

Addressing the threat of Zika Virus to the U.S. Blood Supply: an Update

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Zika Virus and Disease Association

Disclaimers

- Dr. Jeffrey M. Linnen is an employee and stockholder in Grifols.
- The Procleix® Zika Virus assay and the Procleix Panther® system are under development; performance characteristics have not been established.
- The Procleix Zika Virus assay on the Panther system is not available for commercial sale in the US. The assay system is available in the US under an Investigational New Drug (IND) protocol.
- The Procleix Zika Virus assay on the Panther System is CE marked and available for sale in regions that recognize the CE mark.

Zika Virus

- **A virus spread to people primarily through the bite of an infected mosquito**
 - Sometimes called an "arbovirus" (arthropod-borne virus)
 - Transmitted primarily by *Aedes aegypti* and *Aedes albopictus* mosquitos; same mosquitoes spread other arboviral diseases
 - Classified in the *Flaviviridae* family and genus, *flavivirus*; related to Dengue, West Nile, and Yellow Fever viruses
 - Other modes of transmission: sexual, transplacental, laboratory acquired, and blood transfusion
- **Zika virus is not a newly discovered virus**
 - Discovered in 1947; named after the Zika Forest in Uganda
 - 1952: first human cases of Zika were detected; since then, sporadic outbreaks have been reported in tropical Africa, Southeast Asia, and the Pacific Islands

For a comprehensive review see: Musso D, Gubler DJ. Zika Virus. Clin Microbiol Rev. 2016 Jul;29(3):447-524.

Emerging Threats to Blood Safety | Linnen | PFAASB | 28 Aug 2016

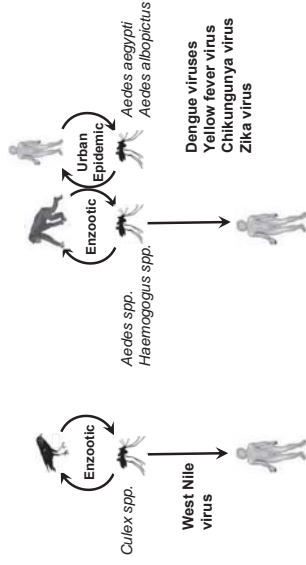


Topics

- Zika virus (ZIKV) and disease association
- History of ZIKV and its movement around the world
- Recent FDA guidance on blood screening by Nucleic Acid Testing (NAT)
- Preliminary performance characteristics of an investigational INAT for blood screening
- Update on ZIKV NAT blood screening in the US

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General Patterns of Mosquito-Borne Arboviral Disease Transmission

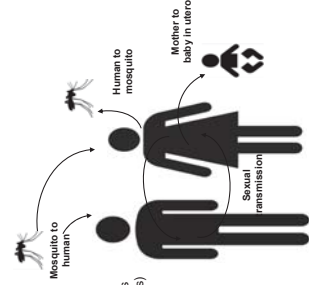


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Zika Virus Documented Transmission Routes

Modes of Transmission:

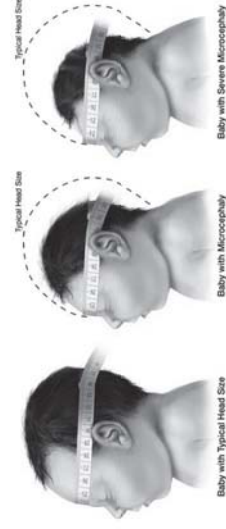
1. Bite by infected mosquito
2. Intrauterine, perinatal
3. Sexual
 - Detected in semen and other body fluids (urine, saliva, cervical/vaginal secretions)
4. Blood transfusion
 - 4 cases/3 donors
 - No ZIKV-related symptoms (from coagulopathy?)
5. Other
 - Lab acquired
 - Close contact



Source: <http://www.cdc.gov/zika/qa/faq-transmission-and-reporting-to-cdc.pdf>

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Microcephaly

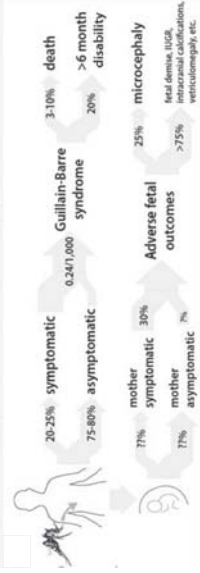


<https://www.cdc.gov/zika/newsroom/infants-with-zika-syndrome-04-16-16-01a.html>

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Individuals with Symptoms Appear to have Highest Risk of Neurologic Complications

Adverse fetal outcomes associated with maternal symptoms

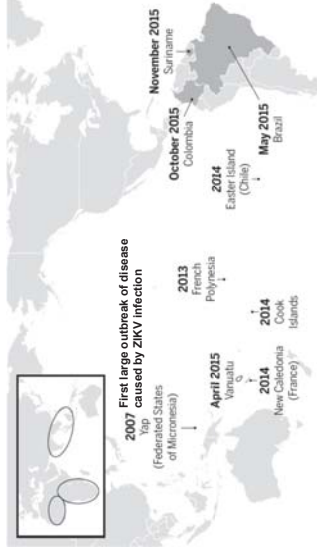


From: Lesler, J. et al. Assessing the global threat from Zika virus. *Science*. 2015 Aug 12;353:1235-36. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4587638/>

75-80% of ZIKV infections are asymptomatic; those with symptoms are at highest risk for neurological complications (e.g., Guillain-Barre)

Zika Congenital Syndrome (and microcephaly) more common when mother is symptomatic

Spread of Zika Virus



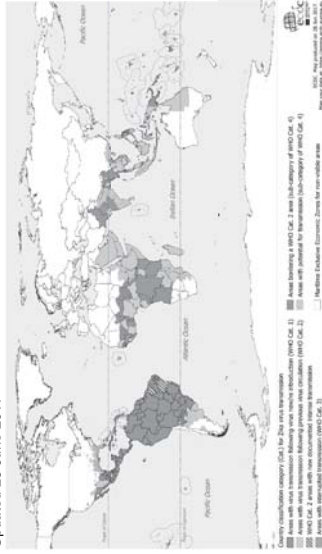
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The Spread of Zika Virus

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Current Zika Virus Transmission: Worldwide

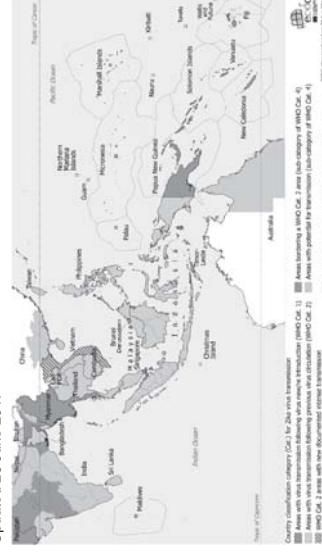
Updated 28 June 2017



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Current Zika Virus Transmission: Southeast Asia

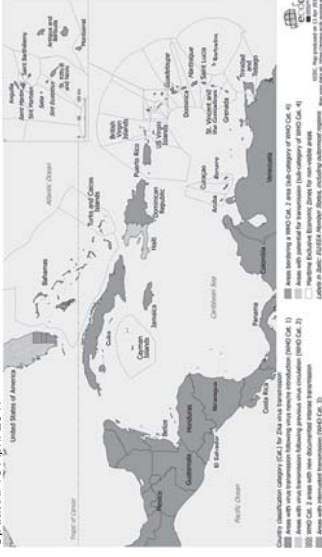
Updated 28 June 2017



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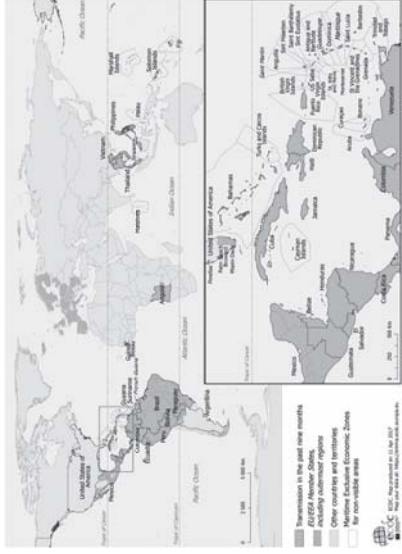
Current Zika Virus Transmission: Central America & Caribbean

Updated 13 April 2017



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Worldwide Zika Virus Transmission in the Past 9 Months



Countries/Territories with Reported Confirmed Autochthonous Vector-Borne Transmission of Zika Virus in Past 9 Months

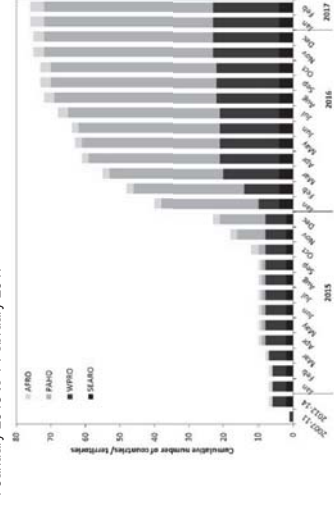
Updated 18 April 2017

American Samoa	Colombia	Madagascar	Saint-Barthelemy
Angola	Costa Rica	Martinique	Samoa
Argentina	Cuba	Mexico	Singapore
Antigua and Barbuda	Curaçao	Micronesia, Federated States of	Sint Eustatius
Aruba	Dominica	Montserrat	Sint Maarten
Bahamas	Dominican Republic	New Caledonia	Solomon Islands
Barbados	Equador	El Salvador	Suriname
Belize	French Guiana	Fiji	Thailand
Bolivia	Grenada	Guadeloupe	Trinidad and Tobago
Brazil	Guatemala	Honduras	Turks and Caicos Islands
British Virgin Islands (UK)	Guinea-Bissau	Jamaica	United States of America
Cape Verde	Guinea	Paraguay	Philippines
Cayman Islands	Haiti	Peru	Polynesia (French Polynesia, Pitcairn Islands, Phoenix Islands)
Chad	Honduras	Philippines	Saba
Chile	Honduras	Philippines	Saint Kitts and Nevis
China	Honduras	Philippines	Saint Lucia
Colombia	Honduras	Philippines	Saint Martin
Costa Rica	Honduras	Philippines	Saint Vincent and the Grenadines
Cuba	Honduras	Philippines	Vietnam
Curaçao	Honduras	Philippines	
Dominica	Honduras	Philippines	
Dominican Republic	Honduras	Philippines	
Equador	Honduras	Philippines	
El Salvador	Honduras	Philippines	
Fiji	Honduras	Philippines	
French Guiana	Honduras	Philippines	
Grenada	Honduras	Philippines	
Guadeloupe	Honduras	Philippines	
Guatemala	Honduras	Philippines	
Guinea-Bissau	Honduras	Philippines	
Guinea	Honduras	Philippines	
Haiti	Honduras	Philippines	
Honduras	Honduras	Philippines	
Jamaica	Honduras	Philippines	
Paraguay	Honduras	Philippines	
Peru	Honduras	Philippines	
Philippines	Honduras	Philippines	
Polynesia (French Polynesia, Pitcairn Islands, Phoenix Islands)	Honduras	Philippines	
Saba	Honduras	Philippines	
Saint Kitts and Nevis	Honduras	Philippines	
Saint Lucia	Honduras	Philippines	
Saint Martin	Honduras	Philippines	
Saint Vincent and the Grenadines	Honduras	Philippines	
Vietnam	Honduras	Philippines	

17 <http://dx.doi.org/10.1186/s12875-017-0582-5>

Cumulative Number of Countries and Territories Reporting Mosquito-borne Zika Virus Transmission for First Time (by year)

1 January 2015 to 1 February 2017

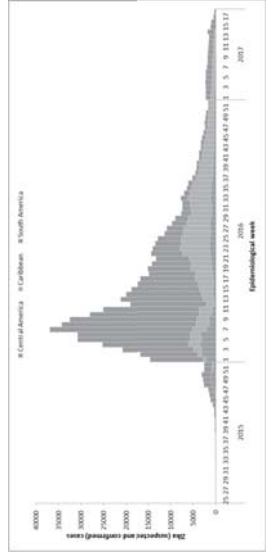


Graph is from the February 2nd WHO Zika Situation Report <http://apps.who.int/inf/1666225497/1616zika022517-eng.pdf?ua=1>

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Distribution of Suspected and Confirmed Zika Cases by Epidemiological Week and Sub-Region

Region of the Americas, 2016 – 2017 (as of EW 18)



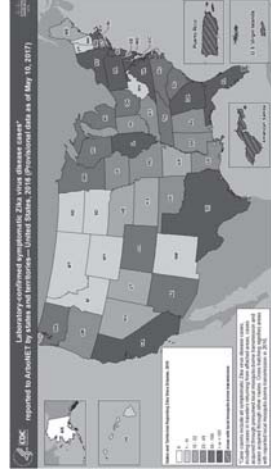
Source: <http://www.paho.org>

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Zika Virus and Blood Safety

2016 Zika Virus CDC Case Counts in the US

Provisional Data for 2016

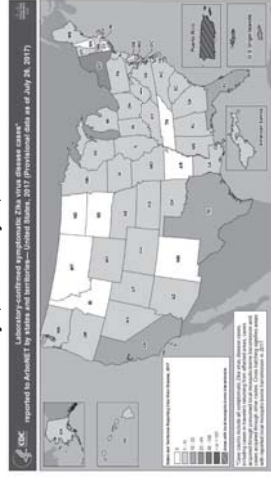


- US States: 5,102 symptomatic cases reported**
- 4,830: travelers returning from affected areas
 - 224: local mosquito-borne transmission
 - 48: other transmission
- <https://www.cdc.gov/zika/reporting/2016-case-counts.html>
- US Territories: 36,079 symptomatic cases reported**
- 142: travelers returning from affected areas
 - 35,937: local mosquito-borne transmission
 - 0: other transmission

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2017 Zika Virus CDC Case Counts in the US

Provisional Data: January 1, 2017 – July 26, 2017



- US States: 181 symptomatic cases reported**
- 180: travelers returning from affected areas
 - 0: mosquito-borne transmission
 - 1: sexual transmission
- <https://www.cdc.gov/zika/reporting/2017-case-counts.html>
- US Territories: 532 symptomatic cases reported**
- 0: travelers returning from affected areas
 - 532: mosquito-borne transmission
 - 0: cases acquired through other routes

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Risk of ZIKV Transmission by Blood Transfusion

Cited by US FDA in revised guidance issued in August 2016

- ZIKV infection asymptomatic in ~80% of individuals; transmitted sexually for unknown duration—as long as 6 months?
- High levels of viremia may occur in pre-symptomatic period which varies from 3-12 days
- ZIKV viremia \geq 8 million copies/mL in plasma, typically lasting ~1-2 weeks
- ZIKV RNA found in asymptomatic blood donors during French Polynesia outbreak (2013-2014); detected in 2016-2107 in asymptomatic blood donors in Puerto Rico and other countries
- Documented transfusion-transmissions of other flaviviruses (e.g., West Nile virus and dengue virus)
- Probable transmission of ZIKV by blood transfusion has been reported*

*Mora LF et al. (2016) Evidence for transmission of Zika virus by platelet transfusion. N Engl J Med. 2016; 374(16):1634-1641.

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Zika Virus Blood Screening in the US: Current Status

- Currently no FDA-licensed tests for Zika virus
- Implementation of investigational screening assays considered necessary because of large proportion of asymptomatic cases in areas of active transmission
- Testing for ZIKV became available through two separate Investigational New Drug (IND) applications for blood collected in Puerto Rico and the continental US
 - Tests became available on April 3, 2016 (Roche Molecular Systems, Inc.) and June 20, 2016 (Hologic, Inc./Gilead Diagnostic Solutions)
 - Based on revised US FDA Guidance of August 26, 2016, all donations collected in US and US territories are being tested using Individual Donor Nucleic Acid Test (ID-NAT) under approved IND applications

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Zika Virus Transfusion Transmitted Infections from Brazil

Four cases from 3 positive donations; all 4 reported via post donation information (3-5 days)

Underlying Condition	Symptoms	Gender	Age	Component
Liver transplant	None	M	55	Platelet pool*
Trauma	Thrombocytopenia	M	38	Red Blood Cells
Myelofibrosis	None	F	54	Apheresis platelets**
AML/ bone marrow transplant	None	F	14	Apheresis platelets** (same donation as above)

AML: acute myeloid leukemia

*Borges-Casero ML et al. Probable transfusion-transmitted Zika virus in Brazil. Transfusion. 2016; Jul;56(7):1694-9.

**Motta U et al. Evidence for Transmission of Zika Virus by Platelet Transfusion. N Engl J Med. 2016 Sep;375(17):1610-3.

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Procleix Zika Virus Assay: Performance Characteristics and Update of Testing in the US

Revised US FDA Guidance of August 26, 2016: Donor Screening and Deferral

- Following the implementation of donor testing and/or pathogen reduction technology, blood establishments may discontinue the following:
 - providing donor educational material for infection
 - screening donors for Zika virus risk factors, such as travel history, and deferring them as previously recommended in the February 2016 guidance
 - Deferral of a donor for 120 days:
 - following a reactive ID-NAT test result based on the date testing or the date of resolution of symptoms, whichever is longer; notify and counsel the donor regarding the deferral and a possible Zika virus infection
- OR—
- If the donor shares a recent history of Zika infection based on the date of resolution of symptoms or the date of the positive viral test, whichever is longer

The complete guidance document can be found at the following site:
<http://www.fda.gov/downloads/Biologics/Blood/Derivatives/Compliance/RegulatoryInformation/Guidance/UCM1518213.pdf>

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Procleix Zika Virus Assay on the Panther System

CE marked NAT for detection of Zika virus RNA



- Qualitative Transcription-Mediated Amplification (TMA) assay on Procleix Panther system
- Assay amplifies and detects 2 regions of Zika Virus genome
- Testing for Zika virus (ZIKV) became available in US through the Grifols (Hologic) Investigational New Drug (IND) protocol June 17, 2016
- Testing started using combination of pools (16-donations) and individual donor nucleic acid testing (ID-NAT), focusing on southern continental US
- Same assay granted Emergency Use Authorization (EUA) by FDA on June 17, 2016 for diagnostic use (marketed as "Aptimer® Zika Virus assay" by Hologic)
- All testing in US now by ID-NAT (based on revised FDA guidance, August 2016) under FDA approved cost recovery
- IND amended February 2017 to include plasma/serum specimens from living donors of human cells, tissues, and cellular and tissue-based products (HCT/TPs)
- Assay was CE marked December 2016

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Analytical Sensitivity: Zika Virus in vitro Transcript (IVT)

Limit of detection (LOD) determination by dilution of IVT

Concentration Copies/mL	Lot 1 (n=72)	Lot 2 (n=72)	Combined (n=144)
90	100 (100%)	100 (100%)	100 (100%)
30	100 (100%)	100 (100%)	100 (100%)
10	100 (100%)	100 (100%)	100 (100%)
3	92 (127.8%)	92 (127.8%)	92 (127.8%)
1	54 (75.0%)	54 (75.0%)	54 (75.0%)
0.3	22 (30.6%)	22 (30.6%)	22 (30.6%)
0.1	3 (4.2%)	3 (4.2%)	3 (4.2%)
0	0 (0%)	0 (0%)	0 (0%)

CI = Confidence Interval
% = 20
% = 40

Data source: Procleix Zika virus assay package insert, AW-15886 Rev. 001 (CE marked assay)

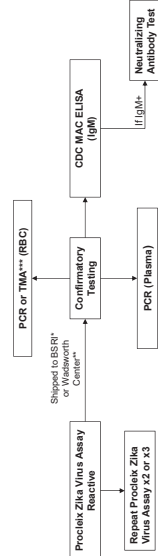
In vitro transcript stock concentration was determined by absorbance at 260 nm

Transcript sequence corresponds to the African strain of Zika virus: isolate MR766 (GenBank accession number AY632635)

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Procleix Zika Virus Assay Currently Used in US for Investigational Screening

Overview of index donation testing algorithm



Follow up testing: about every week until donor is Procleix Zika virus assay non-reactive and:

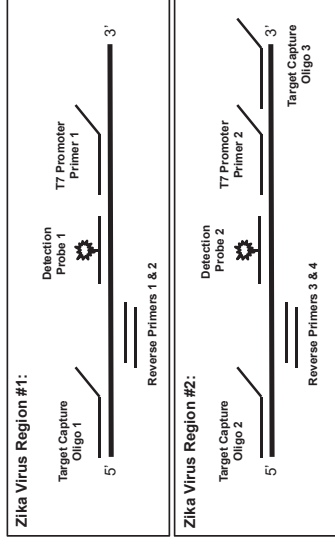
- IgM+ confirmed by neutralizing antibody
- OR
- Alternative NAT and serology results are non-reactive

*Blood Systems Research Institute (San Francisco, CA)
**New York State Public Health, Wadsworth Center (Albany, NY)
***Research and cell TMA performed at Grifols (San Diego, CA)

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Two Region Amplification/Detection of Zika Virus RNA

Redundant design expected to substantially mitigate risk of false negative results



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Procleix Zika Virus Assay: Analytical Sensitivity

Probit analysis of results

Country of Origin	% Positive	95% Fiducial Limits
Colombia	1.1	1.06 (0.92 - 1.20)
Dominican Republic	1.1	2.94 (2.54 - 3.37)
Dominican Republic	1.1	11.71 (9.78 - 14.57)

Detection Probabilities in Copies/mL (95% Fiducial Limits)

50%
95%

Virus (Brazilian Donor Specimen)*
in vitro Synthesized Transcript**

*Virus represents Asian strain
**VY sequence represents African strain

Data source: Procleix Zika Virus Assay package insert, AW-15886 Rev. 001 (CE marked assay)

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Update on Procleix Zika Virus Assay Testing in the US

Results through 22 July 2017 under the Investigational New Drug (IND) protocols

	ARC	Non-ARC	Total*
Individual Donations	3,261,679	2,604,027	5,865,706
Pooled Donations	393,713	--	393,713
Total Donations	3,655,392	2,604,027	6,259,419
Initially Reactive	134	99	233
Confirmed Positive	8	15	23

ARC = Active Release Codes
Non-ARC = Non-Active Release Codes
*Total number of donations derived from some unverified data sets

**ARC: Westborough, MA; Wadsworth Center, Albany, NY; Blood Systems Research Institute (San Francisco, CA); Blood Systems Research Institute (Oakland, CA); Inverness Medical Services (Spring, WA); Blood Assurance (Chattanooga, TN); RhoD Issue Blood Center.

- Total confirmed positive donations: 23 (or -1 per 272,000)
- Estimated overall clinical specificity: 99.987%

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Analytical Sensitivity: Zika Virus from Brazilian Blood Donor

Limit of detection (LOD) determination by dilution of virus

Concentration Copies/mL	Lot 1 (n=72)	Lot 2 (n=72)	Combined (n=144)
90	100 (100%)	100 (100%)	100 (100%)
30	100 (100%)	100 (100%)	100 (100%)
10	100 (100%)	100 (100%)	100 (100%)
3	96 (133.3%)	92 (127.8%)	94 (131.1%)
1	38 (52.8%)	60 (83.3%)	49 (68.1%)
0.3	18 (25.0%)	14 (19.4%)	17 (23.6%)
0.1	1 (1.4%)	0 (0%)	1 (1.4%)
0	0 (0%)	0 (0%)	0 (0%)

CI = Confidence Interval
% = 20
% = 40

Data source: Procleix Zika virus assay package insert, AW-15886 Rev. 001 (CE marked assay)

Virus stock concentration was determined by testing at Blood Systems Research Institute (BSRI, San Francisco, CA) using real-time and digital PCR

Virus tested represents Asian strain of Zika virus

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Procleix Zika Virus Assay: Preliminary Clinical Sensitivity

RNA positive plasma specimens (N = 26) from Colombia and Dominican Republic

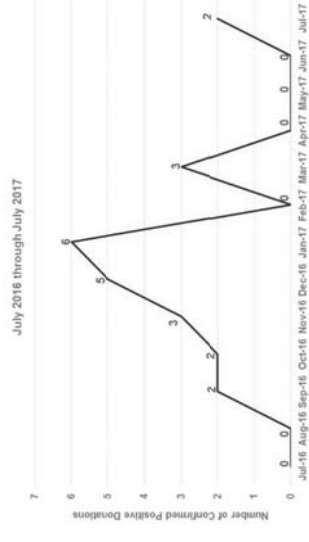
Country of Origin	Reactive/ # tested	% Positive	95% Fiducial Limits	Average t-ROC
Colombia	1/1	100%	100%	30.7
Colombia	1/1	100%	100%	44
Colombia	1/1	100%	100%	31.6
Colombia	1/1	100%	100%	19.7
Colombia	1/1	100%	100%	26.1
Colombia	1/1	100%	100%	30.8
Colombia	1/1	100%	100%	31.1
Colombia	1/1	100%	100%	26.9
Dominican Republic	1/1	100%	100%	27.2
Dominican Republic	1/1	100%	100%	27.6
Dominican Republic	1/1	100%	100%	31.7
Dominican Republic	1/1	100%	100%	30.5
Dominican Republic	1/1	100%	100%	30.5
Dominican Republic	1/1	100%	100%	16.2
Dominican Republic	1/1	100%	100%	27.5
Dominican Republic	1/1	100%	100%	31.3
Dominican Republic	1/1	100%	100%	31.4
Dominican Republic	1/1	100%	100%	26.8
Dominican Republic	1/1	100%	100%	31.9
Dominican Republic	1/1	100%	100%	32.5
Dominican Republic	1/1	100%	100%	31.6
Dominican Republic	1/1	100%	100%	31.6
Dominican Republic	1/1	100%	100%	31.7
Colombia	1/1	100%	100%	32.2
Colombia	1/1	100%	100%	30.2

CI = Confidence Interval
t-ROC = t-ROC
% = 20
% = 40

Data source: Procleix Zika virus assay package insert, AW-15886 Rev. 001 (CE marked assay)

Update on Procleix Zika Virus Assay Testing in the US

Confirmed positive donations identified in ongoing investigational testing



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Examples of Zika Virus RNA Positive Donations

Due to low plasma viral load, not detected reliably with screening NAT

Donor	Collection Site	Collection Date	Reacts: NAT, SCC	Hologic: NAT Repeat Duplicate	ZNY IMA IgG	ZNY ALE NAT RT-PCR	ZNY IMA IgG	Viral Particle NAT, VP1	RBC ALE NAT RT-PCR	Travel/ Location (prior to screening)
1	URS RenoCTS	9/28/2016	R 1.75	NR, NR	Positive, Positive	Negative	Negative, Positive	Zika Neg.	Positive	Scoregona Nicaragua 2016 (41)
1: Follow Up	URS RenoCTS	10/2/2016	NR	NT	Positive, Positive	Negative	Negative, Positive	Zika Neg.	Positive	--
2	New York CenterCTS	9/28/2016	R, 33.08	NR, NR	Positive, Positive	Equivocal	Negative, Positive	Zika Neg.	Positive	Trinidad 2016 (27)
2: Follow Up	New York Blood CenterCTS	10/6/2016	NR	NT	Positive, Positive	Negative	Negative, Positive	Zika Neg.	Positive	--

Donors #1 and #2 reported travel to areas with local transmission 41 and 97 days, respectively, prior to donating blood

Virus detected in RBCs from index and follow up donations

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Conclusions/Summary

- US FDA has classified ZIKV as a relevant transfusion-transmitted infection
- Four probable transfusion transmission cases (in Brazil) have been documented
- NATs from 2 manufacturers (Roche and Grifols) are currently being used to screen individual donations nationwide under Investigational New Drug (IND) protocols
- Preliminary data suggest that NATs used for investigational testing in US are both sensitive and specific
 - Majority of viremic donors in US states acquired ZIKV through travel to areas with local transmission
- Incidence of Zika virus worldwide appears to be decreasing
 - Difficult to predict the course of the outbreak in individual regions
 - Confirmed positive donations continue to be identified (most recent cases in July 2017)



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Additional TMA Testing of RBC Positive Donor Sample

Donor #1 (UBS Reno): Index donation initially reactive but not reliably detected in plasma

Sample	Dilution	# Valid	# Reactive	% Reactive
Plasma	Neat	29	2	7
	1:3	10	10	100
RBC	1:30	10	10	100
	1:300	10	4	40
	1:3000	10	0	0

RBC samples were prepared using "Parasite Transport Medium" (PTM) developed for whole blood sample preparation; tested with the Procleix Zika Virus assay on the Panther system in Grifols R&D Lab (San Diego)

RNA detection observed in RBC specimens down to a 1:300 dilution

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Combined ZIKV IND Data for all sites, US States

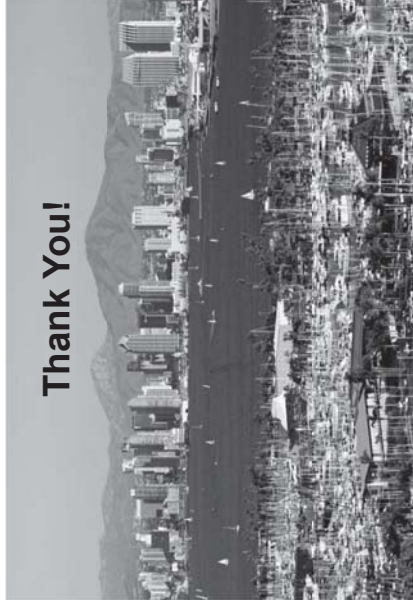
As of June 3, 2017, >8.0 million donations screened

- 47 confirmed positive/549 initially reactive
 - 8.6% Positive Predictive Value (PPV); 99.994% specificity
 - 1:171,000 overall rate
- State of residence for confirmed positive cases (N=47):
 - 25 (53%) FL, 6 CA, 4 NY, 5 TX, 1 NV, 1 AZ, 1 WA, 1 WV, 1 TN, 1 PA, 1 IA
 - Of 25 outside of FL, vast majority had remote travel to an active area
- Summary of confirmatory testing of confirmed positive cases
 - Alt (alternative) NAT pos or eqvl/ Ab neg = 7 (6/6 MP-NAT reactive; 1 QNS)
 - Alt NAT pos/Ab pos = 7 (5/6 MP-NAT reactive; 1 QNS)
 - Alt NAT neg/ Ab pos = 33 (70%) (1 MP-NAT reactive)

<http://www.cdc.gov/zika/genome-and-data.html>
 Danerys, et al. *Emerging Infectious Diseases* 2017, International Society of Blood Transfusion Regional Meeting, Copenhagen, Denmark.

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Thank You!



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Acknowledgements

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Alanna Menez

Grifols Regulatory Affairs

Petra Pavlickova, Ph.D.

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