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Antivenoms: potency or median effective dose, which to use?

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Dear Editor:

In the field of envenomations by poisonous animals, the ability of antivenoms to neutralize the lethal effects of venoms is estimated by a biological assay in which mice are inoculated with a range of venom/antivenom concentrations and the survival/death ratio is recorded. A statistical technique (e.g., Probit) is employed to estimate the amount of antivenom that protects 50% of the animals. This quantity is called median effective dose or effective dose 50 (ED_{50}) and is normally expressed in volume units (i.e., mL or µL). The ED_{50} is used in an expression for the assessment of the potency (P) of the antivenom, as follows (1, 2):

$$P = (\mathbf{n} - 1)LD_{50} / ED_{50} \qquad [1]$$

where "LD_{50}" is the median lethal dose (mass of venom that kills 50% of mice), and "**n**" is the number of LD_{50}s used in the assay. "P" is the amount of venom, expressed in mass units or number of median lethal doses, that is completely neutralized per unit volume of antivenom (the expression "(**n** – 1) LD_{50}" is used instead of the total amount of venom, **n**LD_{50}, because at the endpoint of the neutralization assay, one LD_{50} remains unneutralized and causes the death of 50% of mice). However, in the literature, it is very frequent to find that the same term, ED_{50}, is utilized to represent

the neutralization capacity of antivenoms, typically as "the ratio of the total amount of venom used in the assay to the volume dose of antivenom that keeps alive 50% of the animals", in the same units as potency. The use of this term as a measure of the antivenom neutralization capacity is ambiguous and can generate severe errors, as shown below. To avoid confusion, let us call ER_{50} ("median effective ratio") the ED_{50} (mass/volume) magnitude so that:

$$ER_{50} = \mathbf{n}LD_{50}/ED_{50} \qquad [2]$$

Now, by opening the parentheses in equation [1] and substituting equation [2] we obtain:

$$P = ER_{50} - LD_{50}/ED_{50} \qquad [3]$$

which clearly shows that ER_{50} is larger than P, precisely on the account of the contribution of the last term on the right side of the equation, which stems from the lethal dose that remains unneutralized at the endpoint of the assay. Equation [3] also indicates that P is a property of the antivenom that represents 100% protection. Therefore, in a set of assays where the venom challenge dose is changed, P can be expected to remain constant.

So, to show the behavior of ER_{50} under these conditions, we have carried out simulated assays where when the potency is fixed at 1 LD_{50}/mL; the venom dose challenge, **n**, changes between 2 and 20 and the ED_{50} and ER_{50} are estimated from equations [1] and [2], respectively. The results of the assays are presented in Table 1 in increasing values of **n**. Notice that for low values of **n** (2 or 3), as frequently used in the literature, ER_{50} can differ from P by as much as 100%. Thereafter, ER_{50} decreases with increasing **n** and, for larger values of **n**, ER_{50} and P tend to the same value. On the other hand, for **n** = 5, the value recommended in the Pharmacopoeia (1), there is still a 25% difference between P and ER_{50}.

In conclusion, it can be stated that ER_{50} varies substantially with the venom challenge dose at lower values of **n**. On the other hand, since P is independent of **n**, and the calculation uses the same experimental information, it should be the parameter of choice to report neutralization results.

Table 1. Simulated assays for 50 % survival of mice

n per mouse (challenge)	**n** per mouse (neutralized)	ED_{50} (mL/mouse)	ER_{50} (LD_{50}/mL)	Potency (LD_{50} /mL)
2	1	1	2.00	1
3	2	2	1.50	1
4	3	3	1.33	1
5	4	4	1.25	1
10	9	9	1.11	1
20	19	19	1.05	1

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