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Determination of 50% endpoint titer using a simple formula

Muthannan Andavar Ramakrishnan

Muthannan Andavar Ramakrishnan, Division of Virology, Indian Veterinary Research Institute, Uttarakhand 263138, India

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Correspondence to: Muthannan Andavar Ramakrishnan, Senior Scientist, Division of Virology, Indian Veterinary Research Institute, Mukteswar Campus, Uttarakhand 263138, India. maramakrishnan@gmail.com
Telephone: +91-5942-286346
Fax: +91-5942-286347

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Abstract

Two commonly used methods for calculating 50% endpoint using serial dilutions are Spearman-Karber method and Reed and Muench method. To understand/apply the above formulas, moderate statistical/mathematical skills are necessary. In this paper, a simple formula/method for calculating 50% endpoints has been proposed. The formula yields essentially similar results as those of the Spearman-Karber method. The formula has been rigorously evaluated with several samples.

Key words: Endpoint dilution; TCID50; Spearman-Karber; Reed and Muench

Core tip: The formula described in this manuscript can be used to calculate 50% endpoint titre such as TCID50%, LD50, TD50, *etc*., in addition to the currently existing methods. The proposed formula can be applied without the help of calculator or computer.

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TO THE EDITOR

Currently, there are two methods (formulas) *viz*., Reed and Muench[1] and Spearman-Karber[2,3] are commonly employed for the calculation of 50% endpoint by serial dilution. To understand/apply these methods, moderate mathematical skills along with calculator or computer are essential. Here, I have proposed a simple formula to calculate the 50% endpoint titre and this formula can be used in addition to Reed and Muench or Spearman-Karber, methods but not exclusively at this point. In the following section, the newly proposed method is compared with two commonly used methods viz., Reed and Muench and Spearman-Karber.

Reed and Muench method

$\log_{10}$ 50% end point dilution = $\log_{10}$ of dilution showing a mortality next above 50% - (difference of logarithms × logarithm of dilution factor).

Generally, the following formula is used to calculate "difference of logarithms" (difference of logarithms is also known as "proportionate distance" or "interpolated

Table 1 Calculation of virus titre in mice using the Reed and Muench method

Log_{10} virus dilution	Mice		Cumulative total			Percent mortality
	Died	Survived	Died	Survived	Total	
-1	10	0	57	0	57	57/57 × 100 = 100
-2	10	0	47	0	47	47/47 × 100 = 100
-3	10	0	37	0	37	37/37 × 100 = 100
-4	10	0	27	0	27	27/27 × 100 = 100
-5	10	0	17	0	17	17/17 × 100 = 100
-6	6	4	7	4	11	7/11 × 100 = 63
-7	1	9	1	13	14	1/14 × 100 = 7

Difference of logarithms = (63-50)/(63-7) = 0.23; log_{10} 50% end point dilution = -6 - (0.23 × 1) = -6.23; 50% end point dilution = $10^{-6.23}$; the titre of the virus = $10^{6.23}$ LD_{50}/mL.

Table 2 Calculation of virus titre in mice using the Spearman-Karber method

Log_{10} virus dilution	Mice	
	Died	Inoculated
-1	10	10
-2	10	10
-3	10	10
-4	10	10
-5	10	10
-6	6	10
-7	1	10

x_0 = 5; d = 1; log_{10} of 50% endpoint dilution = - [5 - ½ + 1 (17/10)] = -6.2; 50% end point dilution = $10^{-6.2}$; the titre of the virus = $10^{6.2}$ LD_{50}/mL.

value"): Difference of logarithms = [(mortality at dilution next above 50%)-50%]/[(mortality next above 50%)-(mortality next below 50%)].

Spearman-Karber method

log_{10} 50% end point dilution = - (x_0 - d/2 + d Σ r_i/n_i)

x_0 = log_{10} of the reciprocal of the highest dilution (lowest concentration) at which all animals are positive;

d = log_{10} of the dilution factor;

n_i = number of animals used in each individual dilution (after discounting accidental deaths);

r_i = number of positive animals (out of n_i).

Summation is started at dilution x_0.

Newly proposed method

Formula 1:

log_{10} 50% end point dilution = -[(total number of animals died/number of animals inoculated per dilution) + 0.5] × log dilution factor.

Formula 2 (if any accidental death occurred):

log_{10} 50% end point dilution = -(total death score + 0.5) × log dilution factor.

Table 3 Calculation of virus titre in mice using the new method

Log_{10} virus dilution	Mice		Death score
	Died	Inoculated	
-1	10	10	10/10 = 1
-2	10	10	10/10 = 1
-3	10	10	10/10 = 1
-4	10	10	10/10 = 1
-5	10	10	10/10 = 1
-6	6	10	6/10 = 0.6
-7	1	10	1/10 = 0.1
Total	57		5.7

By using formula 1: log_{10} 50% end point dilution = - (57/10 + 0.5) × 1 = -6.2; 50% end point dilution = $10^{-6.2}$; the titre of the virus = $10^{6.2}$ LD_{50}/mL. By using formula 2: log_{10} 50% end point dilution = - (5.7 + 0.5) × 1 = -6.2; 50% end point dilution = $10^{-6.2}$.

Comparison of the newly proposed and existing methods with an example of virus titration in mice: For simplicity, it is assumed that 1 mL of each dilution was inoculated (Tables 1-3).

The newly proposed formula has been intensively validated with several samples and essentially yields the same results as those by the Spearman-Karber method. Therefore, the newly proposed method can be used in addition to the existing methods but not exclusively at this point.

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