum_{\ell=1}^{M} t_\ell^2 \sum_{\ell=1}^{M} y_\ell^2}}$$

Feller[8] proves the following statements can be made concerning r:

1. $|r| \leq 1$
2. $r = \pm 1$ implies that there exists constants ρ and θ such that $y = \rho t + \theta$ (except for a set of lines which have zero probability of occurring).

In addition, it can be shown that if y and t are independent, then $r = 0$. The converse of this statement is not true, however.

Let us return for a moment to the probability of single spore survival. Most microbiologists are interested in the D-value of the population. We have shown that we can approximate the D-value by

$$D = \frac{1}{b \log_{10} e} = \frac{2.303}{b} .$$

In addition, the standard error in the estimated D is given by

$$S_D = \frac{2.303\, S_b}{b^2} = \frac{D\, S_b}{b} .$$

Another feature which it is sometimes desirable to have available is the confidence band about the curve representing the model. This is also easily computed[6]. Let

$$S_{Z(t_\ell)} = S_{y|t} \sqrt{\frac{1}{M} + \frac{t_\ell - \bar{t}}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}}$$

Then the upper 95% confidence line is given by

$$Z_u(t_\ell) = a + b\, t_\ell + k\, S_{Z(t_\ell)}$$

and the lower by

$$Z_L(t_\ell) = a + b\,t_\ell - k\,S_{Z(t_\ell)}$$

where k is given by the Student's t-distribution of degree M-2. For the .95 confidence interval k is approximated by

$$k = \chi\left[1 + \frac{\chi^2 + 1}{4(M-2)} + \frac{(\chi^2 + 3)(5\chi^2 + 1)}{96(M-2)^2}\right]$$

where $\chi = 1.96$.

The Program

The flow chart for the program is given in Figure 2. This is self-explanatory. The input is prepared in the manner illustrated in Figure 3. The output is described in Figure 4 using the notation of the previous section.

Figure 5 provides an example of the input data while Figure 6 gives the output from the use of the progtam on this example.

Finally, a graphical representation of the data, the model, and the .95 confidence interval is shown graphically in Figure 7.

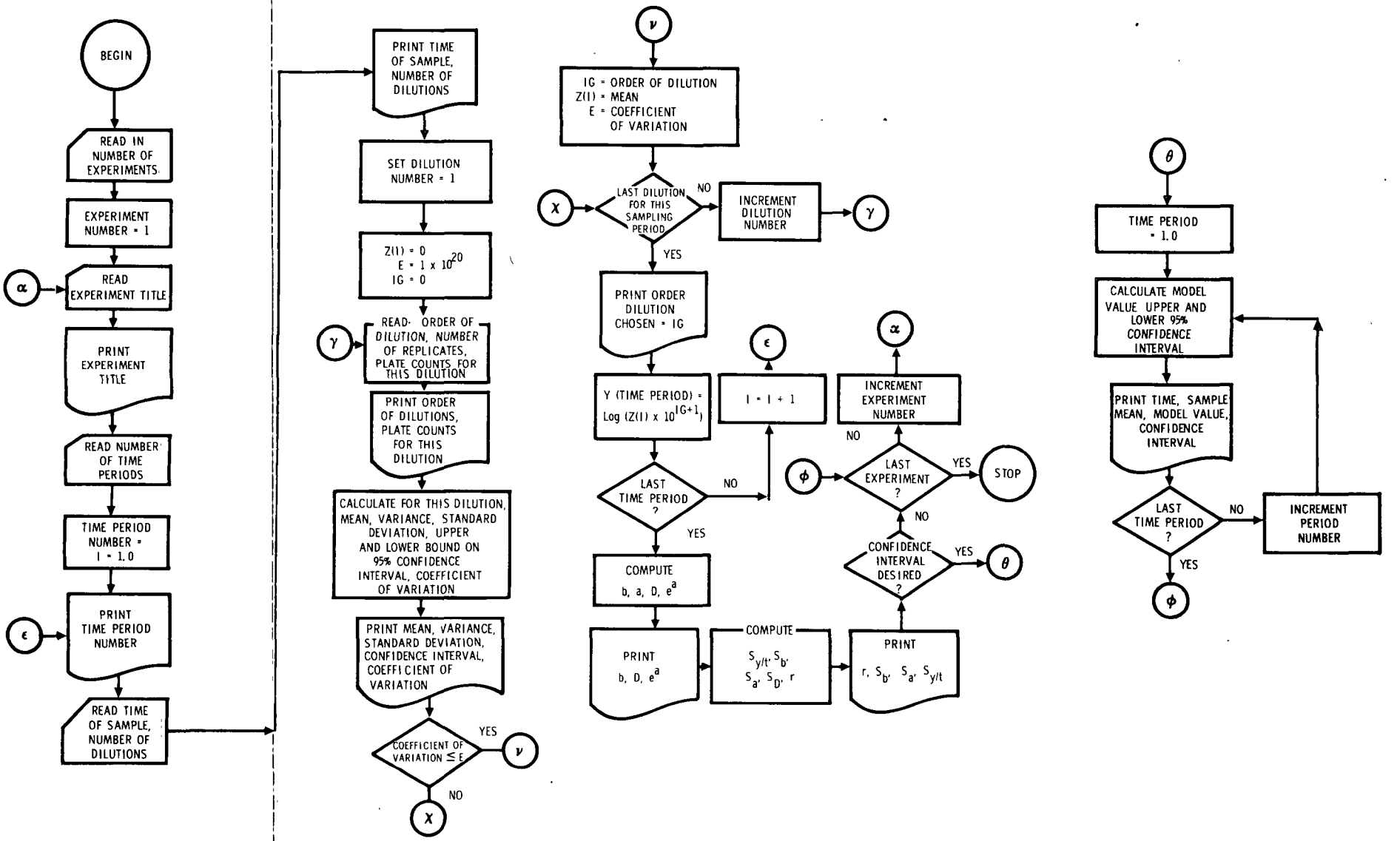

Figure 2. Program Flow Chart

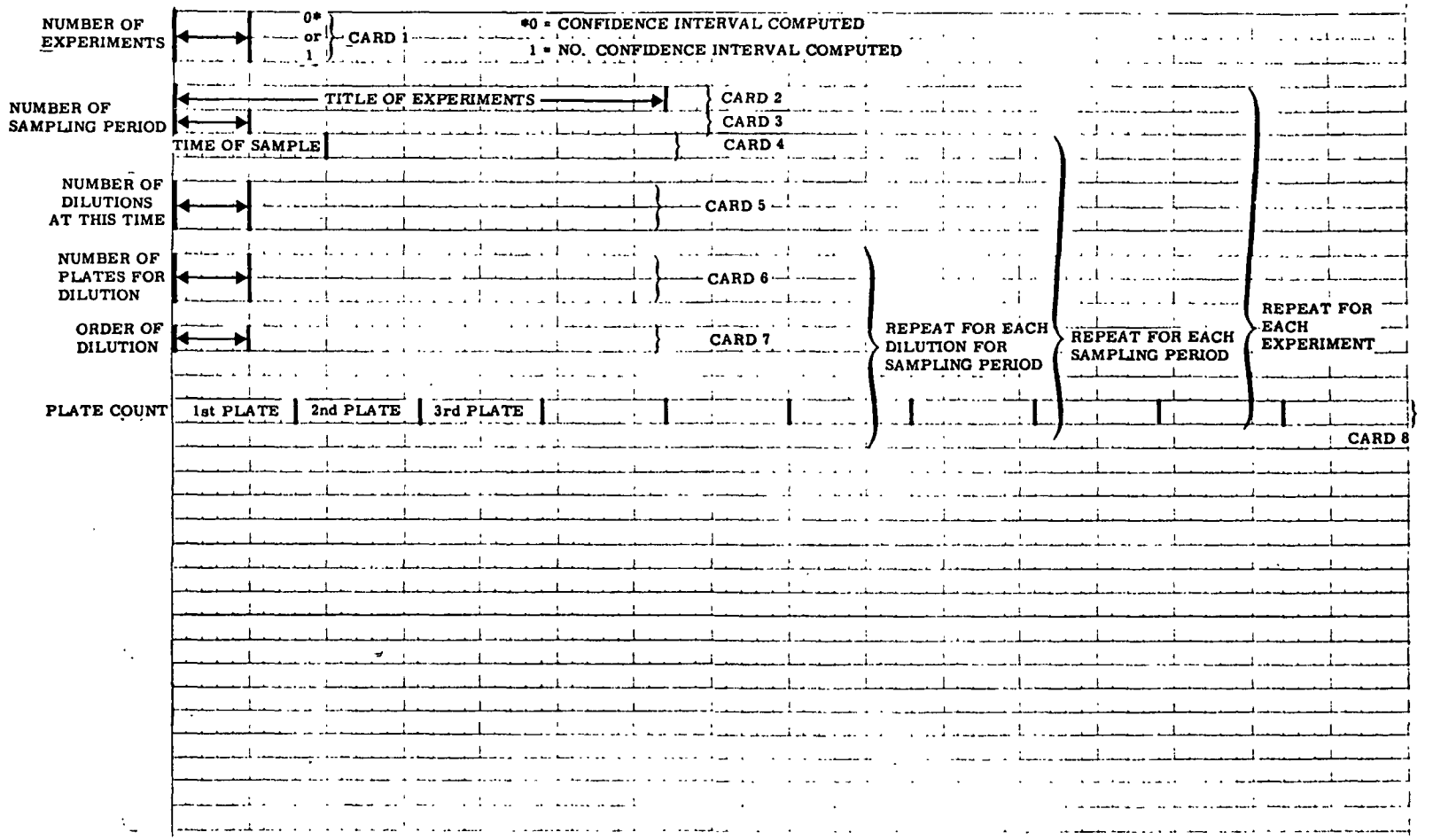

Figure 3. Input Format

TITLE OF EXPERIMENT

Repeat for each sampling time period:

Data Set = ____

Time = ____

Number of Dilutions = ____ (For this time period)

Repeat for each dilution:

Number of Data Points = ____ (Number of plate counts for this time period and dilution)

Order of Dilution = ____

Date (Plate Counts):

Mean = ____ Variance = ____ S.D. = ____ (Standard Deviation) Upper .95 C.I. = ____ (Upper limit of 95% Confidence Interval)(C.I.) Lower .95 C.I. = ____ C.V. = ____ (Coefficient of Variation)

Dilution Chosen = ____ (Dilution chosen for this time period on the basis of the coefficient of variation)

Slope = ____ (Slope of line of best fit to log of selected means) D-Value = ____ Intercept = ____ (Theoretical value of initial population*)

Corr. Coef. = ____ Stand. Err. in Est. Slope = ____ Stand. Err. of Est. = ____ Stand. Err. of Est. Inter. = ____

		.95 Conf. Interval (95% Confidence Interval about Model)		
T (Time)	SAMP (Data)	MODEL (Log Model Value)	Upper (Upper Limit of 95% Confidence Band)	Lower (Lower Limit of 95% Confidence Band)
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

*In computer output, E + 05 means 10^5 e.g., 2.31E + 06 represents 2.31×10^6

Figure 4. Output Format

```
    1
24 NOVEMBER 1970
    8
0.0
    1
    8
    3
265.     282.     299.     267.     250.     277.     265.     285.
3.0
    1
    8
    3
67.      58.      62.      57.      51.      75.      80.      72.
6.0
    1
    8
    2
58.      63.      80.      83.      62.      66.      75.      63.
9.0
    1
    8
    1
278.     212.     197.     201.     214.     255.     236.     231.
12.0
    1
    8
    1
48.      37.      37.      25.      25.      21.      42.      29.
15.0
    1
    8
    0
116.     135.     87.      62.      95.      82.      85.      84.
18.0
    2
    8
    0
26.      16.      12.      14.      11.      10.      18.      9.
    4
   -1
157.     117.     92.      162.
21.0
    1
    4
   -1
51.      50.      93.      89.
```

Figure 5. Example of Input Data

```
                                        24 NOVEMBER 1970

 DATA SET =     1
TIME=    0.000
 NO. DIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     3
DATA
   265.00    282.00    292.00    267.00    250.00    277.00    265.00    285.00
MEAN= 272.875  VARIANCE=    135.0  S.D.=     13.6  UPPER .95 C.I.=    284.2  LOWER .95 C.I.=    261.5  CV =    .0498
 CIL. CHOSEN =     3

 DATA SET =     2
TIME=    3.000
 NO. DIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     3
DATA
    67.00     58.00     62.00     57.00     51.00     75.00     80.00     72.00
MEAN=  65.250  VARIANCE=     99.4  S.D.=     10.0  UPPER .95 C.I.=     73.6  LOWER .95 C.I.=     56.9  CV =    .1528
 CIL. CHOSEN =     3

 DATA SET =     3
TIME=    6.000
 NO. DIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     2
DATA
    58.00     63.00     80.00     83.00     62.00     66.00     75.00     63.00
MEAN=  68.750  VARIANCE=     86.2  S.D.=      9.3  UPPER .95 C.I.=     76.5  LOWER .95 C.I.=     61.0  CV =    .1351
 CIL. CHOSEN =     2

 DATA SET =     4
TIME=    9.000
 NO. CIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     1
DATA
   278.00    212.00    197.00    201.00    214.00    255.00    236.00    231.00
MEAN= 228.000  VARIANCE=    777.7  S.D.=     27.9  UPPER .95 C.I.=    251.2  LOWER .95 C.I.=    204.8  CV =    .1223
 CIL. CHOSEN =     1

 DATA SET =     5
TIME=   12.000
 NO. DIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     1
DATA
    48.00     37.00     37.00     25.00     25.00     21.00     42.00     29.00
MEAN=  33.000  VARIANCE=     99.4  S.D.=      9.5  UPPER .95 C.I.=     40.9  LOWER .95 C.I.=     25.1  CV =    .2866
 CIL. CHOSEN =     1

 DATA SET =     6
TIME=   15.000
 NO. DIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     0
DATA
   116.00    135.00     87.00     62.00     95.00     82.00     85.00     84.00
MEAN=  93.250  VARIANCE=    508.5  S.D.=     22.5  UPPER .95 C.I.=    112.0  LOWER .95 C.I.=     74.5  CV =    .2418
 CIL. CHOSEN =     0

 DATA SET =     7
TIME=   18.000
 NO. DIL.=     2
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     0
DATA
    26.00     16.00     12.00     14.00     11.00     10.00     18.00      9.00
MEAN=  14.500  VARIANCE=     30.9  S.D.=      5.6  UPPER .95 C.I.=     19.1  LOWER .95 C.I.=      9.9  CV =    .3831
NUMBER DATA POINTS=     4
 ORDER OF DIL. =    -1
DATA
   157.00    117.00     92.00    162.00
MEAN= 132.000  VARIANCE=   1116.7  S.D.=     33.4  UPPER .95 C.I.=    183.2  LOWER .95 C.I.=     80.8  CV =    .2532
 CIL. CHOSEN =    -1

 DATA SET =     8
TIME=   21.000
 NO. DIL.=     1
NUMBER DATA POINTS=     4
 ORDER OF DIL. =    -1
DATA
    51.00     50.00     93.00     89.00
MEAN=  70.750  VARIANCE=    549.6  S.D.=     23.4  UPPER .95 C.I.=    106.7  LOWER .95 C.I.=     34.8  CV =    .3314
 CIL. CHOSEN =    -1


SLOPE=    -.521       D VALUE=    4.421       INTERCEPT=     2.3104308639E+06
CORR. COEF.=     .98018 STAND. ERR. IN EST. SLOPE=     .01896 STAND. ERR. OF EST.=     .13595 STAND. ERR. IN EST. INTER.=     .19913
                                          .95 CONF. INTERVAL
         T                    SAMP                  MODEL                 UPPER                 LOWER
    0.                  2.7287500000E+06      2.3104308639E+06      4.1234704598E+06      1.2945626333E+06
    3.0000000000E+00    6.5250000000E+05      4.8409570870E+05      7.7422063309E+05      3.0268975685E+05
    6.0000000000E+00    6.8750000000E+04      1.0143071530E+05      1.4820424844E+05      6.3418995170E+04
    9.0000000000E+00    2.2800000000E+04      2.1252388364E+04      2.9406330336E+04      1.5359414317E+04
    1.2000000000E+01    3.3000000000E+03      4.4529313417E+03      6.1613954984E+03      3.2131991139E+03
    1.5000000000E+01    9.3250000000E+02      9.3300560831E+02      1.3632497272E+03      6.3854732390E+02
    1.8000000000E+01    1.3200000000E+02      1.9548908311E+02      3.1264826143E+02      1.2223314929E+02
    2.1000000000E+01    7.0750000000E+01      4.0960077062E+01      7.3102238390E+01      2.2950431476E+01
```

Figure 6. Example of Output Data

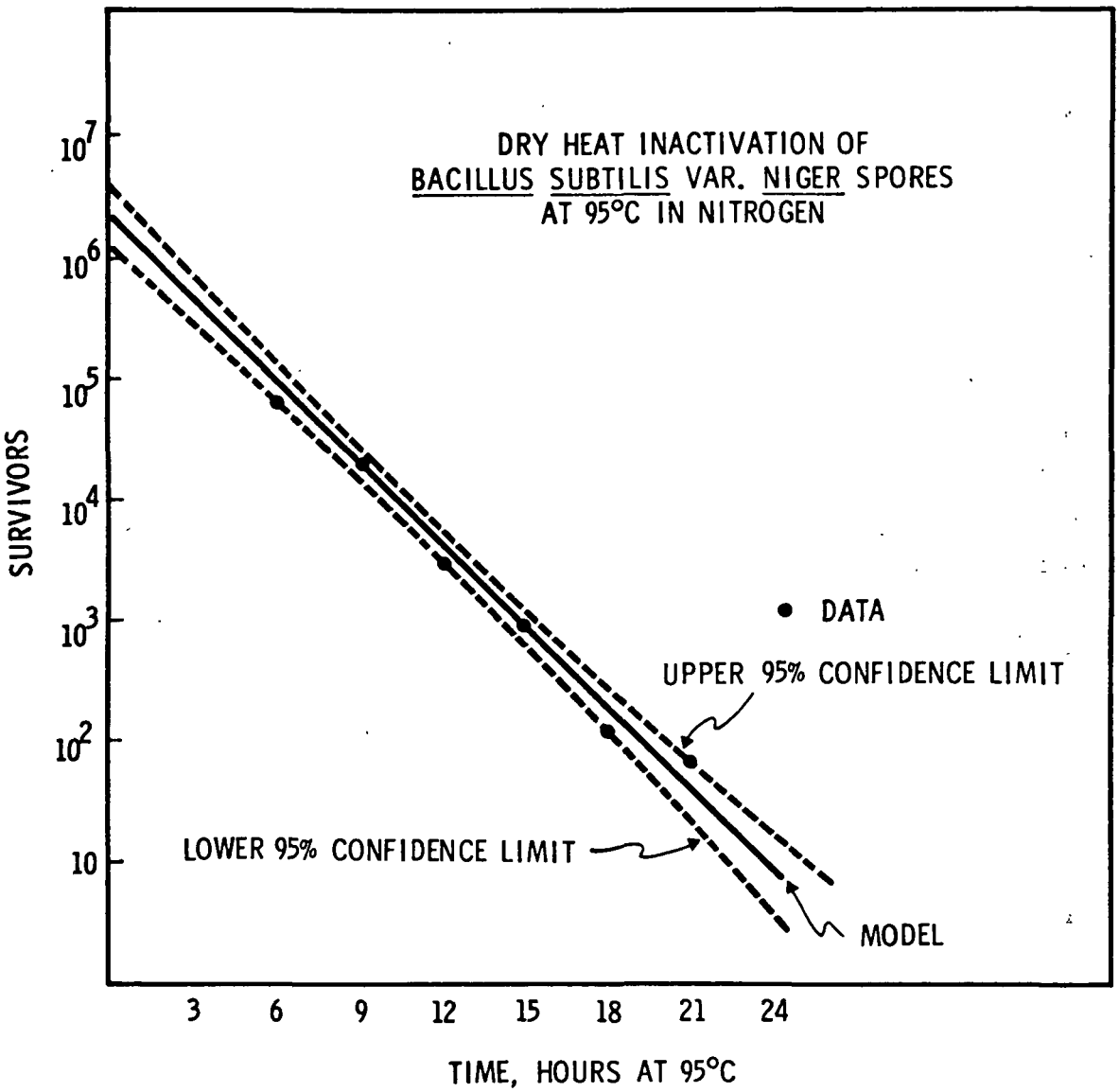

Figure 7. Graphical Representation of Program Output

References

1. NASA Standard Procedures for the Microbial Examination of Space Hardware. NHB-5340-1, National Aeronautics and Space Administration, August 1967.

2. Pelczar, Jr., M. I., Microbiology, McGraw Hill, New York, 1965.

3. B. B. L. Manual of Products and Laboratory Procedures, 5th Edition, BioQuest, Division of Becton, Dickinson and Company, Cockeysville, Maryland, 1968.

4. Brannen, J. P., "A Rational Model for Thermal Sterilization of Microorganisms," Math. Biosci., Vol. 2 (1968), pp. 165-179.

5. Wallis, W. A., and H. V. Roberts, Statistics, a New Approach, The Free Press, Glencoe, Illinois, 1956.

6. Hillier, F. S. and G. J. Lieberman, Introduction to Operations Research, Holden-Day, Inc., San Francisco, California, 1967.

7. Hemmerle, W. J., Statistical Computations on a Digital Computer, Blaisdel Publishing Company, Waltham, Massachusetts, 1967.

8. Feller, W., An Introduction to Probability Theory and Its Applications, Vol. 1, John Wiley & Sons, Inc., New York, 1968.

DISTRIBUTION:
NASA, Code SL
Grants and Contracts
400 Maryland Avenue S. W.
Washington, D. C. 20546 (25)

L. B. Hall, Code SL
NASA Headquarters
400 Maryland Avenue S. W.
Washington, D. C. 20546 (25)

B. W. Colston, Director
Space & Special Programs Division
Office of Operations
U. S. Atomic Energy Commission
Albuquerque, New Mexico 87115

L. P. Daspit, Jr.
Viking Project Quarantine Officer
Viking Project Office, NASA
Langley Research Center
Hampton, Virginia 23365

University of California
P. O. Box 808
Livermore, California 94551
Attn: Tech. Info. Div.
For: Report Librarian

Los Alamos Scientific Laboratory
P. O. Box 1663
Los Alamos, New Mexico 87544
Attn: Report Librarian

Richard G. Bond
School of Public Health
College of Medical Science
University of Minnesota
Minneapolis, Minnesota 55455

John H. Brewer
Mountain View Road
Star Route 2
Brownwood, Texas 76801

Edward B. Kasner
Director of Research Services
Graduate College
University of New Mexico
Albuquerque, New Mexico 87106

Frank B. Engley, Jr., Chairman
Department of Microbiology
School of Medicine
University of Missouri
Columbia, Missouri 65201

Gilbert V. Levin
Biospherics, Inc.
4928 Wyaconda Road
Rockville, Maryland 20853

Irving J. Pflug
Professor of Environmental Health
545 Space Science Center
University of Minnesota
Minneapolis, Minnesota 55455

Gerald J. Silverman
Department of the Army
U. S. Army Natick Laboratories
Natick, Massachusetts 01760

John A. Ulrich
Department of Microbiology
School of Medicine
University of New Mexico
Albuquerque, New Mexico 87106

Samuel Schalkowsky
Exotech Systems, Inc.
525 School Street S. W.
Washington, D. C. 20024

Mark A. Chatigny
Research Engineer
Naval Biological Laboratory
Naval Supply Center
University of California, Berkeley
Oakland, California 94625

Richard G. Cornell
Associate Professor of Statistics
Department of Statistics
Florida State University
Tallahassee, Florida 32306

Dr. Richard C. Corlett
Department of Mechanical Engineering
University of Washington
Seattle, Washington 98105

DISTRIBUTION (Cont)

Martin S. Favero
Department of Health, Education
and Welfare
CDC-Phoenix Field Station
4402 North 7th Street
Phoenix, Arizona 85014

Mr. James Martin
Viking Project Engineer
Langley Research Center, NASA
Langley Station
Hampton, Virginia 23365

Q. Ussery
Code NC3, Quality Assurance Branch
Manned Spacecraft Center, NASA
Houston, Texas

F. J. Beyerle
George C. Marshall Space Flight Center
Manufacturing Engineering Laboratory
Code R-ME-MMC
Huntsville, Alabama 35812

J. Gayle
Code SOP
Kennedy Space Center, NASA
Cape Canaveral, Florida

Murray Schulman
Division of Biology and Medicine
Headquarters, AEC
Washington, D. C. 20545

N. H. MacLeod
Space Biology Branch
Code 624, Bldg. 21, Room 161
Goddard Space Flight Center
Greenbelt, Maryland 20771

Jeptha E. Campbell, Ph. D., Chief
Division of Microbiology
Food and Drug Administration
DHEW, Public Health Service
1090 Tusculum Avenue
Cincinnati, Ohio 54226

G. Rotariu
Process Radiation Staff
Division of Isotopes Development
Headquarters, AEC
Washington, D. C. 20545

Martin G. Koesterer, Microbiologist
Bioscience Operation
Valley Forge Space Technology Center
P. O. Box 8555
Philadelphia, Pennsylvania 19101

Carl W. Bruch, Chief
Drug Microbiology Branch
BD415
Food and Drug Administration
200 C Street S. W.
Washington, D. C. 20204

John W. Beakley
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87106

Loren D. Potter, Chairman
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87106

Loris W. Hughes
Department of Biology
New Mexico State University
University Park, New Mexico

Richard W. Porter
Corporate Engineering Staff
General Electric Company
570 Lexington Avenue
New York, New York 10022

Fred L. Whipple
Smithsonian Astrophysical Observatory
Cambridge, Massachusetts 02138

J. J. McDade
Environmental Research Laboratory
Building 1710
Dow Chemical Company
Midland, Michigan 48640

Otto Hamberg
Aerospace Corporation
Building A2, Room 2019
2350 East El Segundo Blvd.
El Segundo, California

DISTRIBUTION (Cont)

Lawrence P. Chambers
NASA Headquarters
Office of Manned Space Flight
Code MLR
Washington, D. C. 20546

Arthur H. Neill
Code SL
400 Maryland Avenue S. W.
Washington, D. C. 20546

Richard H. Green
Sterilization Group
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, California 91103

Rudy Puleo
Spacecraft Bioassay Unit
Center for Disease Control
USPHS
Cape Kennedy, AFS, Florida 32900

USAEC, Division of Technical Information
P. O. Box 62
Oak Ridge, Tennessee 37830
Attn: Reference Branch
P. E. Postell

Carl Sagan
Cornell University
Center for Radiophysics and Space Research
Space Science Building
Ithaca, New York 14850

Document Library
Lovelace Foundation for Medical
Education and Research
5200 Gibson Blvd. S. E.
Albuquerque, New Mexico 87108

Martin S. Tierney
Group J-10
Los Alamos Scientific Laboratory
Los Alamos, New Mexico 87544

Jack Kaye
11607 Georgetowne Court
Potomac, Maryland 20854

Dr. Robert Angelotti
Office of Food Sanitation, FDA
200 C Street S. W. (BF-201)
Washington, D. C. 20204

Vance I. Oyama, Chief
Life Detection Systems Branch
NASA, Ames Research Center
Moffett Field, California 94035

Byron W. Brown, Jr.
Department of Preventive Medicine
Stanford University School of Medicine
Stanford University Medical School
Palo Alto, California 94304

Don G. Fox
Sterility Control Officer
NASA Headquarters, Code SL
400 Maryland Avenue S. W.
Washington, D. C. 20546

A. A. Rothstein
Manager, Planetary Quarantine
Viking Program
Martin Marietta Corporation
P. O. Box 179
Denver, Colorado 80201

Hillel S. Levinson
U. S. Army Natick Laboratory
Natick, Massachusetts 01760

Dr. Walter M. Urbain
College of Agriculture
Michigan State University
East Lansing, Michigan 48823

H. O. Halvorson
1901 East River Road
Minneapolis, Minnesota 55414

A. Anellis
U. S. Army Natick Laboratories
Natick, Massachusetts 01760

H. W. Johnson, LTC
U. S. Army Medical Research and
Development Command
Washington, D. C. 20314

DISTRIBUTION (Cont)

Donald A. Kautter
Department of HEW
Food and Drug Administration
Division of Microbiology
BF-135
200 C Street S. W.
Washington, D. C. 20204

Lt. Keith C. Hopkins (SAH)
Biomedical Group
Air Force Weapons Laboratory
Kirtland Air Force Base
Albuquerque, New Mexico 87115

Briggs Phillips
Becton, Dickinson Research Center
P. O. Box 11276
Raleigh, North Carolina 27604

Dr. Wolf Vishniac
Department of Biology
University of Rochester
Rochester, New York 14627

F. A. Leone, Program Manager
Radiation Preservation of Foods
Division of Isotope Development
AEC Headquarters, Mail Sta. 255
Washington, D. C. 20545

Dr. Orr Reynolds
American Physiological Society
2134 LeRoy Place N. W.
Washington, D. C. 20008

Dr. Henry Eyring
Chemistry Department
University of Utah
Salt Lake City, Utah 84112

Dr. Allan H. Brown
Department of Biology
University of Pennsylvania
Philadelphia, Pa. 19104

Mr. Marvin Morris
20 Whitman Road, Apt. 1-1
Waltham, Mass. 02154

J. A. Hornbeck, 1
J. M. Wiesen, 100
W. J. Howard, 1000
D. B. Shuster, 1200
C. B. McCampbell, 1310
W. A. Gardner, 1500
H. E. Lenander, 1600
T. M. Burford, 1700
C. Winter, 1710
T. M. Burford (Actg.), 1720
J. M. Worrell, Jr., 1721
D. P. Peterson, 1724
R. G. Clem, 1730
H. E. Sivinski, 1740 (35)
A. A. Lieber, 1750
B. H. Van Domelen, 1913
A. M. Clogston, 5000
L. C. Hebel, 5200
J. V. Walker, 5220
R. M. Jefferson, 5221
J. E. McDonald, 5300
L. M. Berry, 5500
R. E. Henderson, 7000
G. A. Fowler, 9000
J. H. Scott, 9200
L. Hollingsworth, 9300
L. A. Hopkins, Jr., 9400
D. W. Ballard, 9461
R. S. Gillespie, 3151 (3) (For Public Release)
L. S. Ostrander, 8232
W. K. Cox, 3142-1 (15)

q/c