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A MODEL TO ASSESS THE ACCURACY OF DETECTING ARBOVIRUSES IN MOSQUITO POOLS

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Abstract

Vigilant surveillance of virus prevalence in mosquitoes is essential for risk assessment and outbreak prediction. Accurate virus detection methods are essential for arbovirus surveillance. We have developed a model to estimate the probability of accurately detecting a virus-positive mosquito from pooled field collections using standard molecular techniques. We discuss several factors influencing the probability of virus detection, including the number of virions in the sample, the total sample volume, and the portion of the sample volume that is being tested. Our model determines the probability of obtaining at least 1 virion in the sample that is tested. The model also determines the optimal sample volume that is required in any test to ensure a desired probability of virus detection is achieved, and can be used to support the accuracy of current tests or to optimize existing techniques.

Keywords

Surveillance; probability of detection; infection; arboviruses

Arboviral encephalitides such as West Nile, St. Louis encephalitis, eastern equine encephalitis, and western equine encephalomyelitis viruses are threats to human and veterinary health in the United States. Mosquito control and health agencies employ a wide range of surveillance methods for arbovirus detection, including virus testing of field-collected mosquito pools and sentinel chicken serosurveillance (Day and Stark 1996, Day 2001, Blackmore et al. 2003). Common methods for arbovirus testing of mosquito pools include plaque assay, reverse transcriptase polymerase chain reaction (RT-PCR), Rapid Analyte Measurement Platform (RAMP) (Response Biomedical Corporation, Vancouver, BC, Canada), VecTest® (Medical Analysis Systems, Freemont, CA), and quantitative real-time RT-PCR (qRT-PCR) (Blackmore et al. 2003, Bell et al. 2005, Farajollahi et al. 2005, Savage et al. 2006, Turell et al. 2006, Vitek et al. 2008).

Various statistical models have examined how sample size and field infection rate can influence the probability of collecting a virus-positive mosquito from the field sample (Gu and Novak 2004, Lord et al. 2006, Gu et al. 2008). These models assume that biochemical tests can detect all positive mosquitoes in the sample or that testing is done on individual mosquitoes rather than on a pool of mosquitoes. These models also incorporate 2 methods of estimating field infection rates, i.e., minimum field infection rates (MFIR) or maximum likelihood estimates

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(MLE). The MFIR assumes that a single mosquito within a pool is infected (Gu et al. 2003, 2004). However, a more accurate estimate of infection rates is achieved using the MLE for the probable infection rate that is based on the binomial distribution (Gu et al. 2003, 2004). These methods estimate infection rates in field-collected mosquitoes but do not examine the probability of accurately detecting an infected specimen, which is the purpose of our model.

Other factors, including testing method and protocol, influence the probability of identifying a virus-positive sample (Hadfield et al. 2001, Ryan et al. 2003). Virus titers of field-collected mosquitoes also may vary (Nasci and Mitchell 1996), thereby influencing the probability of virus detection because testing sensitivity varies by testing protocol (Nasci et al. 2002).

It is important to understand differences between virus detection methods that may influence detection probability. Due to method-specific limits of detection, samples that may be detected as virus-positive via one method may be classified as virus-negative using an alternative method (Nasci et al. 2002). Even the same testing methods may utilize different protocols, thereby altering the probability of detection. For example, simply combining multiple pools for testing via qRT-PCR may influence the ability to detect a virus-positive sample compared with sampling each pool individually (Chisenhall et al. 2008).

The goal of the current study was to develop a practical mathematical model to estimate the probability of accurately detecting an arbovirus in a sample of mosquitoes. The model we developed can be used to predict the probability of obtaining a virus-positive test result from a known virus-positive sample based on drawing a single aliquot from a larger sample. Current detection methods can be improved using this model to estimate the validity of test results and adjusting methods that will increase detection sensitivity. We also enable a researcher to determine the probability that a negative result is false, and provide a method to decrease the chance of false-negative results. False-negative results have drastic implications on risk prediction and implementation of control measures because responsible agencies would be unaware of virus presence. Many current predictive models assume that a virus-positive sample is correctly identified as positive. For example, Gu and Novak (2004) developed a model to determine the probability of collecting an infected mosquito in a pooled field sample based on infection rates and the total number of mosquitoes collected. However, this model assumes that the detection method accurately identifies the infected mosquito in the sample.

The model we developed examines the probability of correctly detecting a virus from a pool of mosquitoes when only a small aliquot of the pool is tested. Surveillance programs commonly test an aliquot from each mosquito pool. If the entire sample is actually tested, then the probability of detection is limited only by the sensitivity of the detection method. However, testing the entire sample increases the time and cost of surveillance efforts. For the purposes of the current model, we assume that a mosquito pool is prepared in such a way as to provide multiple aliquots for testing. However, we need to determine the probability that any given aliquot from the mosquito pool will contain a virion because only a single aliquot from any given mosquito pool is tested. In this case, the probability of detecting a virus is influenced by the number of virions in the sample, total sample volume, and size of the aliquot taken from the total sample volume. It is assumed for the purposes of this model that the virions are distributed randomly within the sample and that the method of extracting the aliquot from the sample does not influence the probability of obtaining a virion in the aliquot. We also assume that at least 1 virion is required for a virus-positive result, not simply a portion of a virion. Lastly, we are assuming that the testing assays are accurate and specific to the virus for which it is being tested, and that they are capable of detecting a single virion.

Consider a pool of mosquitoes or sample containing 1 virion. If an aliquot is taken from this sample for testing, the probability of getting 1 virion in any single aliquot of size a is

$$a/V,$$

where V is the total sample volume. As a increases relative to V , the probability of obtaining 1 virion in the aliquot approaches 100%. Of course, it follows that the probability of *not* getting the virion in our aliquot is

$$1 - a/V.$$

When there is >1 virion in V , we can calculate the probability of not getting a single virion in a single aliquot of size a . For each virion, Eq. 2 must be multiplied by itself, or $([1 - a/V] \times [1 - a/V] \times [1 - a/V] \times \dots)$ for all virions. This can be rewritten as Eq. 2, raised to the power of the total number of virions in V . The total number of virions in the initial sample is written as the density of virions (d) multiplied by V . Density of virions equals the virus titer of the sample. The probability of getting no virions in a with dV virions is

$$(1 - a/V)^{dV}.$$

We are interested in the probability of getting at least 1 virion in the test. In order to calculate the probability of at least 1 virion in a , we subtract Eq. 3 from 1:

$$1 - (1 - a/V)^{dV}.$$

This equation incorporates the density of virions in the sample (d), total sample volume (V), and size of the aliquot taken from the total sample volume (a).

This model assumes that the virus detection method can identify 1 virion. While RT-PCR and qRT-PCR are able to detect a single virion in the tested aliquot (Lanciotti et al. 2000, Weilke et al. 2006), other detection methods may not be as sensitive. The current model shows the probability of drawing an aliquot containing at least 1 virion and does not differentiate between 1 or multiple virions. The probability of aliquots containing 2 or more virions requires a different approach and can be determined using a Poisson distribution, but this is also dependent on the density of virions in the original sample, sample volume, and the volume and numbers of aliquots being tested.

Since virus titer is sometimes measured using plaque forming units (PFUs), the current model assumes that 1 virion = 1 PFU. The current model also assumes that only 1 aliquot was taken from the original sample because this action alters the virus titer and volume of the original sample. In certain circumstances when arboviral titer is very low, one improves detection by the repeated testing of multiple aliquots. If a sample with a large volume contains a single virion, the probability of getting the single virion in a small aliquot is very low than when the same aliquot is taken from a sample with a small volume. Each subsequent aliquot improves the probability that the virion is being sampled in an aliquot because the total sample volume decreases. If a second aliquot is removed, new parameters for total sample volume and virus titer would need to be calculated to determine the probability that subsequent aliquots contain the virion when using this model.

Aliquots from a sample with a high viral density have a greater probability of containing a virion, thereby increasing the probability of virus detection (Fig. 1). Figure 1 also shows that smaller aliquots have greater probability of missing a virion even if the original sample is positive. Aliquots of 25 μ l approach 100% detection when the sample contains 2 logs virions/ml, while 2- μ l aliquots require sample titers ≥ 3 logs virions/ml to provide near 100% accuracy in detecting a virion.

The variables in this model may be adjusted for specific protocols because different detection methods utilize varying aliquot volumes and total starting sample volumes may vary. In addition, the model can be solved for different variables to allow for different parameter requirements. For example, this model can show the appropriate aliquot volume to ensure that viruses will be detected accurately. An agency may require 100% (or close to 100%) accuracy of detecting a virus-positive sample when it is present. We can calculate the required aliquot volume for any total sample volume and specific viral density using

$$V \left[1 - (1 - p)^{(1/dV)} \right],$$

where the parameters are the same as the previous equation, p is the required probability of detection (in decimal form), V is the total sample volume, and d is the density of virions, i.e., virus titer. This equation was derived from Eq. 4, simply manipulating the formula to solve for aliquot volume (a) instead of probability (p). For example, if a surveillance program requires a 95% probability of detection for any given viral titer from a 1-mL sample, the required aliquot volume can be calculated as shown in Fig. 2. This figure shows that as the titer in the sample decreases, the size of the aliquots tested must increase to minimize the probability of a false-negative result. One of the primary advantages of using this model rather than a binomial distribution is the ability to algebraically manipulate the model to calculate different parameters beyond the probability of detection.

It is possible to determine if current methods provide the suitable probability of detection, or if protocols need to be altered to increase testing accuracy. If a testing agency is concerned with detecting a certain arboviral titer, the optimal aliquot volume can be calculated based on their current protocols.

The approaches described here may also be useful for designing experiments. An investigator might determine that the experimental protocol being used provides a 90% probability of detection. If greater accuracy is required, then the protocols can be adjusted as described above or the 90% accuracy may be incorporated into the statistical analysis. The approaches described here can also be expanded to compare detection probabilities of commonly used virus detection methods.

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Fig. 1.

Probability of obtaining at least 1 virion from a 1-ml sample, with aliquots of 25 µl, 10 µl, and 2 µl. For testing methods such as reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative real-time RT-PCR (qRT-PCR) that are able to detect a single virion, this equals the probability of detection. As the concentration of virions increases (increased titer in the original sample), the probability of virus detection increases and reaches almost a 100% probability of obtaining at least 1 virion from the original sample.

Fig. 2.

The required aliquot volume from a 1-ml sample for an 85%, 90%, or 95% detection probability at varying viral densities using either reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative real-time RT-PCR (qRT-PCR) (which can detect a single virion). To detect virus from a 1-ml sample that has a titer of 1 log virions/ml with 90% probability, an aliquot of 200 μ l is required. This model shows how to determine a minimum aliquot volume for virus detection for any desired probability of detection. The lines approach but never reach the x -axis, indicating that as titer in the infected sample increases, the required aliquot volume decreases.