

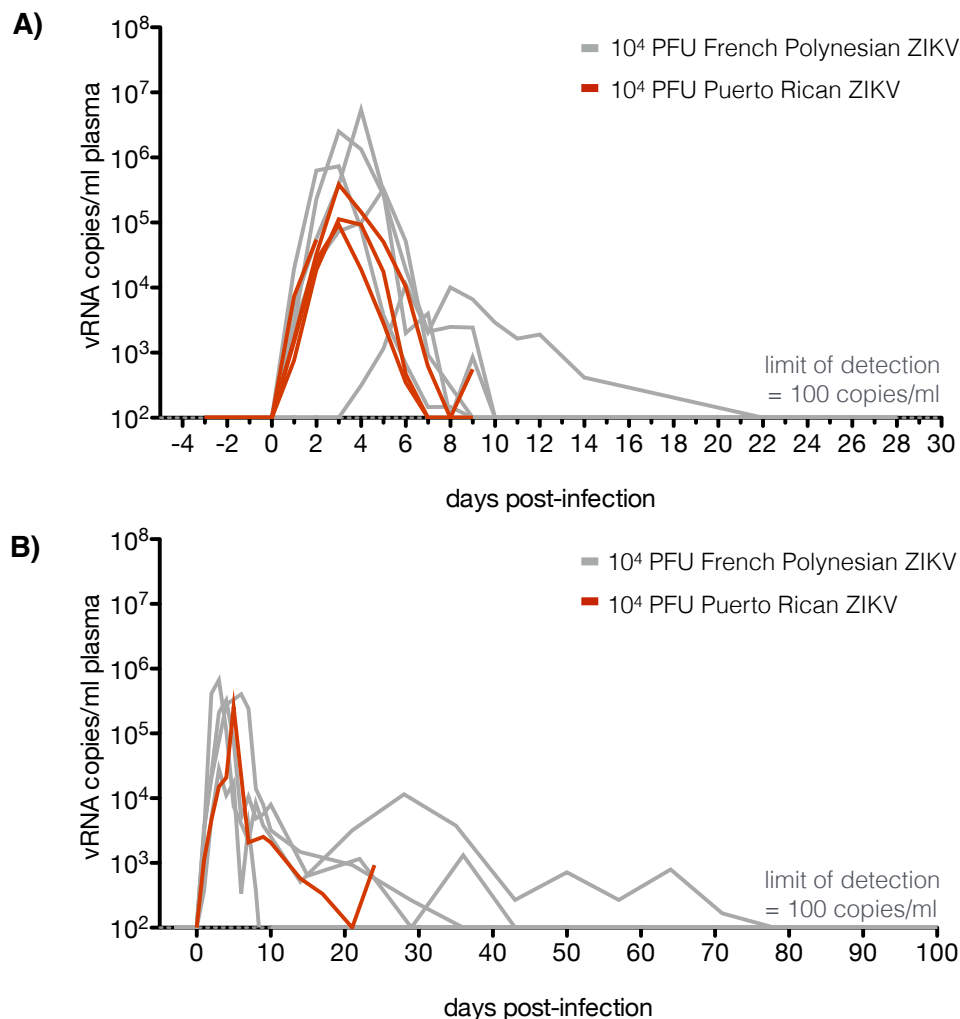
## Virus stock info: Zika virus/H.sapiens-tc/PUR/2015/PRVABC59-3329

Real-time data for in vivo experiments is accessible via [zika.labkey.com](http://zika.labkey.com)

ZIKV strain PRVABC59 (GenBank:KU501215), was originally isolated from a traveler to Puerto Rico. The seed stock passaged with three rounds of amplification on Vero cells was obtained from Brandy Russell (CDC, Ft. Collins, CO). Virus stocks were prepared by inoculation onto a confluent monolayer and passaged two times on C6/36 mosquito cells.

Infectious Titer	Stock Viral Load	Ratio of Titer to vRNA Content
$1.58 \times 10^7$ PFU/ml	$2.01 \times 10^{10}$ copies/ml	1:1272

Indian rhesus macaques challenged with  $10^4$  PFU PRVABC59 achieve peak viral load within the acute timeframe previously seen in inoculations with the French Polynesian ZIKV strain, albeit at a slightly lower peak (**Figure 1a**). Prolonged viremia has been detectable in pregnant animals, and is a consistent finding in an animal inoculated with PRVABC59 (**Figure 1b**).



**Figure 1. PRVABC59 virus stock *in vivo* rhesus challenge. A)** Indian rhesus macaques were challenged subcutaneously with  $10^4$  PFU of either the French Polynesian ZIKV strain or the Puerto Rican ZIKV strain (PRVABC59). **B)** Prolonged viremia defined by positive viral load after 14dpi in pregnant rhesus macaques. Viral load assay by qRT-PCR has a limit of detection of 100 vRNA copies/ml of plasma.

Additionally, 4 IFNAR<sup>-/-</sup> mice were inoculated in the left hind footpad with 10<sup>6</sup> PFU (in 50ul) PRVABC59. At 2 days post-infection, the mice were all successfully infected with viremias of 5.72, 5.51, 5.51, and 5.81 log<sub>10</sub> PFU/ml (**Figure 2**). Mosquitoes were allowed to feed on the mice at this time point and then will be allowed to feed on macaques for a future transmission study.

Animal	Challenge Stock	Challenge Dose (in 50ul)	Viral Load (log <sub>10</sub> PFU/ml)
Mouse 1	PRVABC59	10 <sup>6</sup> PFU	5.72
Mouse 2	PRVABC59	10 <sup>6</sup> PFU	5.51
Mouse 3	PRVABC59	10 <sup>6</sup> PFU	5.51
Mouse 4	PRVABC59	10 <sup>6</sup> PFU	5.81

**Figure 2. PRVABC59 virus stock *in vivo* mouse challenge** IFNAR<sup>-/-</sup> mice were inoculated with 10<sup>6</sup> PFU (in 50ul) PRVABC59) and viremia was assessed at 2dpi.