# The Spearman-Karber Method of Estimating 50% Endpoints

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A suitable test dose of virus is selected, for example 300 $ID_{50}$, and falling dilutions of serum are added in equal amounts to the appropriate virus dilution. The lesions in the inoculated eggs are recorded and the neutralizing endpoint is expressed as that dilution of serum which "protects" 50% of the eggs against the test dose of virus used.

| Dilution | Number of Eggs Tested | Number of Eggs Infected | Proportion of Eggs Infected |
|---|---|---|---|
| $10^{x}$ | $n_0$ | $r_0$ | $r_0/n_0$ |
| $10^{d+x}$ | $n_1$ | $r_1$ | $r_1/n_1$ |
| $10^{2d+x}$ | $n_2$ | $r_2$ | $r_2/n_2$ |
| $\vdots$ | $\vdots$ | $\vdots$ | $\vdots$ |
| $10^{(m-1)d+x}$ | $n_{m-1}$ | $r_{m-1}$ | $r_{m-1}/n_{m-1}$ |
| $10^{md+x}$ | $n_m$ | $r_m$ | $r_m/n_m$ |
| | | | Sum = S |

Then the 50% endpoint is found using the formula below:

$$\log 50\% \text{ endpoint} = x+d(m+1/2-S)$$

where

- $x$ is $\log_{10}$ of the lowest dilution
- $d$ is $\log_{10}$ of the dilution factor
- $m$ is one less than the number of dilutions used
- $S$ is the sum of proportion of eggs infected

Example: (equal numbers of eggs at each dilution)

| Dilution | Number of Eggs Tested | Number of Eggs Infected | Proportion of Eggs Infected |
|---|---|---|---|
| $10^{-1}$ | 5 | 0 | 0/5 |
| $10^{-2}$ | 5 | 2 | 2/5 |
| $10^{-3}$ | 5 | 4 | 4/5 |
| $10^{-4}$ | 5 | 5 | 5/5 |
| | | | S = 11/5 |

Here we have:

$$x = -1,\ d = -1,\ m = 3$$

so

$$\log 50\% \text{ endpoint} = -1 -(3 + \frac{1}{2} - \frac{11}{5})$$

$$= -1 -(3.5 - 2.2) = -2.3$$

Therefore, the 50% endpoint is $10^{-2.3}$.

Suppose there are unequal numbers of eggs at the different dilutions as

| Dilution | Number of Eggs Tested | Number of Eggs Infected | Proportion of Eggs Infected |
|---|---|---|---|
| $10^{-1}$ | 3 | 0 | 0/3 |
| $10^{-2}$ | 5 | 1 | 1/5 |
| $10^{-3}$ | 4 | 3 | 3/4 |
| $10^{-4}$ | 5 | 5 | 5/5 |
| | | | S = 39/20 |

Here

$$x = -1,\ d = -1,\ m = 3$$

so

$$\log 50\% \text{ endpoint} = -1 -(3 + \frac{1}{2} - S)$$

$$= -2.55$$

For two-fold dilutions the same procedure is used; we might have, for example:

| Dilution | Number of Eggs Tested | Number of Eggs Infected | Proportion of Eggs Infected |
|---|---|---|---|
| 1/2 | 5 | 0 | 0/5 |
| 1/4 | 5 | 1 | 1/5 |
| 1/8 | 5 | 3 | 3/5 |
| 1/16 | 5 | 4 | 4/5 |
| 1/32 | 5 | 5 | 5/5 |
| | | | S = 13/5 |

We have:

$$x = -.3,\ d = -.3,\ m = 4$$

so

$$\log 50\%\ \text{endpoint} = -.3 - .3(4 + \tfrac{1}{2} - 2.6)$$

$$= -.87$$

For $ID_{50}$ titer for virus dilutions the same procedure is used except the number of eggs showing no lesions are used.

Example:

| Dilution | Number of Eggs Tested | Number of Eggs Not Infected | Proportion of Eggs Not Infected |
|---|---|---|---|
| $10^{-1}$ | 5 | 0 | 0/5 |
| $10^{-2}$ | 5 | 2 | 2/5 |
| $10^{-3}$ | 5 | 4 | 4/5 |
| $10^{-4}$ | 5 | 5 | 5/5 |
| | | | S = 11/5 |

Here

$$x = -1,\ d = -1,\ m = 3$$

so

$$\log 50\%\ \text{endpoint} = -1 - (3 + \tfrac{1}{2} - 2.2)$$

$$= -2.30$$

In general we have:

| Dilution | log Dilution | Number of Eggs Tested | Number Eggs Infected | Proportion of Eggs Infected |
|---|---|---|---|---|
| $Y_1$ | $x_1$ | $n_1$ | $r_1$ | $r_1/n_1$ |
| $Y_2$ | $x_2$ | $n_2$ | $r_2$ | $r_2/n_2$ |
| $Y_3$ | $x_3$ | $n_3$ | $r_3$ | $r_3/n_3$ |
| ⋮ | ⋮ | ⋮ | ⋮ | ⋮ |
| $Y_k$ | $x_k$ | $n_k$ | $r_k$ | $r_k/n_k$ |
| | | | | Sum = S |

Then we have

log 50% endpoint =

$$\frac{x_k+x_{k-1}}{2} - \frac{1}{2}\left[\frac{r_2}{n_2}(x_3-x_1)+\frac{r_3}{n_3}(x_4-x_2)+\frac{r_4}{n_4}(x_5-x_3)+\cdots+\frac{r_{k-1}}{n_{k-1}}(x_k-x_{k-2})\right]$$

If the dilutions are equally spaced on the log scale then this reduces to the formula previously given. If the dilutions are unequally spaced, as, for example, when all eggs at one dilution die, then the above formula must be used.

| Dilution | log Dilution $x_i$ | Number of Eggs Tested $n_i$ | Number of Eggs Infected $r_i$ | Proportion of Eggs Infected $r_i/n_i$ |
|---|---|---|---|---|
| $10^{-1}$ | -1 | 4 | 0 | 0/4 |
| $10^{-2}$ | -2 | 5 | 1 | 1/5 |
| $10^{-4}$ | -4 | 4 | 2 | 1/2 |
| $10^{-5}$ | -5 | 3 | 2 | 2/3 |
| $10^{-6}$ | -6 | 5 | 5 | 5/5 |

log 50% endpoint

$$= \frac{-5-6}{2} - \frac{1}{2}\left[\frac{1}{5}(-4+1)+\frac{1}{2}(-5+2)+\frac{2}{3}(-6+4)\right]$$

$$= \frac{-11}{2} + \frac{1}{2}\left[\frac{3}{5}+\frac{3}{2}+\frac{4}{3}\right]$$

$$= \frac{-11}{2} + \frac{103}{60} = \frac{-227}{60} = -3.78$$

Reference:

Finney, D. J., 1952. Statistical Method in Biological Assay. New York: Hafner Publishing Company.