

SCIENTIFIC NOTE

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

Christopher J. Vitek<sup>1</sup>, Stephanie L. Richards<sup>2</sup>, Heather L. Robinson<sup>2</sup>, Chelsea  
T. Smartt<sup>2</sup>

<sup>1</sup>Department of Biology, University of Texas Pan American

1201 W. University Drive, Edinburg, TX 78539

<sup>2</sup>Florida Medical Entomology Laboratory, University of Florida - IFAS

200 9<sup>th</sup> Street SE, Vero Beach, FL 32962

Correspondence/Reprints:

Chelsea T. Smartt

[ctsmart@ufl.edu](mailto:ctsmart@ufl.edu)

Florida Medical Entomology Laboratory

University of Florida - IFAS

200 9<sup>th</sup> Street SE, Vero Beach, FL 32962

Phone: 772-778-7200

Fax: 772-778-7205

**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 allows determining the probability of obtaining at least one virion in the sample that is tested. The model allows one to determine the optimal sample volume that is required in any test to ensure a desired probability of detecting viruses in a sample and can be used to support the accuracy of current tests, or to optimize existing techniques.

**KEY WORDS:** Surveillance, probability of detection, infection, arboviruses

Arboviral encephalitides such as West Nile, St. Louis, Eastern equine encephalitis, and Western equine encephalitis 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 1996, 2001; Blackmore et al. 2003). Common methods for arbovirus testing of mosquito pools include: plaque assay, reverse transcriptase polymerase chain reaction (RT-PCR), 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 and that testing is done on individual mosquitoes. Other factors, including testing method and protocol, will 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), which may influence the probability of virus detection since 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 to 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 actually a false-negative, 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 since 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 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 actually tested. Surveillance programs commonly test an aliquot representing a 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. We also assume that at least one 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 capable of detecting a single virion.

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

(Equation 1) 
$$a/V$$

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

(Equation 2)

$$1-a/V$$

When there are more than one virion in  $V$ , we can calculate the probability of not getting each of the virions in a single aliquot of size  $a$ . For each virion, Equation 2 must be multiplied by itself, or  $((1-a/V)*(1-a/V)*(1-a/V)* \dots)$  for all virions. This can be rewritten as Equation 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 can also be considered at the titer of the arboviral sample. The probability of getting no virions in  $a$  with  $dV$  virions is:

(Equation 3)

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

Since we are engaged in detecting viruses in an aliquot, we are interested in the probability of getting at least one virion in the test. In order to calculate the probability of at least one virion in  $a$ , we subtract Equation 3 from one:

(Equation 4)

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

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 ( $a$ ).

The current model assumes that the virus-detection method can identify an aliquot that contains one 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 detecting at least one virion; it does differentiate between one or multiple virions. The probability of aliquots containing two or more virions requires a different approach but this is also dependent on the numbers of virions in the original sample, the volume of this sample and the volume and numbers of the aliquots being tested. The probability of 1, 2, 3 or more virions in aliquots of size  $a$  is distributed according to a Poisson distribution. For example if the probability of a single virion is  $p$  (.01) in any aliquot and there are  $V/a$  (100) aliquots, then only 26% of the 100 aliquots will have 2 or more virions. By chance alone the detection system will have a false negative in 37% of the aliquots with a single virion, and 37% will be truly negative with no virions. For the purposes of the current model, a virus-detection method must be capable of detecting at least one virion.

Since virus titer is sometimes measured using plaque forming units (pfu), the current model assumes that one virion = one pfu. The current model also assumes that only one aliquot was taken from the original sample since this action alters the virus titer and volume of the original sample. If a second aliquot is removed, new parameters for total sample volume and virus concentration would need to be calculated. In certain circumstances when arboviral titer is very low, one improves detection by repeated testing of multiple aliquots. For example, consider a large volume containing a single virion. The detection of this virion in a small aliquot has a very low probability as we have seen above. Now consider how many aliquots might need to be sampled to improve the ability to detect the virion. In an example with a single virion somewhere among 100 aliquots, one

approaches 100% as one approached sampling all aliquots. However, in the same situation described above for the Poisson distribution, 37% of the 100 aliquots will not have a single virion in them and the original sample would be considered a false negative. Under this situation a second aliquot would have a 63% probability of being positive. The probability of 2 false negative aliquots under these circumstances is 13%, with 0.2% probability that 3 aliquots would be negative. Increasing the number of aliquots will assure that there are few false negatives. Aliquots from a sample with a high viral density have a greater probability of containing a virion, thereby increasing the probability of virus detection (Figure 1). This figure also shows that smaller aliquots have greater probability of missing a virion even if the original sample is positive. Aliquots of 25  $\mu\text{L}$  approach 100% detection at 2 logs per mL, while 2  $\mu\text{L}$  aliquots require titers of over 3 logs/mL to provide near 100% accuracy in detecting a virion.

The variables in this model may be adjusted for specific protocols since different detection methods utilize varying aliquot sizes and total starting sample volumes may vary. This model can show the appropriate aliquot size to ensure that viruses will be detected when present in the original sample. For example, 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 size for any sample volume and specific viral density using

(Equation 5)

$$V(1-(1-p)^{(1/dV)})$$

where  $p$  is the required probability of detection (in decimal form),  $V$  is the total sample volume, and  $d$  is the density of virions. 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 size can be calculated as shown in Figure 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.

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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### References Cited

Bell JA, Mickelson NJ, Vaughan JA. 2005. West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Ford, North Dakota. *Vector-Borne Zoon Dis* 5: 373-382.

Blackmore CG, Stark LM, Jeter WC, Oliver RL, Brooks RG, Conti LA, Wiersma ST. 2003. Surveillance results from the first West Nile virus transmission season in Florida, 2001. *Am J Trop Med Hyg* 69: 141-159.

Chisenhall DM, Vitek CJ, Richards SL, Mores, CN. 2008. A method to increase efficiency in testing pooled field-collected mosquitoes. *J Am Mosq Control Assoc* 23: 311-314.

Day JF, Stark LM. 1996. Transmission patterns of St. Louis encephalitis and Eastern equine encephalitis viruses in Florida: 1978-1993. *J Med Entomol* 33: 132-139.

Day JF. 2001. Predicting St. Louis encephalitis virus epidemics: lessons from recent, and not so recent outbreaks. *Ann Rev Entomol* 2001 46: 111-138.

Farajollahi A, Crans WJ, Bryant P, Wolf B, Burkhalter KL, Godsey MS, Aspen SE, Nasci RS. 1995. Detection of West Nile virus RNA from an overwintering pool of *Culex pipiens* (Diptera: Culicidae) in New Jersey, 2003. *J Med Entomol* 42: 490-494.

Gargan TP, Bailey CL, Higbee, GA, Gad, A, El Said S. 1983. The effect of laboratory colonization on the vector-pathogen interactions of Egyptian *Culex pipiens* and Rift Valley fever virus. *Am J Trop Med Hyg* 32: 1154-1163.

Gu W, Novak RJ. 2004. Short report: detection probability of arbovirus infection in mosquito populations. *Am J Trop Med Hyg* 71: 636-636.

Gu W, Unnasch TR, Katholi CR, Lampman R, Novak RJ. 2008. Fundamental issues in mosquito surveillance for arboviral transmission. *Trans R Soc Trop Med Hyg* 102: 817-822.

Hadfield TL, Turell M, Dempsey MP, David J, Park J. 2001. Detection of West Nile virus in mosquitoes by RT-PCR. *Mol and Cell Probes* 15: 147-150.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe K, Davis BS, Roehrig JT. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan Reverse Transcription-PCR Assay. *J Clin Microbiol* 38: 4066-4071.

Lord CC, Rutledge CR, Tabachnick WJ. 2006. Relationships between host viremia and vector susceptibility for arboviruses. *J Med Entomol* 43: 623-630.

Nasci RS, Mitchell CL. 1996. Arbovirus titer variation in field-collected mosquitoes. *J Am Mosq Control Assoc* 12: 167-171.

Nasci RS, Gottfried KL, Burkhalter KL, Kulasekera VL, Lambert AJ, Lanciotti RS, Hunt AR, Ryan JR. 2002. Comparison of vero cell plaque assay, Taqman reverse transcriptase polymerase chain reaction RNA assay, and VecTest antigen assay for detection of West Nile virus in field-collected mosquitoes. *J Am Mosq Control Assoc* 18: 294-300.

Ryan J, Dave K, Emmerich E, Fernandez B, Turell M, Johnson J, Gottfried K, Burkhalter K, Kerst A, Hunt A, Wirtz R, Nasci R. 2003. Wicking assays for rapid detection of West Nile and St. Louis encephalitis viral antigens in mosquitoes (Diptera: Culicidae). *J Med Entomol* 40: 95-99

Savage HM, Anders M, Gordon E, McMillen L, Colton L, Charnetzky D, Delorey M, Aspen S, Burkhalter K, Biggerstaff BJ, Godsey M. 2006. Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae). *J Med Entomol* 43: 1227-1238.

Turell MJ, Mores CN, Dohm DJ, Komilov N, Paragas J, Lee JS, Shermuhemedova D, Endy TP, Kodirov A, Khodvaev S. 2006. Laboratory transmission of Japanese encephalitis and West Nile viruses by molestus form of *Culex pipiens* (Diptera: Culicidae) collected in Uzbekistan in 2004. J Med Entomol 43: 296-300.

Vitek CJ, Richards SL, Mores CN, Day JF, Lord CC. 2008. Arbovirus transmission by *Culex nigripalpus* in Florida, 2005. J Med Entomol 45: 483-493.

Weilke C, Tellmann G, Hoffmann M. 2006. Lightcycler 480 system: a new standard for accurate high-throughput qPCR. Biochemica 1: 4-7.

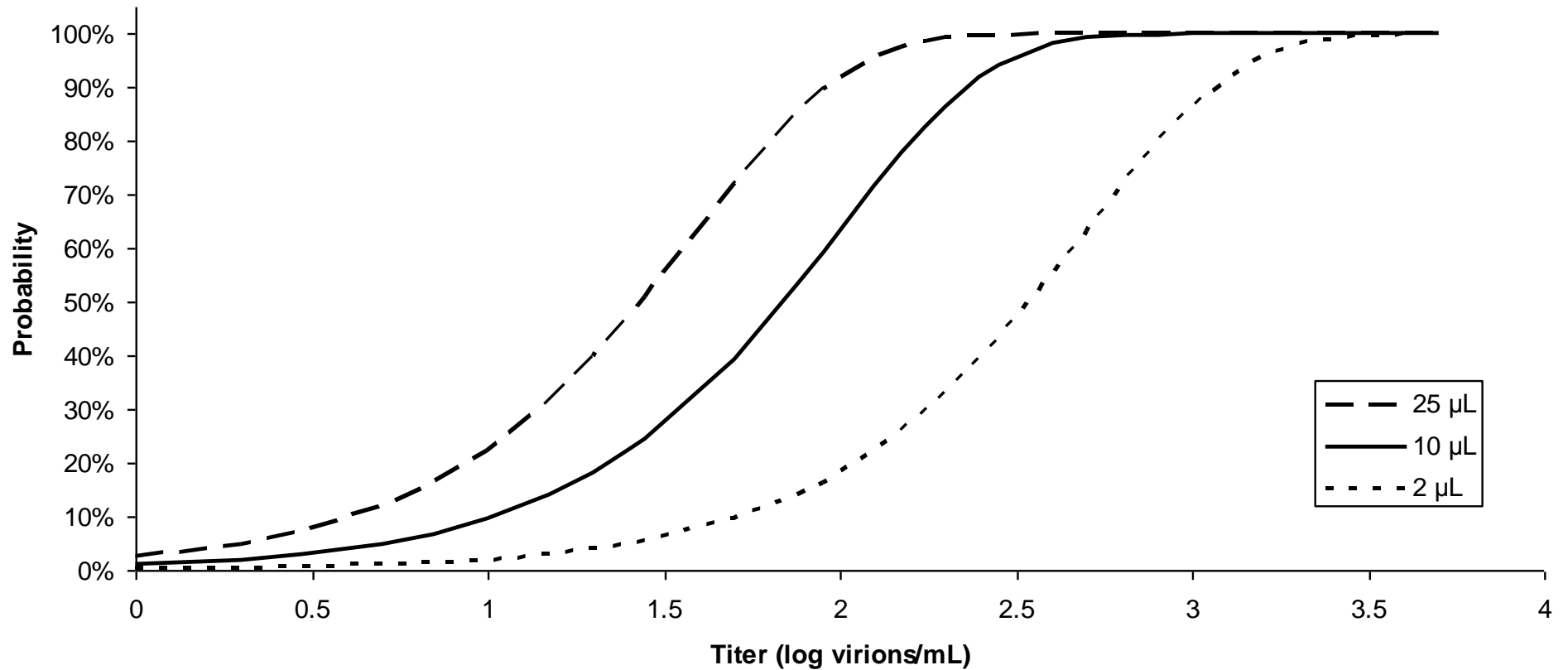


Figure 1. Probability of obtaining at least one virion from a 1 mL sample, with aliquots of 25 microliters ( $\mu\text{L}$ ), 10  $\mu\text{L}$ , and 2  $\mu\text{L}$ . For testing methods such as qRT-PCR and RT-PCR that are able to detect a single virion, this equals the probability of detection. As 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 one virion from the original sample.

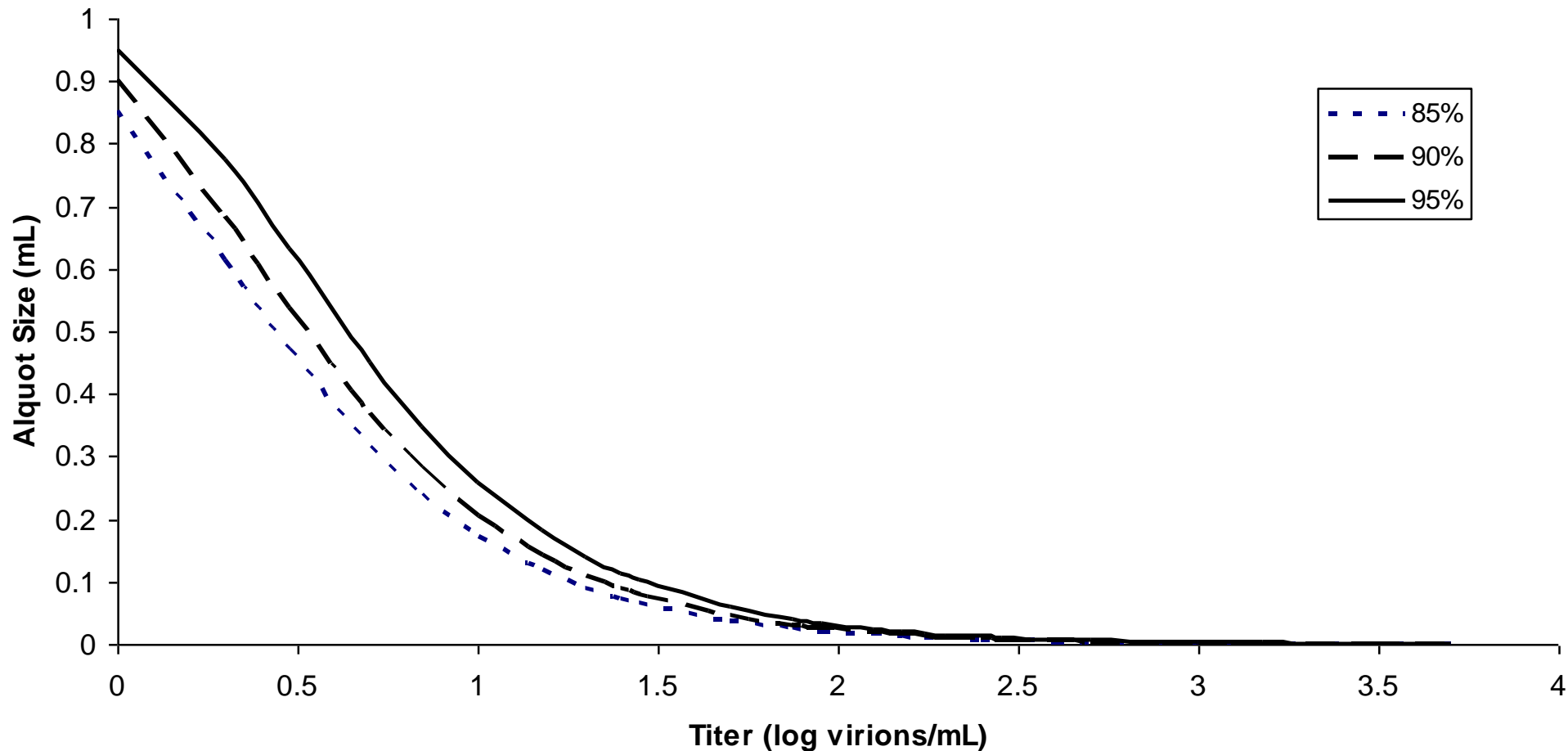


Figure 2. The required aliquot size from a 1 mL sample for either an 85%, 90%, or a 95% detection probability at varying viral densities using either qRT-PCR or RT-PCR (which can detect a single virion). To detect virus from a 1 mL sample of a titer of 1 log virions/mL with 90% probability, an aliquot of 0.2 (200  $\mu$ L) is required. This model shows how to determine a minimum aliquot size 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 size decreased.

