THE DILUTION ASSAY OF VIRUSES. II

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If λ is the average density of virus particles per unit volume and a^m ($a > 1$, $m = \dots -2, -1, 0, 1, 2, \dots$) are the dilution levels, the proportion of eggs remaining sterile at dilution a^m (if eggs are used as the material to be infected) will be $e^{-\lambda a^m}$ if the ordinary assumptions of dilution assay are valid. In many cases this is not true because the eggs vary amongst themselves in their infectibility. If p is the probability of any one particle (when present) being infective for an individual egg, and if p does not vary from particle to particle the probability of the egg remaining sterile is $e^{-p\lambda a^m}$. If p varies from egg to egg so that it has a probability distribution $f(p)$, the probability of an egg chosen at random remaining sterile is

$$P_m = \int_0^1 e^{-p\lambda a^m} f(p) dp.$$

If a series of dilutions is taken and P_m plotted against m , this will give a flatter curve than the exponential. No valid estimate of λ can then be made, but in order to study the effect of various chemical substances on the infectibility of the egg it is very important to be able to recognize this phenomenon quickly when it occurs.

One method of doing this is to attempt to fit an exponential curve by maximum likelihood methods and use χ^2 as a test of goodness of fit. This is laborious if it has to be done on a large number of such experiments and in a previous paper (Moran, 1954) I have proposed a simple test which can be carried out in less than five minutes and which is probably more powerful than the χ^2 test in testing for deviations of this kind.

Let n eggs be tested at each dilution and let f_i remain sterile at the i th dilution. Calculate the quantity $T = \sum f_i(n - f_i)$. Then it is easy to show that

$$E(T) = n(n-1) \sum P_i(1 - P_i),$$

$$\text{var}(T) = n(n-1) \{ (n-1) \sum P_i(1 - P_i) - (4n-6) \sum P_i^2(1 - P_i)^2 \}.$$

The distribution of T , being the sum of a number of independent variates, will not be very far from normal so that we can test the deviation of T from $E(T)$ as if it were normally distributed with zero mean and standard error equal to $\sqrt{\text{var}(T)}$ provided we can calculate $\sum P_i(1 - P_i)$ and $\sum P_i^2(1 - P_i)^2$. This assumes that the series is sufficiently long for the probabilities to be effectively zero and unity at the two ends. Also as our alternative hypothesis is that the curve is flatter than the exponential, we need only a one-sided test, a standardized deviation from expectation being significant at the 5 and 1 % levels if it exceeds 1.645 and 2.326 respectively.

For a twofold dilution series this test was given in a previous paper (where the

formula for the variance occurs in a slightly different form). In this case $\Sigma P_i(1-P_i)$ is identically equal to unity because $P_{i+1}=P_i^2$. $\Sigma P_i^2(1-P_i)^2$, however, depends on λ , and if $\lambda=a^\mu$, is a periodic function of μ . Its mean value is 0.169925 and by calculating it for a series of typical values of μ it can be shown to lie always in the range 0.169915 to 0.169935.

The purpose of the present note is to give the results for other dilution series and tables for the rapid application of the test. For a fourfold dilution series $\Sigma P_i(1-P_i)$ is also a periodic function of μ . Its mean value is 0.5 and it always lies in the range 0.497 to 0.503. Similarly, the mean value of $\Sigma P_i^2(1-P_i)^2$ is 0.0850 and it always lies in the range 0.082 to 0.088.

Table 1. Values of $E(T)$

n	Twofold	Fourfold	$\sqrt{\text{Tenfold}}$	Tenfold
5	20	10	12.04	6.02
6	30	15	18.06	9.03
7	42	21	25.29	12.64
8	56	28	33.72	16.86
9	72	36	43.35	21.67
10	90	45	54.19	27.09
11	110	55	66.23	33.11
12	132	66	79.47	39.74
13	156	78	93.92	46.96
14	182	91	109.58	54.79
15	210	105	126.43	63.22
16	240	120	144.50	72.25
17	272	136	163.76	81.88
18	306	153	184.23	92.12
19	342	171	205.91	102.95
20	380	190	228.79	114.39
30	870	435	523.80	261.90
40	1560	780	939.23	469.61

For a $\sqrt{\text{tenfold}}$ dilution series the mean value of $\Sigma P_i(1-P_i)$ is 0.60207, and it always lies between 0.601 and 0.603. $\Sigma P_i^2(1-P_i)^2$ has a mean value 0.1023 and lies between 0.1013 and 0.1033.

For the tenfold dilution the mean value of $\Sigma P_i(1-P_i)$ is 0.301, and $\Sigma P_i(1-P_i)$ lies between 0.27 and 0.33, whilst the mean value of $\Sigma P_i^2(1-P_i)^2$ is 0.0512, lying between 0.035 and 0.067. For this series the variation is now getting large and the test somewhat dubious.

In the above calculations the mean values were found theoretically by explicit evaluation of the corresponding integrals and the ranges within which the sums lie were found by calculating them for ten values of μ between 0 and 1. Thus for all dilution series other than the twofold $E(T)$ has a periodic component in μ . It is interesting to verify mathematically that $E(T)$, averaged over μ , will always be greater than the average value given above if the probability distribution of p is not concentrated in a single point. By elementary but somewhat complicated algebra this can in fact be proved.

Table 1 gives the values of $E(T)$ (averaged over μ if necessary) for twofold, fourfold, $\sqrt{\text{tenfold}}$ and tenfold dilution series, whilst Table 2 gives corresponding value of $\sqrt{\text{var}(T)}$.

Since T is the sum of a number of independent bounded variates we can expect its distribution to be close to normality. The accuracy of the approximation will be better for large n and small dilution. The exact distribution for small μ can be found numerically (for a given value of μ) and this was done for $n = 5$ and fourfold dilution. Only fair agreement was found at the one-sided 5 and 1 % points but the closeness of the approximation should increase rapidly with n , and in any case should be much better for twofold dilutions.

The average expectation of T can be evaluated for various assumptions about the distribution of p , e.g. for a rectangular distribution. Similarly in the case where the distribution of p is approximated by a gamma type distribution so that P has the form of the zero term of a negative binomial, the average expectation of T can be expressed as the difference of two digamma functions.

Table 2. *Values of $\sqrt{\text{var}}(T)$*

n	Twofold	Fourfold	$\sqrt{\text{Tenfold}}$	Tenfold
5	5.69	4.02	4.42	3.12
6	7.63	5.40	5.92	4.19
7	9.75	6.89	7.57	5.35
8	12.02	8.50	9.33	6.60
9	14.46	10.22	11.22	7.93
10	17.03	12.04	13.21	9.34
11	19.74	13.96	15.32	10.83
12	22.58	15.97	17.52	12.39
13	25.55	18.07	19.82	14.02
14	28.63	20.24	22.21	15.71
15	31.83	22.51	24.70	17.74
16	35.14	24.85	27.27	19.28
17	38.56	27.27	29.92	21.16
18	42.07	29.75	32.64	23.08
19	45.70	32.31	35.46	25.08
20	49.41	34.94	38.34	27.11
30	91.53	64.72	71.02	50.22
40	141.49	100.05	109.79	77.63

REFERENCE

MORAN, P. A. P. (1954). *J. Hyg., Camb.*, **52**, 189.

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