A LARGE SAMPLE BIO ASSAY DESIGN

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TECHNICAL REPORT NO. 17

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STANFORD, CALIFORNIA
A Large Sample Bio Assay Design

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Summary:

A large sample locally optimal design is presented for a bio-assay problem in the presence of Poisson dose error and an unknown amount of dose material.

The conclusions given are that for the exponential model, (i) only one dose level designs are optimal, (ii) if the cost of experimental animals is negligible then as many animals as are available should be used in the experiment, (iii) a very good approximation to the proportion of dose material which is to be injected in test animals is

\[ \beta = \min \left[ \frac{1}{1 + \frac{1}{\alpha}}, \frac{(1.6)s}{\alpha \lambda} \right] \]

Introduction:

A bio-assay of a strain of lethal or infectious bacteria consists in performing injections, in test animals, of various doses of the bacteria and using the resulting data to estimate the dose response relationship peculiar to the particular bacteria strain - animal type used in the experiment.

One of the models in current use states that the probability of a negative response following the injection of n bacteria in a given test animal is \( (1 - \alpha)^n \). When individual doses are obtained by diluting a
portion of a concentrated mixture of bacteria, the actual dose used will be a Poisson random variable. The expected dose in this case will be referred to as the nominal dose. The probability that a dose of nominal dose \( N \) will produce a negative reaction is

\[
p_N(\alpha) = e^{-\alpha N}
\]

The problem of concern here is one of design. Suppose that a vessel holds, in solution, an unknown number \( N \) of infections bacteria. Assume that \( N \) is a random variable obeying an unknown Poisson law of probability. Let \( \lambda \) be the expectation of \( N \). It is also assumed that the experiment is to be carried out in two parts: (i) An assay to estimate the unknown Poisson parameter \( \lambda \); and (ii) injection of experimental animals to determine the dose response curve. What then is an allocation of the bacterial material to each of these subexperiments which provides an optimal estimate for \( \alpha \)? (It is shown above that \( \alpha \) characterizes the dose response curve.)

**Allocation of doses:**

Denote by \( \beta \) the fraction of the bacterial material to be reserved for part (ii), the injection part of the experiment, and by \( s \) the number of animals to be injected. If \( r \) dose levels are to be used, and \( \beta_i \) is the fraction of the bacterial material to be used for the \( i^{th} \) dose level, \( s_i \) the number of animals receiving a dose of the \( i^{th} \) level,

\[
s = \sum_{i=1}^{r} s_i, \quad \beta = \sum_{i=1}^{r} \beta_i
\]

then the nominal dose received by each animal at the \( i^{th} \) dose level will be

\[
\frac{\beta_i}{s_i} \lambda
\]

We first inquire as to which value of \( r \) is optimal. Since the result is to be local and for large samples, we define the optimal \( r \) to be that which provides the maximum information matrix. (The information
matrix defined by Fisher is

$$
\left( \frac{2}{\theta} \frac{\partial}{\partial \theta_i} \frac{\partial}{\partial \theta_j} \ln f(x, \Theta) \right)
$$

where $f$ is the probability density and $\Theta = (\alpha, \lambda)$ is the vector parameter to be estimated. Denoting by $Z$ the random variable observed during the assay, then $Z$ has a Poisson law of probability with expectation $(1-\beta) \lambda$. Letting $X_{ij} = 1$ if the $j$th animal at the $i$th dose level gives a positive response and $X_{ij} = 0$ if a negative response, we see that the joint probability density of $(Z, X_{11}, X_{12}, \ldots, X_{rs}, \ldots)$ is

$$
P[Z = z, X_{11} = x_{11}, \ldots, X_{rs}, = \ldots, \alpha, \beta, \lambda, s] = e^{-\lambda(1-\beta)} \frac{\lambda^z(1-\beta)^{\alpha-1}}{z!} \prod_{i=1}^{r} \prod_{j=1}^{s} (1-p_i) X_{ij} p_i^{1-X_{ij}},
$$

where

$$
\beta = \frac{s_1 \lambda}{\alpha}
$$

$p_i = e^{-\beta}$, since all these random variables are independent.

Fixing $\beta$ and $s$ the information matrix when $r$ dose levels are used is

$$
\mathbf{X}_r = \left( \begin{array}{cc} 0 & 0 \\ 0 & 1-\beta \end{array} \right) + \left( \begin{array}{cc} \lambda^2 & \alpha \lambda \\ \alpha \lambda & \alpha^2 \end{array} \right) \sum_{i=1}^{r} \frac{\beta_i^2}{s_i} \frac{p_i}{1-p_i}
$$

We use the customary ordering for semi-positive definite matrices, that is $X_r' = X_r''$ means that $X_r' = X_r''$ is a semi-positive definite matrix. From this definition and $X_r$, we see that $X_r' = X_r''$ if and only if

$$
\sum_{i=1}^{r} \frac{\beta_i^2}{s_i} \frac{p_i}{1-p_i} = \sum_{i=1}^{r''} \frac{\beta_i^2}{s_i} \frac{p_i}{1-p_i}
$$

where the two experiments use nominal dose levels

$$
\frac{\beta_1'}{s_1'}, \lambda, \ldots, \frac{\beta_r'}{s_r'}, \lambda
$$
and \( \frac{\beta_1}{s_1}, \ldots, \frac{\beta_r}{s_r} \) respectively, with \( \beta = \sum_{i=1}^r \beta_i = \sum_{i=1}^r \beta_i \) and
\[
s = \sum_{i=1}^r s_i = \sum_{i=1}^r s_i.
\]
Since for fixed \( \beta, s \), inequality (3) depends only on the injection part of the experiment, we might inquire as to whether or not \( r = 1 \) is optimal, for if it were the entire experiment would be a mixture of two experiments used in a two parameter problem.

Now \( X = X_r \) for any \( r = 1, 2, \ldots \) if and only if

\[
\sum_{j=1}^r \frac{\beta_j}{s_j} \frac{p_j}{1-p_j} \leq \frac{\beta}{s} \frac{p}{1-p}.
\]

A sufficient condition for inequality (4) is that the function \( f(\mu) = \frac{\mu^2}{e^{\mu} - 1} \) be concave for positive values of \( \mu \). This can be seen by identifying \( \theta_i = \frac{s_i}{s}, \mu_i = \frac{\beta_i}{s_i} \alpha \lambda \) and then (4) becomes equivalent to

\[
\sum_{i=1}^r \theta_i f(\mu_i) = f\left(\sum_{i=1}^r \theta_i \mu_i\right).
\]

Considering the function \( f(\mu) \) we compute

\[
f'(\mu) = (e^{\mu} - 1)^{-3} \left[ e^{2 \mu}(\mu-4, \mu+2) + e^{\mu}(\mu^2+4 \mu-4) + 2 \right].
\]

Expanding \( e^{2 \mu} \) and \( e^{\mu} \) in Taylor Series we get

\[
f''(\mu) = (e^{\mu} - 1)^{-3} \sum_{j=2}^{\infty} A_j \frac{\mu^j}{j!}
\]

with \( A_j = j^2 (j-9) + 4(2 j-1) + j^2 \)

so that \( \alpha_j < 0 \) if \( j = 2, 3, \ldots, 8 \)

\[
\alpha_j < 0 \text{ if } j = 9, 10, \ldots
\]

from this it can be shown that \( f(\mu) \) has only one positive root which is computed to be approximately 3.086. If then \( 0 < \mu < 3.086 \) the function \( f \) is concave. We have proven the
**Lemma:** If all nominal doses proposed are less than \( ED_{95} \), the optimal number of dose levels is one.

Suppose further that a nominal dose greater than \( ED_{95} \) is proposed for use. Because the function \( f \) has a unique maximum near \( \mu = 1.6 \) and is zero for \( \mu = 0 \) there will always exist a nominal dose less than \( ED_{95} \) (in fact less than \( ED_{80} \) which would be optimal if enough material was available) which will give the same information as the proposed dose but this lesser dose would require less material for injection and hence the value of \( 1 - \beta \) could be increased with no loss of information from injection; however the information contributed by the assay is \( \begin{pmatrix} 0 & 0 \\ 0 & 1 - \beta \end{pmatrix} \) and an increase in \( 1 - \beta \) gives an increase in this information matrix. The total information from the entire experiment would then be increased by using the lesser nominal dose in place of the proposed nominal dose greater than \( ED_{95} \). This result is stated in the

**Lemma:** To achieve maximum information only nominal dose less than \( ED_{80} \) need be considered. Putting the two lemmas together produces the

**Theorem:** The optimal number of dose levels, for the model considered here is \( r = 1 \).

In the following paragraphs we will consider only the case \( r = 1 \).

Suppose further that the maximum number of animals available is \( s_0 \).

From the probability density (1) we compute the maximum likelihood estimates of \( \lambda, \alpha \) to be \( \hat{\lambda} = \frac{2}{1 - \beta}, \hat{\alpha} = -\frac{s}{\beta} \frac{1}{\lambda^2} \log (1 - \tilde{x}) \), so that \( \hat{\alpha} = -\frac{s}{2} \frac{(1 - \beta)}{\lambda} \log (1 - \tilde{x}) \).

The asymptotic variance of \( \hat{\alpha} \) is

\[
\sigma^2(\hat{\alpha}) = \frac{\hat{\alpha}^2}{\lambda(1 - \beta)} + \frac{s}{\beta^2} \frac{1}{\lambda^2} (e^{-\frac{\lambda}{\beta}} - 1)
\]

\[
= \frac{\alpha^2}{\lambda(1 - \beta)} + \frac{s}{\beta^2} \frac{1 - p}{p} \left( \frac{1}{p} \right)
\]

where \( p = \frac{e^{-\frac{\lambda}{\beta}}}{\beta} \).
Differentiating with respect to $s$

$$\frac{\partial \sigma^2(\hat{\lambda})}{\partial s} = \frac{1}{p \beta^2 \lambda^2} (1-p + \log p)$$

expanding $\log p$ and collecting terms

$$\frac{\partial \sigma^2(\hat{\lambda})}{\partial s} = \frac{-1}{p \beta^2 \lambda^2} \sum_{k=1}^{\infty} (1-p) \frac{2k}{2k(2k+1)} < 0$$

so that $\sigma^2(\hat{\lambda})$ is a decreasing function of $s$. Since $s$ is the number of experimental animals used the design will be improved if $s$ is increased, so that $s = s_0$ will be optimal.

**Allocation of Bacterial Material:**

Since any optimal value of $\beta$ depends on the values of $\lambda$, $\alpha$, designs of this type can only be hoped to have local optimal properties. However in many situations some a priori knowledge of $\lambda$, $\alpha$ is available which should enable one to get a fairly efficient design.

Examining $\sigma^2(\hat{\lambda})$ as a function of $\beta$ we see that for small values of $\frac{\alpha \lambda}{s}$

$$\sigma^2(\hat{\lambda}) = \frac{s}{\beta^2 \lambda^2} \left[ \frac{\beta \lambda}{s} \alpha \right] + \frac{\alpha^2}{\lambda(1-\beta)}$$

$$= \frac{\alpha}{\lambda} \left\{ \frac{1}{\beta} + \frac{\alpha}{1-\beta} \right\}$$

which is a minimum for

$$\beta = \frac{1}{1 + \sqrt{\alpha}}$$

For large values of $\lambda$ there should be enough material to give each animal a dose of approximately $ED_{80}$ which is the optimal single dose as can be determined again from the information function. If this is the case

$$\frac{\beta \lambda}{s} \alpha \approx 1.6$$
so that \( \beta = \frac{(1.6)s}{\alpha \lambda} \) should be close to the optimal value.

Let \( \beta^* = \min \left[ \frac{1}{1 + \frac{1}{\alpha}}, \frac{(1.6)s}{\alpha \lambda} \right] \).

Table 1. gives for various values of \( \alpha, \lambda \) the actual value of \( \beta \) minimizing \( \sigma^2(\hat{\lambda}) \) for \( s = 30 \), the upper and lower limits of the values of \( \beta \) giving at least 80% efficiency, and the minimum value of \( \sigma^2(\hat{\lambda}) \).

If Table 1. is to be used for values of \( s \) other than \( s = 30 \) the conversion formula

\[
\sigma^2(\hat{\lambda}) = \frac{1}{\gamma^2} \sigma^2(\hat{\lambda}) \quad \text{for all } \alpha, \beta, s, \lambda, \text{is useful.}
\]

For example, if for \( s = 60, \lambda = 500, \alpha = .0392 \), the minimum variance and optimal \( \beta \) is desired, we see from Table 1. that for \( s = 30, \lambda = 2500, \alpha = .0392 \) the optimal \( \beta \) is \( \beta = .48 \). From equation (8)

\[
\sigma^2(\hat{\lambda}) = \frac{1}{2} \sigma^2(\hat{\lambda}) \quad \text{for } s = 60, \lambda = 500, \alpha = .0392; \beta = .48 \quad \text{30, \lambda = 2500, } \alpha = .0392; \beta = .48
\]

\[
= \frac{1}{2} \times 10^{-4}(.803300)
\]

\[
= 10^{-4}(.40165)
\]

The optimal value of \( \beta \) remains at \( \beta = .48 \) and the upper and lower 80% efficiency limits on \( \beta \) remain at .78 and .26 respectively. If \( \beta = \beta^* \) is used as an approximation to the optimal value of \( \beta \) we see that this gives a design of at least 80% efficiency and an extremely efficient design for small \( \lambda, \alpha \), and large \( \lambda \) as can be seen in two instances from figures 1 and 2.

Conclusion:

It should be emphasized that there is no provision for testing the exponential model in this design. The result of one dose level being optimal is a direct consequence of the one parameter nature of the true dose-response curve given in the introduction.
Comparison of $\beta$ Values as a Function of $\lambda$

$\alpha = .1305$

$s = 30$

Figure 1
Various $\beta$ Values and the Minimum Asymptotic Variance of $\hat{\alpha}$.

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*Key*

- $\beta$ Lower 80% Efficiency Limit
- $\beta^o$ Optimal Value of $\beta$
- $\beta^o$ Upper 80% Efficiency Limit
- $\sigma^2(\hat{\alpha})$ Minimum Asymptotic Variance of $\hat{\alpha}$. 
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