Persistence of Zika in Red Blood Cells, Plasma and Other Compartments

Documentation for Public Use Data files

Zika virus (ZIKV) is a mosquito-borne arbovirus that can also be transmitted congenitally and through transfusion and sexual contact. It was first identified in the Zika forest in Uganda in the 1950's. More recently, it spread to Malaysia and Indonesia, then to Micronesia, French Polynesia and, in 2014 to Brazil. Although asymptomatic or mildly symptomatic in most cases, ZIKV can cause Guillain-Barré syndrome and infection during pregnancy has been associated with intrauterine fetal death and congenital Zika syndrome. The goal of this study was to characterize viral persistence and immune responses to Zika infection following acute infection. This information is needed to inform blood donor and diagnostic testing policies and understand the natural history of ZIKV infection.

In this study, which was supported with funds from the Recipient Epidemiology and Donor Evaluation Study-III (REDS-III), blood donors in Puerto Rico and Florida were screened with nucleic acid testing to identify individual infected with Zika. Infected donors were invited to participate in a one-year follow-up study to document viral dynamics and immune response to infection. Eight study visits, including the enrollment or index visit, were planned, with follow-up visits taking place at 1, 2, 6, 12, 24, 36 and 52 weeks after enrollment. At each visit, samples of blood, urine and saliva were obtained from the participant. Semen was collected at some visits from male donors but collection was less complete than collection of other sample types. Viral load in plasma, red blood cells, whole blood, PBMCs, urine and saliva and was measured by quantitative PCR for zika RNA (Musso et al. 2017; Stone et al. 2017). Eight replicates of each sample of plasma, two of whole blood and two of urine were tested by transcription mediated amplification (TMA) using an assay from Grifols (Williamson et al. 2017). TMA results are reported as the number and percentage positive for each sample type in each sample.

Antibody titers were measured for Zika IgM and IgG using a modified ELISA developed by the CDC (Galel et al. 2017; Williamson et al. 2017). Cases of previous dengue infection were identified using the InBios Detect IgG ELISA. The results are listed as VRI_dengue_IgG in the data file. The file also includes a consensus dengue IgG result that is based on results from the InBios Detect assay, other serological testing and the results from neutralization assays. The consensus result has been used to classify subjects as positive or negative for dengue IgG in previous publications of these data (e.g. Stone et al. 2019 submitted). In addition to the quantitative results, the results of PCR and serological test were classified as positive, negative or equivocal. In published analyses of these data, equivocal results have been treated as positive.

In the public use file, results for Zika RNA by PCR are listed as negative at the enrollment visit for 22 of 53 participants and equivocal for 8 more. All participants were positive for Zika RNA in plasma on the nucleic acid test that was used to identify potential study participants. The screening test is more sensitive than the quantitative PCR test, which accounts for the difference in results.

Investigators have recognized five stages of Zika infection in the table below. This scheme was used to classify each participant at the enrollment visit.

Stage	Definition
I	Zika IgM negative, viral load <300 IU/mL
II	Zika IgM negative, viral load ≥300 IU/mL
III	Zika IgM positive or equivocal, viral load ≥300 IU/mL
IV	Zika IgM positive or equivocal, viral load <300 IU/mL, repeat reactive on NAT
V	Zika IgM positive or equivocal, viral load <300 IU/mL, repeat nonreactive on NAT

Because of some anomalies in the data, classification into one of these stages is not always possible. Because of these problems, a few study participants are listed as IV/V.

Symptom data

It is very common for zika-infected individuals to remain asymptomatic or to experience only mild symptoms. To study the incidence of symptoms further, all study participants were asymptomatic at the time of index donation as required by AABB and FDA policies and donor center screening procedures. A ZIKV Symptom Questionnaire, that was developed in collaboration with the US Centers for Disease Control and Prevention, was administered at each visit to record symptoms in the two weeks prior to the study visit (Appendix A). It was designed to capture symptoms ranging from mild ZIKV-compatible but nonspecific symptoms (e.g., fever, conjunctivitis, headache, and myalgia) to more severe neurologic disorders. The public use dataset includes information on six symptoms that the CDC labels the most common symptoms of zika infection: fever, rash, joint or bone pain, body or muscle pain, painful or red eyes and headache. In previous analyses of these data, a cutoff of 3 or more symptoms was used to define symptomatic infection. The same definition was used in studies of symptoms of West Nile Virus (Custer et al. 2019; Lanteri et al. 2019).

Changes to the data

Five variables were deleted during preparation of the public use files: Associated Index ID, Sample ID, Donation Identification Number, Donor ID and Questionnaire Number. Participant ID and study site were replaced with random numbers. Date of collection was replaced with time in days since the enrollment visit. The enrollment visit is labeled visit 0 in the data file.

Literature Cited

Custer B, Kamel H, Kiely NE, Murphy EL, Busch MP. Associations between West Nile virus infection and symptoms reported by blood donors identified through nucleic acid test screening. *Transfusion* 2009; **49**(2): 278-88.

Galel SA, Williamson PC, Busch MP, et al. First Zika-positive donations in the continental United States. *Transfusion* 2017; **57**(3pt2): 762-9.

Lanteri MC, O'Brien KM, Purtha WE, et al. Tregs control the development of symptomatic West Nile virus infection in humans and mice. *The Journal of clinical investigation* 2009; **119**(11): 3266-77.

Musso D, Richard V, Teissier A, et al. Detection of ZIKV RNA in semen of asymptomatic blood donors. *Clinical Microbiology and Infection* 2017; **23**(12): 1001.e1-.e3.

Stone M, Lanteri MC, Bakkour S, et al. Relative analytical sensitivity of donor nucleic acid amplification technology screening and diagnostic real-time polymerase chain reaction assays for detection of Zika virus RNA. *Transfusion* 2017; **57**(3pt2): 734-47.

Stone M, Bakkour S, Lanteri MC, et al. Persistence in blood compartments and body fluids: a prospective observational study. Lancet Infectious Disease 2019 (submitted).

Williamson PC, Linnen JM, Kessler DA, et al. First cases of Zika virus–infected US blood donors outside states with areas of active transmission. *Transfusion* 2017; **57**(3pt2): 770-8.

Appendix A

Post-Donation Symptoms Questionnaire

TO BE ADMINISTERED AT EACH FOLLOW-UP VISIT

SECTION A Patient Information: To be completed by research staff only.

A1. Blood Donor ID: __ __ __ __ __ __ __ __ __ __

A3. Specimen ID:

A2. Informed Consent Obtained: ____YES _____ NO

A4. Date of Interview: __ __/__ __/__ __ __ __

A5. Date of Index Donation: __ __/__ __/__ __ __ __

A6. Date of Last Study Visit:__ __/__ __/__ __ __ __ (not applicable for first study visit)

A7. Initials of person conducting the interview: __ __ __ __ __

Please Read: This study has been approved by Institutional Review Boards in the U.S. This study also has also received a Clinical Exemption by the U.S. National Institutes of Health.

Please Read: Thank you for agreeing to complete this survey. The purpose of this interview is to ask you about symptoms you may or may not have had. The data gathered from your answers will help to improve the safety of the blood supply. We would like to remind you that your answers are confidential and will be reported together with all other participants who complete this interview. Please, respond as truthfully as you can. I will read the questions to you and you can answer them. If there is any question you do not understand, please ask me to explain. Please answer each question as best you can. If you do not recall or do not know, please answer "don't' know". You may also refuse to answer any question you do not want to answer. Do you have any questions for me before we begin?

SECTION B Symptoms: The following questions are about symptoms that you may have had after your last visit to the blood center.

Since your last visit have you had any of the following?

	Yes	No	Don't Know
B1. Feverish	○	○	○
B2. Night sweats	○	○	○
B3. Chills	○	○	○
B4. Rash or red skin	○	○	○
B5. Tired with no energy; weak	○	○	○
B6. Joint pain	○	○	○
B7. Body pain	○	○	○
B8. Muscle pain	○	○	○
B9. Bone pain	○	○	○
B10. Painful eyes (pain behind your eyes or when moving your eyes).	○	○	○
B11. Red eyes	○	○	○
B12. Change in vision	○	○	○
B13. Headache	○	○	○
B14. Edema (swollen legs/feet/arms)	○	○	○
B15. Swollen lymph nodes	○	○	○
B16. Loss of appetite	○	○	○
B17. Abdominal pain	○	○	○
B18. Diarrhea	○	○	○
B19. Vomiting	○	○	○
B20. Mild bleeding (e.g., nose or gum bleeds, red or purple spots on patches of your skin, or easy bruising)	○	○	○
B21. (Males only) Have you noticed blood in your semen?	○	○	○
B22. Any other symptoms	○	○	○

B22. If Yes to B22, can you describe the other symptoms you have had?

_____

_____

SECTION C Zika virus History:

C1. Has a physician or other health care provider ever told you that you had Zika virus infection (other than the notification you received from the blood bank)?

○ **NO** ○ **YES** ○ **Don't Know**

C2. Since your last study visit, did you see a doctor or other health care provider?

○ **NO** ○ **YES**

C3. If yes to C2:

a.) When? ___/___/___

b) What was the reason for your contact with a doctor or other health care provider?

_____

_____

c) What was the diagnosis?

_____

_____

C4. Since your last study visit, were you hospitalized?

○ **NO** ○ **YES** ○ **Declined to state**

C5. If yes to C4:

a.) When were you admitted to the hospital? ___/___/___

b.) When were you discharged from the hospital? ___/___/___

C6. If yes to C4: What was your diagnosis when admitted to the hospital?

_____

► **If you have any questions or concerns, please talk to the research assistant or nurse.**