Reverse Transcription–Polymerase Chain Reaction Testing on Filter Paper–Dried Serum for Laboratory-Based Dengue Surveillance—American Samoa, 2018

Emily J. Curren,^{1,2} Aifili John Tufa,³ William Thane Hancock,⁴ Brad J. Biggerstaff,¹ June S. Vaifanua-Leo,³ Catherine A. Montalbo,³ Tyler M. Sharp,¹ Marc Fischer,¹ Susan L. Hills,¹ and Carolyn V. Gould^{1*}

¹Division of Vector-Borne Diseases, Centers for Disease Control and Prevention (CDC), Fort Collins, Colorado; ²Epidemic Intelligence Service, CDC, Atlanta, Georgia; ³American Samoa Department of Health, Pago Pago, American Samoa; ⁴Division of State and Local Readiness, CDC, Hagatna, Guam

Abstract. Laboratory-based surveillance for arboviral diseases is challenging in resource-limited settings. We evaluated the use of filter paper–dried sera for detection of dengue virus (DENV) RNA during an outbreak in American Samoa. Matched liquid and filter paper–dried sera were collected from patients with suspected dengue and shipped to a reference laboratory for diagnostic testing. RNA was extracted from each sample and tested for DENV RNA by real-time reverse transcription–polymerase chain reaction (RT-PCR). Of 18 RT-PCR–positive liquid specimens, 14 matched filter paper–dried specimens were positive for a sensitivity of 78% (95% CI, 55–91%). Of 82 RT-PCR–negative liquid specimens, all filter paper–dried specimens were negative for a specificity of 100% (95% CI, 96–100%). Shipping of filter paper–dried specimens was similarly timely but less expensive than shipping liquid sera. Using filter paper–dried serum or blood can be a cost-effective and sustainable approach to surveillance of dengue and other arboviral diseases in resource-limited settings.

American Samoa has experienced multiple recent outbreaks of arboviral disease, including dengue, chikungunya, and Zika virus disease.^{1,2} During these outbreaks, laboratory testing was a public health challenge because of complex logistics and costs of storing and sending serum specimens overseas. In May 2018, during an outbreak of dengue virus (DENV) serotype 2,¹ the American Samoa Department of Health requested assistance from the U.S. CDC to evaluate the capacity and sustainability of their arboviral disease surveillance system, including an assessment of filter paper–dried serum for dengue diagnostic testing. The use of dried blood spots for detection of DENV RNA in various resource-limited settings has shown promise in previous evaluations; however, the study methods varied in the use of filter paper products, elution methods, assays, storage times, transport conditions, and other factors.^{3–8} Only one study evaluated the use of filter paper–dried serum.⁴

Serologic test results can be difficult to interpret because of cross-reactivity in areas endemic for multiple flaviviruses⁹; therefore, we sought to assess molecular diagnosis of acute DENV infection using filter paper–dried serum. We evaluated the sensitivity and specificity of DENV real-time reverse transcription–polymerase chain reaction (RT-PCR) on filter paper–dried serum compared with matched liquid serum specimens collected in American Samoa. During June 15–July 12, 2018, we collected 100 acute serum specimens from patients in American Samoa who had 1) a positive DENV non-structural protein-1 (NS1) antigen test (Dengue Duo Panel, Diagnostic Automation/Cortez Diagnostics, Inc., Calabasas, CA) or 2) a negative DENV NS1 antigen test but met the clinical criteria for suspected dengue (i.e., fever and one or more of headache, myalgia, arthralgia, or rash). Filter paper–dried serum spots were created using 100- μ L aliquots of liquid serum pipetted onto Whatman FTA Micro Cards (GE Healthcare Life Sciences, Pittsburgh, PA). The dried serum specimens were shipped to the CDC Arboviral Diseases Branch in Fort Collins,

CO, without refrigerant via U.S. Postal Service priority mail. Corresponding liquid sera were shipped cold as per the established protocols of the Pacific Island Health Officers Association's specimen transport mechanism, which includes shipping specimens from American Samoa to Hawaii at 2–6°C on frozen ice packs and then repacking them on dry ice in Hawaii for shipment to CDC in Fort Collins. At CDC, total RNA was extracted from a 5-mm-diameter disk hole punched from each filter paper–dried serum card by adding 300 μ L AVL buffer (QIAGEN Inc., Germantown, MD) and incubating for 1 hour at 56°C followed by completion of the QIAGEN QIAamp Viral RNA Mini Kit protocol as described by the manufacturer. RNA was extracted from 140 μ L of corresponding liquid serum using the same protocol. The resulting RNA from both sample types was tested in parallel by real-time RT-PCR¹⁰ using the same volume (10 μ L). The protocol for this investigation went through the institutional review process at the CDC and was determined to be non-research public health surveillance and program evaluation.

The envelope with filter paper–dried serum specimens arrived at the CDC in 4 days and cost US\$8.20 to ship. The box of liquid serum specimens arrived in 3 days and cost US\$629. Of the 100 liquid serum specimens, 18 had DENV RNA detected by RT-PCR (median cycle threshold [Ct] value, 29.3 [range, 23.2–36.3]). Of the 18 liquid serum specimens that were positive by RT-PCR, 14 matched filter paper–dried serum specimens had a positive RT-PCR result (median Ct value, 31.6 [range, 31.1–34.4]). Of the 82 RT-PCR–negative liquid serum specimens, all matched filter paper–dried serum specimens had a negative result.

The 14 specimens that were RT-PCR–positive on both liquid and filter paper–dried serum were collected a median of 1 day post-illness onset (range, 0–4 days) compared with a median of 2.5 days post-illness onset (range, 2–3 days) for the four dried serum specimens that were PCR–negative. Compared with liquid serum, the sensitivity of RT-PCR on filter paper–dried serum was 78% (95% CI, 55–91%) and specificity was 100% (95% CI, 96–100%).

In this investigation, using filter paper–dried serum was more cost-effective but lower in sensitivity for dengue surveillance compared with liquid serum. The precision of our

* Address correspondence to Carolyn V. Gould, Division of Vector-Borne Diseases, CDC, 3156 Rampart Rd., Mail Stop P-02, Fort Collins, CO 80521. E-mail: cgould@cdc.gov

sensitivity estimate was limited by the small number of RT-PCR-positive liquid serum specimens. We attempted to increase the likelihood of obtaining serum from viremic patients by preferentially selecting specimens from NS1 antigen-positive patients; however, as the outbreak was waning at the time of the investigation, only 10 of the 100 specimens we obtained were NS1-positive.

There are several possible reasons for our observation of relatively modest sensitivity of RT-PCR on filter paper-dried serum. Varying degrees of time- and temperature-dependent RNA degradation have been observed with filter paper cards and other RNA-stabilizing products.⁵ Also, RNA elution from filter papers might be less efficient than from liquid serum, and efficiency may vary by the elution method.⁵ Another factor likely impacting sensitivity was the lower volume of the sample collected on the filter paper and used for RNA extraction compared with liquid serum.¹¹ In our analysis, RNA quantity, as approximated by the inverse of the Ct values of the RT-PCR assay, was generally lower for the filter paper-dried serum compared with liquid, an observation reported in other studies.^{3,5} Therefore, the use of filter paper-dried specimens might reduce the sensitivity of RT-PCR to detect infection in patients with low-level viremia. Eluting RNA from multiple hole punches from a single filter paper card or from multiple filter paper cards collected from a single patient might improve the sensitivity of RT-PCR.

Similarly, the use of whole blood spots rather than serum might have improved sensitivity. We used pre-collected clinical serum specimens spotted onto filter paper cards in the laboratory rather than venous or capillary whole blood to minimize impact on patients and providers. Most previous studies evaluating DENV testing of filter paper-dried specimens used venous or capillary whole blood. Three studies evaluating filter paper-dried venous whole blood collected in Vietnam, Peru, and Cuba found high sensitivities for RNA detection ranging from 93% to 97% compared with various liquid specimens (plasma, whole blood, and serum).^{3,5,8} A study evaluating filter paper-dried capillary whole blood collected from patients during dengue outbreaks in French Guiana found a sensitivity of 91% for DENV RT-PCR testing compared with liquid venous blood.⁶ One study, conducted on specimens from Pacific Island countries, evaluated filter paper-dried serum and found robust detection of DENV RNA, but sensitivity estimates were not performed because only a small subset of matched, frozen sera was tested.⁴ The investigators noted that the quantity of RNA obtained from dried serum was adequate for genotype and serotype testing of the circulating DENV strains, which is useful for epidemiologic surveillance.

Filter paper-dried serum or blood has been successfully used for the molecular diagnosis of multiple other viral (e.g., hepatitis C virus and HIV) and parasitic pathogens, with important applications to large-scale epidemiologic field studies.^{12–14} Filter paper-dried capillary blood also was found to be an effective sampling method for molecular detection of chikungunya virus in Madagascar, with a sensitivity and specificity compared with liquid serum of approximately 93% and 94%, respectively.¹⁵ The advantages of using filter paper-dried serum or blood for arboviral surveillance include the possibility of simple and cost-effective collection, storage, and long-distance transport of specimens in ambient conditions, particularly in remote settings. In addition, small volumes

of blood can be collected by finger prick, which does not require trained phlebotomists or cold storage and transport. This is especially advantageous for testing young children.^{5,6,8} Because testing of filter paper-dried serum also appears promising, it would be convenient for laboratories in resource-limited settings to spot serum that is positive on initial dengue rapid diagnostic testing onto filter paper cards for molecular confirmation and serotyping in a reference laboratory. Additional evaluation, including stability studies in specific conditions, should be performed on the use of filter paper-dried serum or blood as a feasible and sustainable approach to testing for dengue and other arboviral diseases for surveillance in resource-limited settings.

Received October 28, 2019. Accepted for publication November 23, 2019.

Published online January 13, 2020.

Acknowledgments: We thank Gilberto Santiago and Holly Hughes for scientific input and critical review of the manuscript.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Authors' addresses: Emily J. Curren, Brad J. Biggerstaff, Marc Fischer, Susan L. Hills, and Carolyn V. Gould, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention (CDC), Fort Collins, CO, E-mails: ecurren@cdc.gov, bbiggerstaff@cdc.gov, mfischer@cdc.gov, shills@cdc.gov, and cgould@cdc.gov. Aifili John Tufa, June S. Vaifanua-Leo, and Catherine A. Montalbo, American Samoa Department of Health, Pago Pago, AS, E-mails: a.tufa@doh.as, jleo@doh.as, and c.montalbo@doh.as. W. Thane Hancock, Division of State and Local Readiness, Centers for Disease Control and Prevention, Hagatna, GU, E-mail: whancock@cdc.gov. Tyler M. Sharp, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention (CDC), San Juan, PR, E-mail: tsharp@cdc.gov.

REFERENCES

1. Cotter CJ, Tufa AJ, Johnson S, Matai'a M, Sciulli R, Ryff KR, Hancock WT, Whelen C, Sharp TM, Anesi MS, 2018. Outbreak of dengue virus type 2—American Samoa, November 2016–October 2018. *MMWR Morb Mortal Wkly Rep* 67: 1319–1322.
2. Healy JM et al., 2016. Notes from the field: outbreak of Zika virus disease—American Samoa, 2016. *MMWR Morb Mortal Wkly Rep* 65: 1146–1147.
3. Anders KL et al., 2012. An evaluation of dried blood spots and oral swabs as alternative specimens for the diagnosis of dengue and screening for past dengue virus exposure. *Am J Trop Med Hyg* 87: 165–170.
4. Aubry M et al., 2012. Use of serum and blood samples on filter paper to improve the surveillance of dengue in Pacific Island Countries. *J Clin Virol* 55: 23–29.
5. Dauner AL, Gilliland TC, Mitra I, Pal S, Morrison AC, Hontz RD, Wu SL, 2015. Evaluation of nucleic acid stabilization products for ambient temperature shipping and storage of viral RNA and antibody in a dried whole blood format. *Am J Trop Med Hyg* 93: 46–53.
6. Matheus S, Meynard JB, Lacoste V, Morvan J, Deparis X, 2007. Use of capillary blood samples as a new approach for diagnosis of dengue virus infection. *J Clin Microbiol* 45: 887–890.
7. Matheus S, Meynard JB, Lavergne A, Girod R, Moua D, Labeau B, Dussart P, Lacoste V, Deparis X, 2008. Dengue-3 outbreak in Paraguay: investigations using capillary blood samples on filter paper. *Am J Trop Med Hyg* 79: 685–687.
8. Prado I, Rosario D, Bernardo L, Alvarez M, Rodriguez R, Vazquez S, Guzman MG, 2005. PCR detection of dengue virus using dried whole blood spotted on filter paper. *J Virol Methods* 125: 75–81.

9. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, Brandt WE, 1989. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *J Gen Virol* 70: 37–43.
10. Johnson BW, Russell BJ, Lanciotti RS, 2005. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. *J Clin Microbiol* 43: 4977–4983.
11. Santiago GA et al., 2018. Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses. *Nat Commun* 9: 1391.
12. Abe K, Konomi N, 1998. Hepatitis C virus RNA in dried serum spotted onto filter paper is stable at room temperature. *J Clin Microbiol* 36: 3070–3072.
13. Beck IA, Drennan KD, Melvin AJ, Mohan KM, Herz AM, Alarcon J, Piscoya J, Velazquez C, Frenkel LM, 2001. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 subtype B DNA in dried blood samples for diagnosis in infants in the field. *J Clin Microbiol* 39: 29–33.
14. Cox-Singh J, Mahayet S, Abdullah MS, Singh B, 1997. Increased sensitivity of malaria detection by nested polymerase chain reaction using simple sampling and DNA extraction. *Int J Parasitol* 27: 1575–1577.
15. Andriamandimby SF, Heraud JM, Randrianasolo L, Rafisandratantsoa JT, Andriamamonjy S, Richard V, 2013. Dried-blood spots: a cost-effective field method for the detection of chikungunya virus circulation in remote areas. *PLoS Negl Trop Dis* 7: e2339.