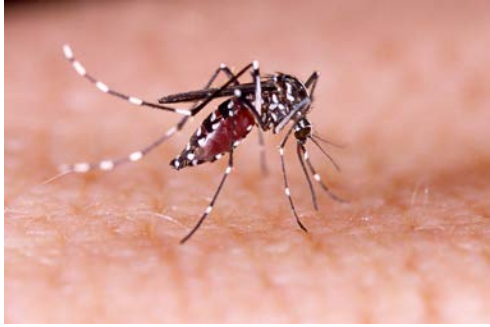

Zika Virus and Blood Safety in the US

Update on the Procleix® Zika Virus Assay

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BPAC Meeting
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Disclosure / Disclaimer

Procleix® Zika Virus assay is in Development

- The Procleix Zika virus assay is in development and available for use under an Investigational New Drug (IND) protocol in the United States; the assay is for Investigational Use Only in the US. The performance characteristics of this product have not been established.

  The Procleix Panther® system is not available for commercial use in the US; the system is for Investigational Use Only. Performance characteristics of the Procleix Panther system have not been established.

- Conflict of interest: the presenter is an employee and stockholder of Hologic Inc., which develops and manufactures NAT blood screening assays in partnership with Grifols Diagnostic Solutions Inc.

Topics

- Review of design goals for Procleix Zika Virus assay, an investigational NAT
- Preliminary assay performance characteristics
- Additional applications of the technology used in the blood screening investigational NAT
- Current status of testing under the Hologic-Grifols Investigational New Drug (IND) protocols in the US

Risk of ZIKV Transmission by Blood Transfusion

Outlined by FDA in revised guidance issued in August 2016*

- ZIKV infection asymptomatic in ~80% of individuals; transmitted sexually for unknown duration, presence of viral RNA as long as 6 months has been reported
- Infectious virus, with high levels of viremia, may be present during asymptomatic period
- Virus titer can reach levels ≥ 8.1 million copies/mL in serum/plasma
- Probable transmission of ZIKV by blood transfusion has been reported**

*The complete guidance document can be found at the following site:
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM518213.pdf

**Motta IJF et al. (2016) Evidence for transmission of Zika virus by platelet transfusion. N Engl J Med.; Barjas-Castro, M. L. et al. (2016), Probable transfusion-transmitted Zika virus in Brazil. Transfusion, 56: 1684–1688.

Zika Virus Blood Screening in the US: Current Status

- Currently no FDA-licensed NAT blood screening tests for Zika virus
- Implementation of investigational screening assays considered necessary because of potential for large proportion of asymptomatic cases in areas of active transmission (Feb. 26, 2016 FDA Guidance)
- Testing for ZIKV RNA became available through the Hologic-Grifols Investigational New Drug (IND) protocol on June 20, 2016
  - Testing initiated using a combination of pools (16-donations) and individual donor nucleic acid testing (ID-NAT), focusing on southern continental US
- FDA issued revised guidance in August 2016 (superseded Feb. 2016 Guidance):
  - Donations from US and US territories should be tested using ID-NAT under an approved IND application or pathogen reduction technology for platelets and plasma using an FDA-approved pathogen reduction device (phased implementation allowed)
  - Efforts to implement ID-NAT nationwide underway; currently implemented at 12 sites with some additional sites under consideration

Zika Virus Screening: IND Protocols

- Current IND Protocols
  - Direct detection of ZIKV RNA in plasma specimens from donors
  - Alt NAT and Serology used for confirmation of reactives
  - Non-reactive donations accordingly labeled for use
  - Update to protocols (currently under Agency review) - adding option of testing of red blood cells and/or whole blood and proposed changes to confirmatory algorithm
- Additional protocols in preparation
  - Organ/tissue and cellular products from living donors
  - Cadaveric specimens

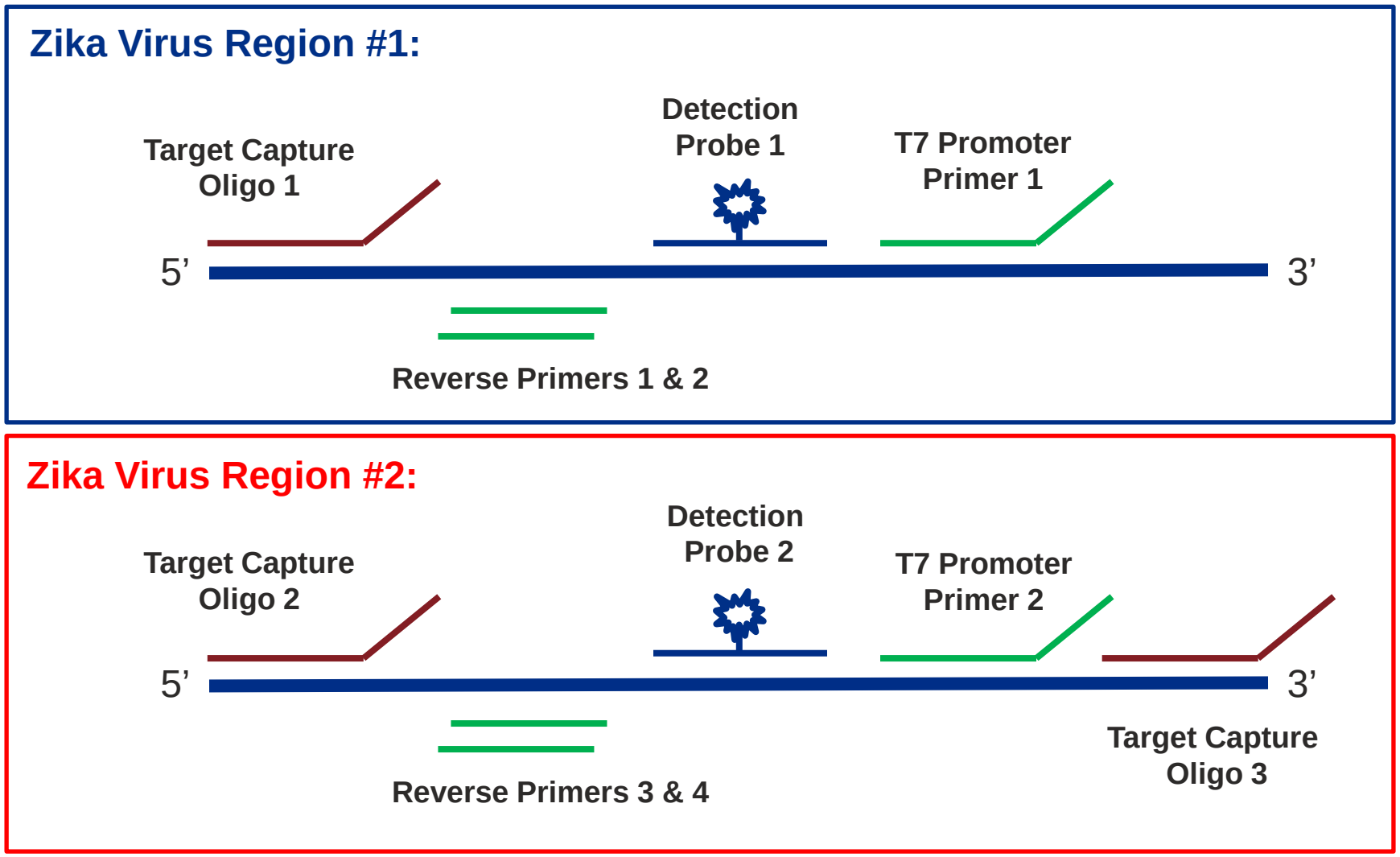
Procleix Zika Virus Assay on the Procleix Panther System

Assay design goals

- Qualitative transcription-mediated amplification (TMA) assay designed to run on the fully automated Procleix Panther system
- Analytical sensitivity
  - Comparable to other TMA blood screening assays: 95% detection of 10-30 copies/mL
- Assay specificity
  - Comparable to current blood screening nucleic acid tests (NATs): ≥ 99.90%
- Detection of genetic variants of Zika virus
  - Including African and Asian strains
- Two region amplification/detection expected to enhance sensitivity and reduce risk of false negative results

Two Region Amplification/Detection of Zika Virus RNA

Redundant design expected to substantially mitigate risk of false negative results



Procleix Zika Virus Assay: Analytical Sensitivity

Hologic in vitro synthesized transcript (African strain) diluted in buffer

Copies/mL	# Reactive / Tested	% Reactive (95% CI)	S/CO	
			Average*	%CV
90	20/20	100 (84-100)	33.5	4.3%
30	72/72	100 (95-100)	33.2	4.7%
10	66/72	92 (83-96)	32.0	13.6%
3	39/72	54 (43-65)	28.7	23.3%
1	16/72	22 (14-33)	27.7	26.4%
0.3	2/72	3 (1-10)	24.5	50.7%
0	0/72	0 (0-5)	0.0	N/A

*Average of reactive replicates unless all tests were nonreactive, in which case the average analyte signal of nonreactive replicates is shown.
CI=confidence interval; S/CO=signal to cutoff

Procleix Zika Virus Assay: Analytical Sensitivity

Brazilian blood donor specimen (Asian strain) diluted in plasma

Virus, Copies/mL	# Reactive / Tested	% Reactive (95% CI)	S/CO	
			Average*	%CV
90	20/20	100 (84-100)	32.5	3.3%
30	72/72	100 (95-100)	32.6	4.1%
10	72/72	100 (95-100)	31.5	9.7%
3	62/72	86 (76-92)	25.8	29.3%
1	27/72	38 (27-49)	24.1	37.1%
0.3	14/72	19 (12-30)	17.7	32.8%
0.1	1/72	1 (0-8)	15.7	N/A
0	0/72	0 (0-5)	0.0	N/A

*Average of reactive replicates unless all tests were nonreactive, in which case the average analyte signal of nonreactive replicates is shown.
CI=confidence interval; S/CO=signal to cutoff

Aptima Zika Virus Assay: Analytical Sensitivity in Processed Urine*

Additional application of the technology using the Aptima Zika Virus assay (TMA assay with FDA Emergency Use Authorization)*

Copies/mL	# Reactive / Tested	% Reactive	S/CO	
			Average*	%CV
90	30 / 30	100	35.26	4%
30	30 / 30	100	34.16	13%
10	30 / 30	100	28.70	27%
3	14 / 30	46.7	22.12	33%
1	6 / 30	20	29.40	28%
0.3	3 / 30	10	29.75	37%
0	0 / 30	0	0.00	N/A

*Average of reactive replicates unless all tests were nonreactive, in which case the average analyte of nonreactive replicates is shown.
CI=confidence interval; S/CO=signal to cutoff

Virus spiked in urine was added to Urine Transport Medium (UTM) prior to testing; UTM increases stability of Zika virus in urine specimens

*For more information about EUA assays for Zika virus: http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika

Procleix/Aptima Zika Virus Assay: Analytical Sensitivity

Probit analysis of results

	Detection Probabilities in Copies/mL (95% Fiducial Limits)	
	50%	95%
Virus (Brazilian Donor Specimen)	1.0 (0.8-1.2)	5.9 (4.3-8.9)
in vitro Synthesized Transcript	2.4 (2.0-2.9)	13.4 (9.9-20.3)
Virus in Processed Urine*	2.6 (1.9-3.4)	8.5 (6.0-15.3)

***testing of Zika virus in urine was done with the Aptima Zika Virus assay, diagnostic version of the assay which is available under an FDA EUA (Emergency Use Authorization)**

Repeatability of Procleix Zika Virus Assay

Carried out by 2 operators using 2 Panther Systems

Copies/ mL	N	#R	%A	Analyte S/CO	Inter-Day		Inter-Operator		Inter-Instrument		Intra-Run		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
18	108	108	100%	33.12	0.15	0%	0.15	0%	0.25	1%	1.29	4%	1.33	4%
30	108	108	100%	33.24	0.24	1%	0.16	0%	0.18	1%	1.32	4%	1.36	4%
60	54	54	100%	33.20	0.24	1%	0.19	1%	0.25	1%	1.20	4%	1.26	4%
600	54	54	100%	32.96	0.24	1%	0.21	1%	0.27	1%	1.32	4%	1.38	4%
6000*	54	54	100%	32.97	0.33	1%	0.21	1%	0.23	1%	1.27	4%	1.35	4%

*in vitro synthesized transcript was used for this panel member

A: agreement; S/CO: signal to cut-off; SD: standard deviation; CV: coefficient of variability

Intra-run factor was the largest source of variability

Procleix Zika Virus Assay: Preliminary Clinical Specificity

RUO results: Individual Donor specimens from non-endemic regions* in US

	Hologic (San Diego, CA)		American Red Cross (Gaithersburg, MD)	Combined Results
	Plasma	Serum	Plasma	
N	775	240	9,000	**10,015**
Non-Reactive	775	240	8,999	**10,014**
Initially Reactive	0	0	1	**1**
True Positive	0	0	0	**0**
False Positive	0	0	1	**1**
Specificity (95% CI)	100% (99.53-100)	100% (98.47-100)	99.99% (99.94-100)	**99.99% (99.94-100)**

Data from testing two master lots under an Research Use Only (RUO) protocol; CI=confidence interval

*States included in ARC RUO testing: Nebraska, Georgia, Florida, South Carolina, Mississippi, and Alabama (note: at the time of the study there were no locally transmitted ZIKV cases in these states).

Procleix Zika Virus Assay: Preliminary Sensitivity and Specificity in Cadaveric Specimens

Comparison of plasma and serum cadaveric specimens

Sensitivity: ZIKV spiked into cadaveric plasma/serum specimens at 18 copies/mL of virus

		Plasma		Serum		Combined	
		Control	Cadaveric	Control	Cadaveric	Control	Cadaveric
N		30	20	30	20	60	40
# Reactive		30	20	30	20	60	40
Reactive		100%	100%	100%	100%	100%	100%
Analyte S/CO	Average	32.61	31.72	32.72	32.01	32.66	31.86
	%CV	9%	4%	3%	3%	7%	4%

Specificity: Non-spiked cadaveric plasma/serum specimens

		Plasma		Serum		Combined	
		Control	Cadaveric	Control	Cadaveric	Control	Cadaveric
N		30	20	30	20	60	40
# Reactive		0	0	0	0	0	0
Specificity		100%	100%	100%	100%	100%	100%
IC S/CO	Average	1.91	1.96	1.92	1.95	1.91	1.96
	%CV	2%	3%	2%	5%	1%	3%

Update on Blood Screening under the Hologic-Grifols Investigational New Drug (IND) Protocols

Summary of Testing Implementation under Hologic-Grifols IND protocol in US (as of 10 November 2016)

Testing Site	Testing Start Date
American Red Cross (ARC), Charlotte, VA	6/20/2016
Creative Testing Solutions (CTS), Tempe, AZ	7/18/2016
Creative Testing Solutions (CTS), Bedford, TX	7/18/2016
American Red Cross (ARC), St. Louis, MO	8/29/2016
American Red Cross (ARC), Portland, OR	9/6/2016
Oklahoma Blood Institute (OBI), Oklahoma City, OK	9/21/2016
MD Anderson (MDA), Houston, TX	9/21/2016
Blood Assurance (BASS), Chattanooga, TN	9/25/2016
Inova Blood Donor Services (IBDS), Sterling, VA	9/28/2016
Rhode Island Blood Center, Providence, RI	11/19/2016
Labs Inc., Centennial, CO	11/19/2016
Interstate Blood, Memphis, TN	*12/12/2016*

Update on ZIKV Testing Results under the IND Protocol

Available results as of November 5

Testing Site	# Pools Tested*	# IDS Tested	Total Donations Tested	# Initially Reactive Results**	# Confirmed Positive Results
ARC	9,000	126,582	270,565	3	*1*
CTS/BASS/ MDA/OBI/ IBDS	0	234,175	234,175	18	3
Total			**504,740**	**21**	**3 (+1)**

ARC: American Red Cross; CTS: Creative Testing Solutions; BASS: Blood Assurance; MDA: MD Anderson; OBI: Oklahoma Blood Institute, IBDS: Inova Blood Donor Services
IDS: Individual donor samples
*16-donation pools
**Includes false-positives defined as non-repeating initially reactive, seronegative, ALT NAT negative and confirmed positives

Summary of Repeat Reactive Procleix ZIKV NAT or Serologically Confirmed Donations- As of November 5

Donor	Collection Site/Testing Site	Collection Date	Hologic NAT, S/CO	Hologic NAT Retest in Duplicate	ZIKV IgM, IgG	ZIKV Alt. NAT (RT-PCR)	DENV IgM, IgG	Viral Particle Neut. (VPN)*	RBC Alt. NAT (RT-PCR)	Travel/ Location/ Date (days prior)
1	UBS, Reno/CTS	9/26/2016	R, 18.75	NR, NR	Positive, Positive	Negative	Negative, Positive	Zika Neut.	Positive	Yes/ Nicaragua 5/21- 8/16, 2016 (41)
1: Follow Up 1	UBS, Reno/CTS	10/5/2016	NR	NT	Positive, Positive	Negative	Negative, Positive	Zika Neut.	Positive	--
2	New York Blood Center/CTS	9/26/2016	R, 30.68	NR, NR	Positive, Positive	Equivocal	Negative, Positive	Zika Neut.	Positive	Yes/ Trinidad 6/8-6/21, 2016 (97)
2: Follow Up 1	New York Blood Center/CTS	10/6/2016	NR	NT	Positive, Positive	Negative	Negative, Positive	Zika Neut.	Positive	--
3	UBS, Arizona/CTS	10/14/2016	R, 21.61	R, NR	Positive, Positive	Negative	NT, Positive	*Pending*	Positive	Yes/Mexico (Pinotepa, Oaxaca) 8/17-8/24 2016 (51)
3: Follow Up 1	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	--
4	*Texas Hospital/ ARC St. Louis*	*11/2/2016*	*R, 32.4*	*R, R, R*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Yes, Puerto Rico (28)*
4: Follow up 1	*Texas Hospital/ ARC St. Louis*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	*Pending*	--

For a description of the VPN technology see: Song et al. BMC Microbiology2014 **14**:44

Speaker Notes for Slide 19

First ZIKV+ donors detected using Hologic's assay

First ZIKV+ donors detected outside PR and FL

All three donors had travel histories 2-3 months prior to donation, and two developed symptoms c/w Zika shortly after returning from travel to ZIKV risk countries

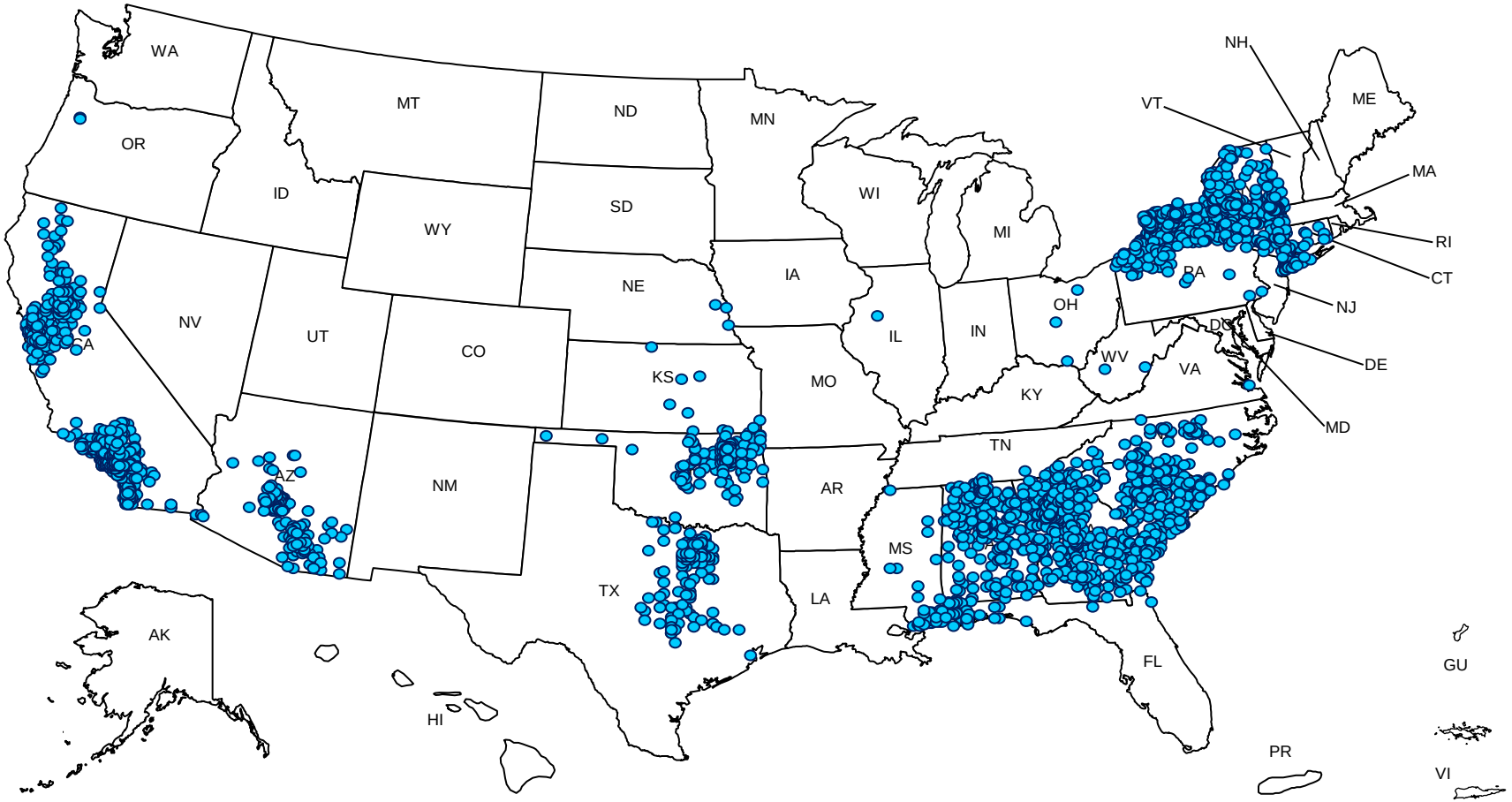
Extremely low plasma VL (based on replicate NAT and PCR/VL testing so barely detected by the Hologic's assay on plasma

High levels of ZIKV RNA associated with RBC from index donation EDTA pilot tubes and for 2 cases in LR-pRBC components, c/w our now extensive index donation and follow-up data for ZIKV+ PR donors on persistence of ZIKV RNA in the RBC compartment after clearance from plasma

Strong ZIKV IgM, IgG and neutralizing activity (still increasing after 2-3 months for the NY and Reno cases with serial samples) and absence of DENV IgM and IgG (aside from very weak cross reactivity on the IgG ELISA)

RVPs are pseudo-infectious virions produced by complementation of a self-replicating flavivirus replicon with the DENV structural genes in trans. RVPs harvested from transfected cells are capable of only a single round of infection and encapsidate replicon RNA that encodes a reporter gene used to enumerate infected cells. RVPs may be produced using the structural genes of different DENV serotypes, genotypes, and mutants by changing plasmids used for complementation. Further modifications are possible including generating RVPs with varying levels of uncleaved prM protein, which resemble either the immature or mature form of the virus. Neutralization potency is measured by incubating RVPs with serial dilutions of antibody, followed by infection of target cells that express DENV attachment factors. Enhancement of infection is measured similarly using Fc receptor-expressing cells capable of internalizing antibody-virus complexes.

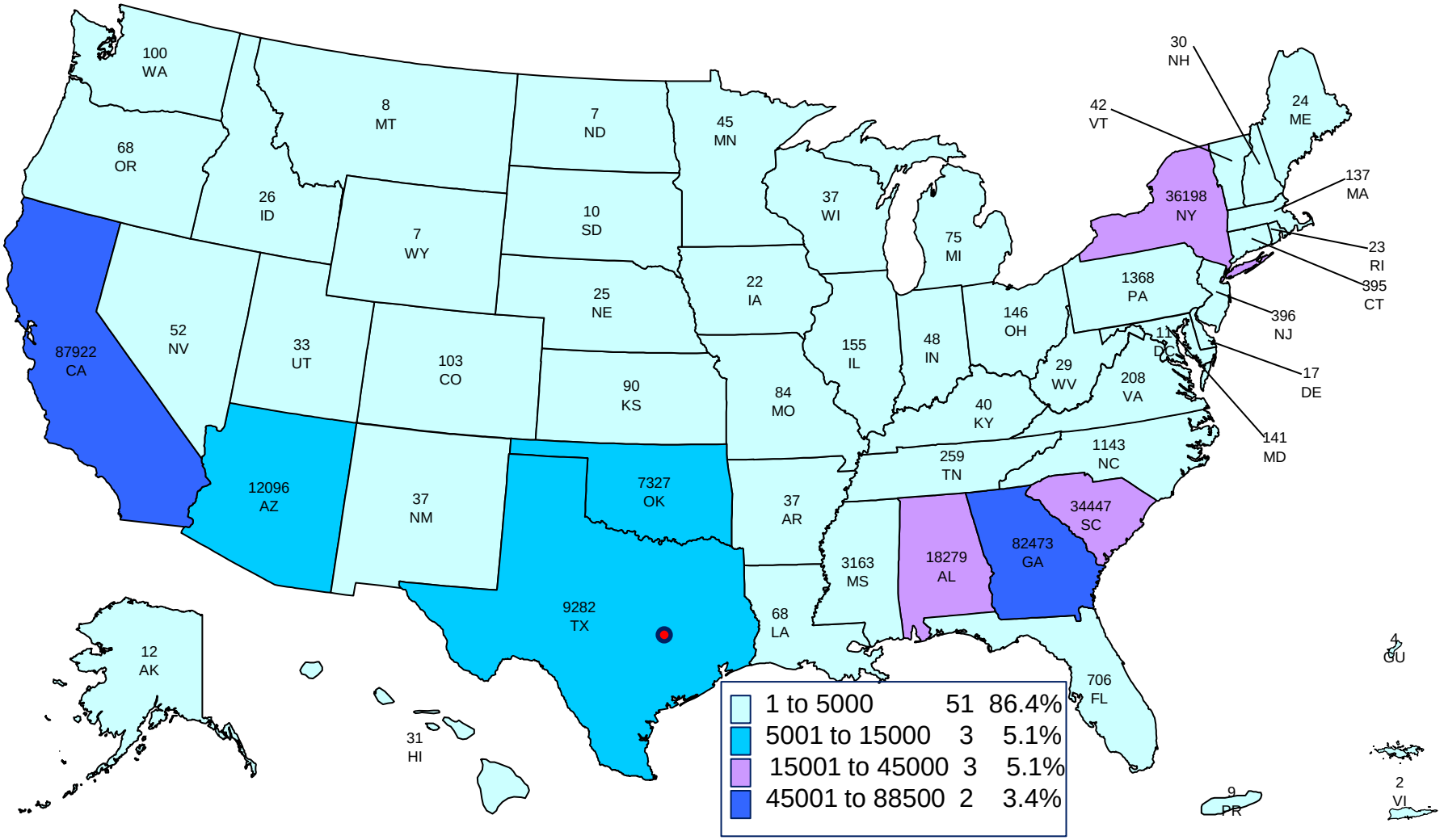
American Red Cross: ZIKV Testing by Blood Drive Zip Code
June – November 12 2016



Slide courtesy of Dr. Susan Stramer

3,205 zip codes with 1 - 4,936 donations per zip code

ARC: ZIKV Tested Samples by State of Donor Residence
June - November 12 2016

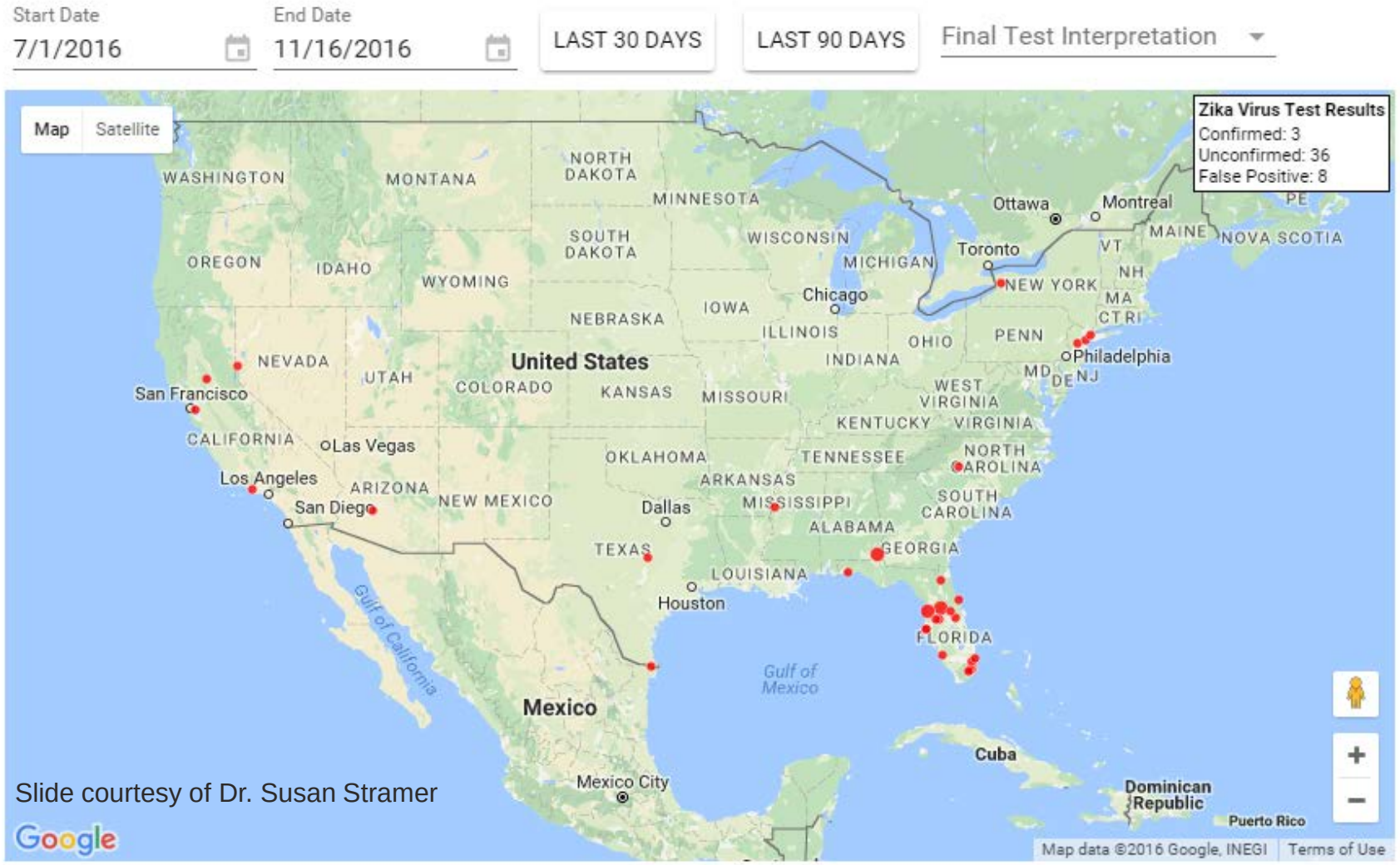


Slide courtesy of Dr. Susan Stramer

297,519 donations tested including 143,983 MP-NAT and 153,533 ID-NAT; 3 nonRR; 1RR

AABB ZIKV Biovigilance Site

U.S. Map: ZIKV-Reactive Blood Donors by Postal Code



Slide courtesy of Dr. Susan Stramer

Collection Sites for Testing at CTS (Dallas and Phoenix)

Testing being done on individual donor samples (IDS) under Hologic IND Protocol

Slide courtesy of Phillip Williamson, CTS

CTS: Creative Testing Solutions; Phoenix: **blue**; Dallas: **red**

Additional Procleix 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
RBC	1:3	10	10	100
	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 Hologic R&D Lab (San Diego)

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

Summary of ZIKV Positive Donations

- Three (plus 1) ZIKV confirmed positive donors detected in the Hologic-Grifols IND protocols
- ZIKV positive donors originated from collections outside of areas with active transmission: Nevada, New York, Arizona, and Texas
- All four donors had travel histories, ranging from 28 to 97 days prior to donation; two developed symptoms consistent with ZIKV infection shortly after returning from travel to ZIKV risk countries
- Extremely low plasma viral load levels (based on replicate NAT and PCR/Viral load testing) for 3 out of 4 donors
- High levels of ZIKV RNA associated with RBCs from index donation
- Strong ZIKV IgM, IgG and neutralizing activity (still increasing after 2-3 months for the NY and Reno cases with serial samples) and absence of DENV IgM but very weak cross reactivity with Dengue IgG ELISA
- ARC case of donor from Texas IR and RR 3 times with consistent high S/CO values; further testing pending

Overall Summary/Conclusions

- Procleix assay design for 2-region amplification/detection expected to substantially reduce the chance of false negative results
- Demonstrated very sensitive detection of Zika virus RNA (95% detection at ~6 to 13 copies/mL)
- Preliminary data indicated that the specificity of the assay was >99.90%; confirmed by testing under IND protocol
- Preliminary testing of cadaveric specimens showed assay sensitivity/ specificity were not affected by these potentially problematic samples (showed robustness of the assay)
- All Procleix repeat reactive or serologically confirmed donations were linked to travel to areas with local transmission and 3/4 contained very low viral titers
- Efforts are underway to support the US FDA revised guidance for nationwide individual donor NAT

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