

Transforming Molecular Diagnostics with the GenArraytion MultiFLEX™ Bioassay for Zika, Chikungunya, Dengue, and Yellow Fever Viruses

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About ATCC

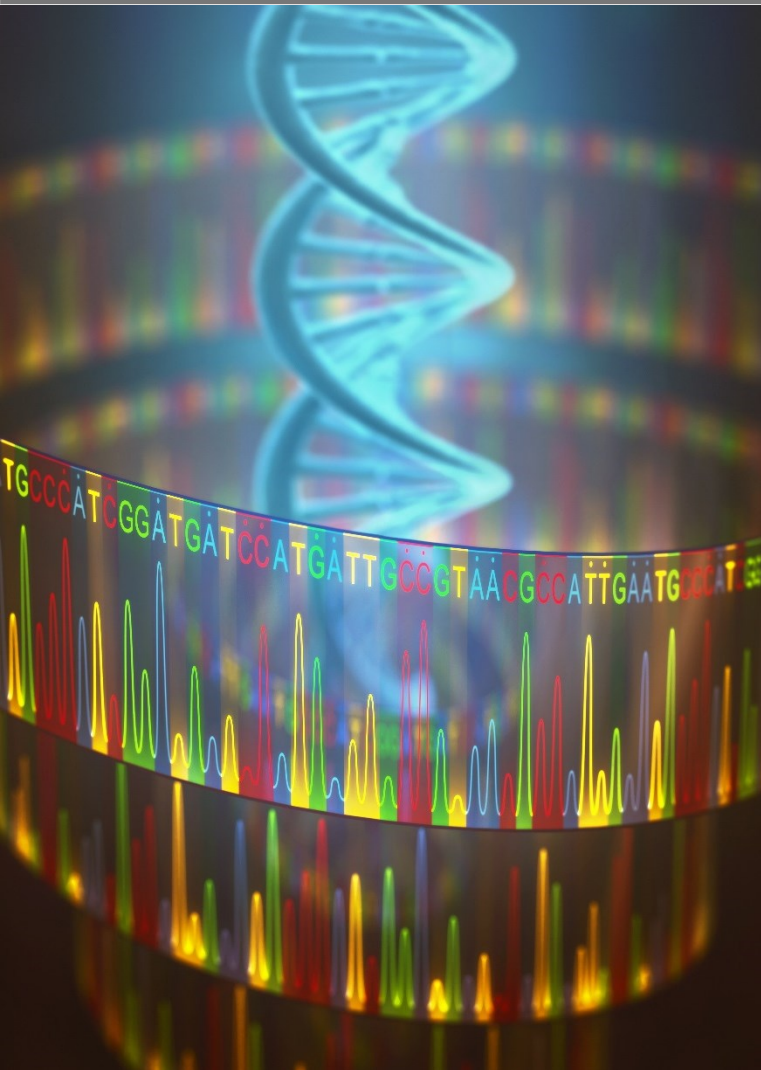
- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees



Established partner to global researchers and scientists



Agenda



- **The impact of vector-borne diseases and factors contributing to their spread**
- **Methods of detection and current challenges**
- **The need for authenticated control materials in assay development**
- **GenArraytion's molecular assay development approach**
- **GenArraytion MultiFLEX™ vector-borne disease panels**
 - Platform compatibility
 - Assay sensitivity
 - Assay specificity

Vector-borne disease

The World Health Organization estimates that vector-borne diseases account for **17%** of the estimated global burden of all infectious diseases, resulting in more than **1 million** deaths annually

Mosquitoes are one of the best known vectors

- *Aedes* – Zika, Dengue, Chikungunya, Yellow fever, Rift Valley fever
- *Anopheles* – Malaria
- *Culex* – Japanese encephalitis, Lymphatic filariasis, West Nile fever



Image of *Aedes aegypti*
courtesy of James Gathany

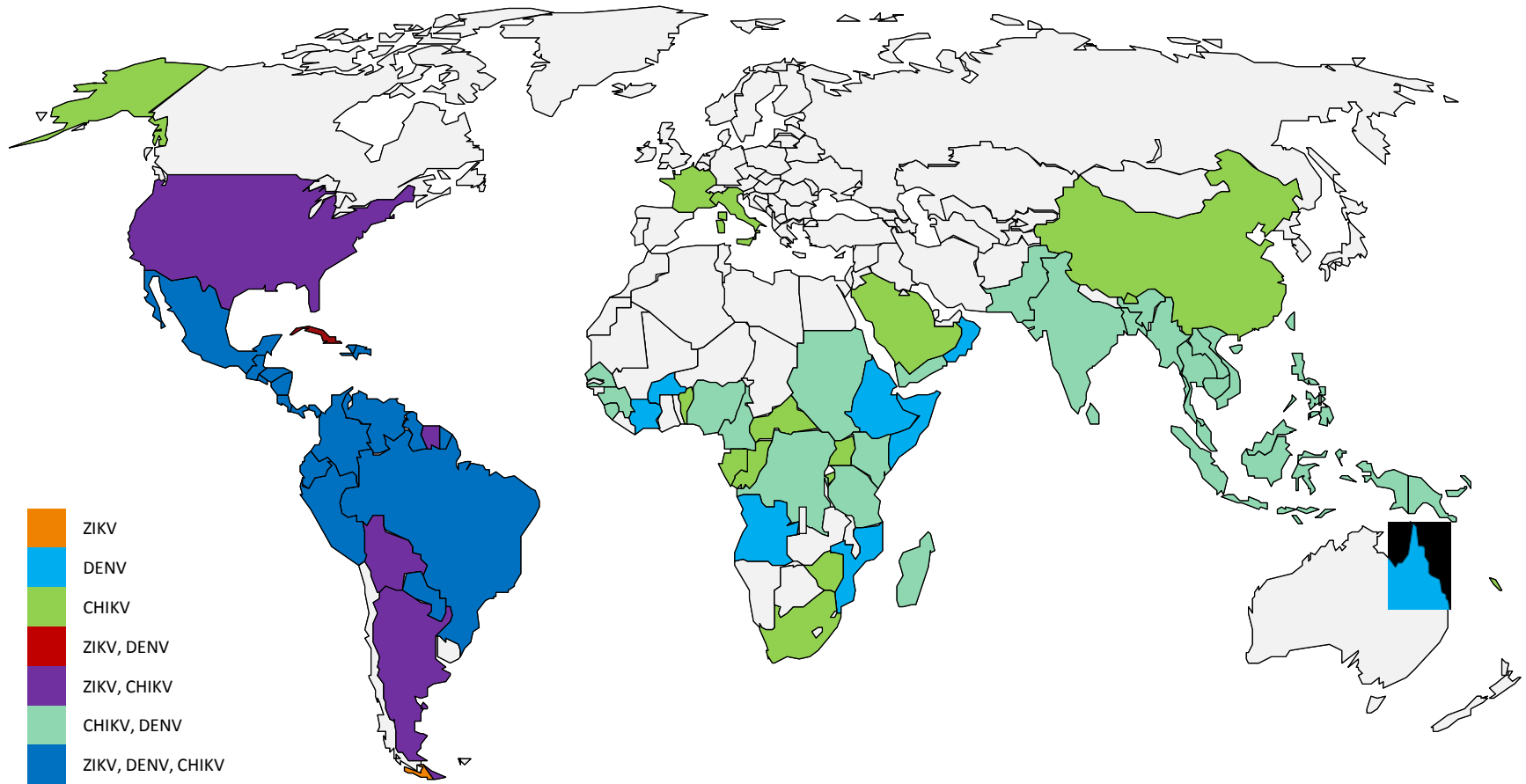
Factors contributing to the spread of disease

- Environmental changes
- Global transportation and trade
- Demographic and societal changes
- Poor waste disposal and water storage
- Changes in agricultural practices
- Pathogen evolution



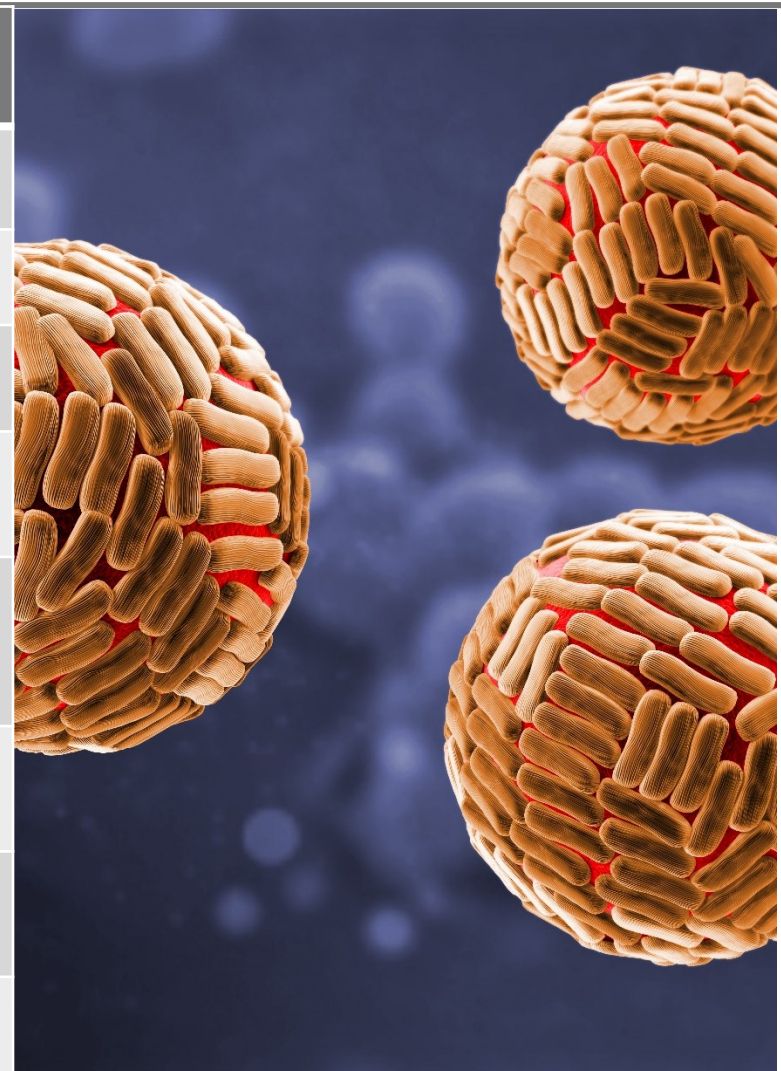
Image of *Culex* larvae in standing water courtesy of James Gathany

Areas of risk - Dengue, Chikungunya, & Zika virus



Symptoms - Dengue, Chikungunya, & Zika virus

Symptoms	Dengue	Chikungunya	Zika
Fever	X	X	X
Headache	X	X	X
Skin rash	X	X	X
Muscle and joint pain	X	X	X
Nausea and/or vomiting	X	X	
Bleeding or bruising	X		
Pain behind the eyes	X		
Conjunctivitis		X	X



Methods of detection and current challenges

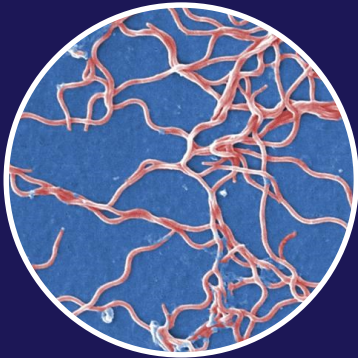
	Advantages	Challenges
Viral isolation	<ul style="list-style-type: none">• Identifies active infection• Sensitive and specific	<ul style="list-style-type: none">• Laborious• Time intensive• Requires expertise and appropriate BSL infrastructure
Serological tests	<ul style="list-style-type: none">• Identifies recent infection• Can measure a rise in microbial-specific IgM• Rapid• Often the go-to method of diagnosis	<ul style="list-style-type: none">• Less sensitive and specific• Antibodies may take several days before they are detectable• Antibodies may be at appreciable levels months following clearance• Serological cross-reactivity• Anamnestic response
Molecular-based detection	<ul style="list-style-type: none">• Enables early detection• Rapid and reliable• Sensitive and specific• Allows for quantification of microbial load• Multiplexing available	<ul style="list-style-type: none">• Requires expertise and specialized equipment• False positives can occur due to contamination

ATCC vector-borne disease solutions

- *In vivo* and tissue culture-adapted viral strains
- Pathogenic bacteria and protozoa
- Genomic and synthetic nucleic acids preparations
- Host cell lines and reagents
- Custom solutions for expansion, titering, and banking

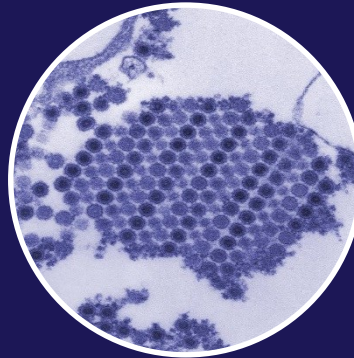


ATCC Genuine Cultures[®]



Bacteria

Anaplasma
Borrelia
Ehrlichia
Rickettsia



Viruses

Chikungunya virus
Dengue virus
La Crosse virus
Western equine
encephalitis virus
West Nile virus
Zika virus



Protozoa

Babesia
Leishmania
Plasmodium
Trypanosoma

Visit us online at www.atcc.org/vectorborne

ATCC® Genuine Nucleics



Genomic Nucleic Acids

Babesia
Borrelia
Leishmania
Plasmodium
Trypanosoma
Yersina pestis
Zika virus



Synthetic Nucleic Acids

Chikungunya virus
Dengue virus I-IV
Eastern equine encephalitis virus
St. Louis encephalitis virus
West Nile virus
Yellow fever virus
Zika virus

Visit us online at www.atcc.org/vectorborne

ATCC Synthetic Molecular Standards

Can be used as a positive control for:

- Difficult-to-culture or unculturable strains
- Strains requiring BSL-3 containment
- Strains on the commerce control list

Advantages of synthetic nucleic acids:

- Eliminate the need to culture microorganisms
- Can be used in a BSL-1 facility
- No shipping restrictions
- Manufactured under ISO 13485:2003
- Quantified using ddPCR™
- Useful for monitoring assay-to-assay or lot-to-lot variation



Summary

- Vector-borne diseases are a major cause of morbidity and mortality
- Due to limited preventative and therapeutic treatment options, accurate detection methods are critical
- ATCC offers authenticated strains and nucleic acids, related reagents, and customized solutions that support the development, validation, and employment of novel detection methods

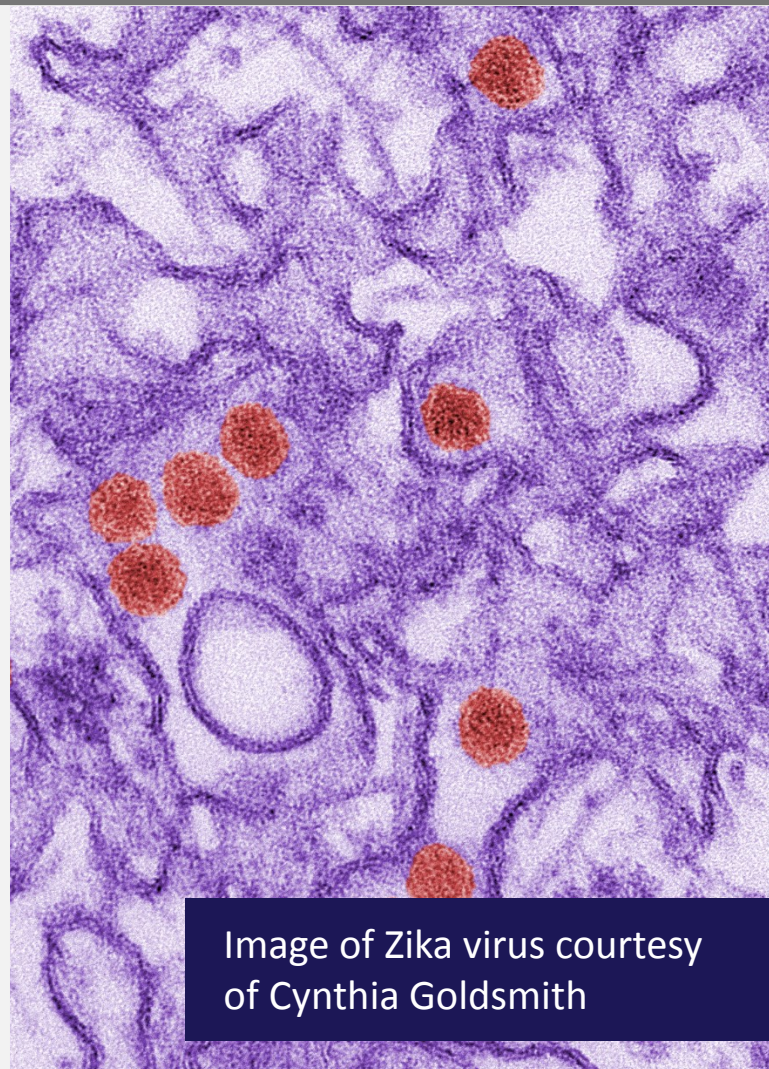
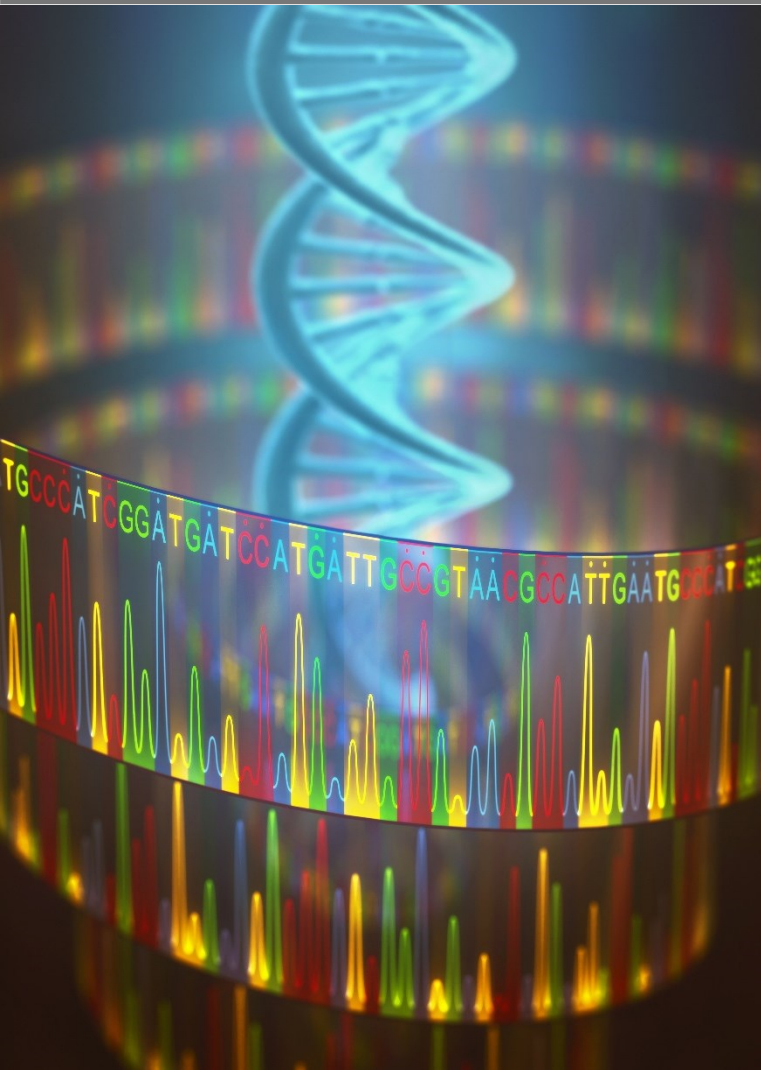


Image of Zika virus courtesy of Cynthia Goldsmith

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- **GenArraytion's molecular assay development approach**
- **GenArraytion MultiFLEX™ vector-borne disease panels**
 - **Platform compatibility**
 - **Assay sensitivity**
 - **Assay specificity**



GenArraytion Inc

NEXT GENERATION DIAGNOSTICS

R. Paul Schaudies, Ph.D.

GenArraytion, Inc.

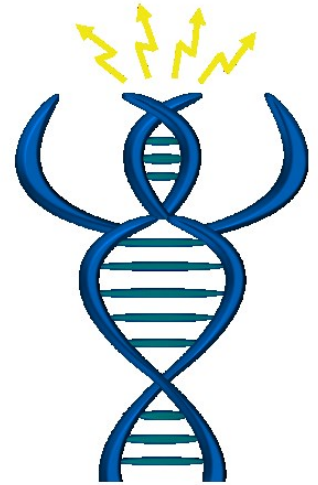
Rockville, MD

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GenArraytion, Inc.

- Veteran-owned small business in Rockville, MD
- Dedicated to improving global healthcare through development and distribution of molecular infectious disease assays on a variety of platforms
- Target markets include clinical & veterinary diagnostics, biosurveillance, biodefense, and forensics



Transforming Molecular Diagnostics

- Rapid development and distribution of infectious disease assay panels
 - Highly sensitive and specific multiplexed assays
 - New assays within weeks
 - Identification of established and emerging pathogens
 - Demonstrated compatibility with numerous platforms, including FDA approved platforms (real-time PCR and bead platforms)
- Assays for any DNA or RNA containing organism at the desired level of resolution
- Large database of molecular targets for rapid development and validation of expanded panels

GenArraytion MultiFLEX™ Molecular Assays

“Your Platform,
Your Targets,
Your Fluors,
No Ceiling”™

All targets within a particular panel mix and match on the platform of choice

GenArraytion 2014-2016

Assay development team for two major DoD programs

- Joint Biological Tactical Detection System (JBTDS)
 - 17 targets (RNA and DNA)
- Global Biosurveillance Technology Initiative (GBTI)
 - 64 targets (RNA and DNA)
- Luminex licensed 14 target *Aedes aegypti* mosquito panel and obtained FDA EUA for 6 target Zika subset assay
- End point and real-time assays developed for all targets

What Makes GenArraytion Unique?

Similarities

- At the end of the day, all PCR reactions have a primer pair and something in between to demonstrate presence of target

Discriminators

- **Unique Development Process**
 - High number of candidate target sequences validated
 - Bioinformatic and laboratory validation iterations identify highly specific targets with low LOD that “work and play well together”
 - Highly parallel processes, generating inclusivity and exclusivity data at each step
- **Extensive Database** Unique and functional oligo targets at various levels of validation
- **MultiFLEX™ Assays** for multiple real-time and end point platforms
- **Speed** *Aedes aegypti* panel with Zika test developed in three weeks

Zika Diagnostic Timeline (2016)

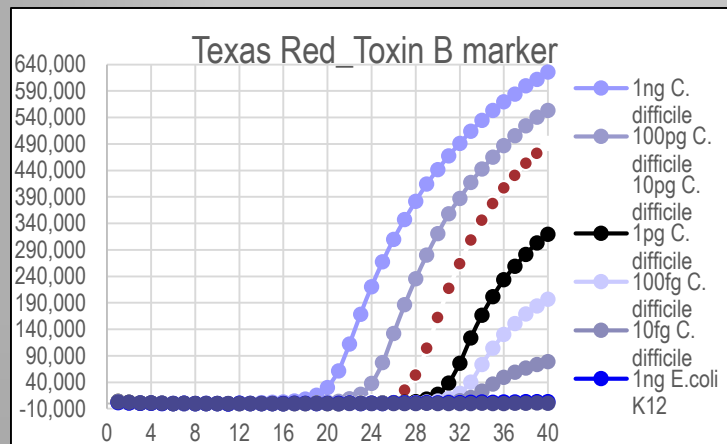
- 8 Jan Decision to develop assay
- 8-11 Jan Bioinformatics for screening array
- 11 Jan Zika (African) ordered from ATCC
- 13 Jan Array order submitted
- 20 Jan Array delivered – Hybridized with Zika NA
- 22 Jan Capture oligos and primers ordered
- 26 Jan Oligos and primers delivered
- 28 Jan 4-plex Zika test incorporated into panel
- 29 Jan Press release
- 4 Feb Washington Post Section A article
- March Panel licensed to Luminex for human IVD
- August FDA granted EUA for Zika virus

PCR Assay Protocols

End Point (Luminex MagPIX)	Real-Time (ABI 7500)
Isolate and purify nucleic acids	
Perform PCR amplification using appropriate enzyme, RNA and DNA, or DNA only Follow machine and enzyme protocols	
Product of PCR amplification is hybridized with complete bead mixture (up to 50 bead sets)	Place in PCR machine (up to 7 targets per assay) and run program
Incubate with reporter dye to label positive (hybridized) beads	Machine provides data
Place in MagPIX and run program	
Machine provides data	

PCR Assay Comparison

	End Point Assay	Real-Time Assay
Targets per reaction	1-23 ⁺ demonstrated, max 50	1-7, Instrument dependent
Optimal Sensitivity	< 10 copies demonstrated	< 10 copies demonstrated
% of target in each reaction	100%	Divide by number of reactions
Semi-quantitative	No	Yes
Instrument options	Few	Many
Reagents	Primers, beads & capture oligos	Primers and FRET probes
Time to answer	3-4 hours	30 min to 2 hours
Data generation	End of PCR cycles	Every PCR cycle



Template	Analyte 20 BaChrom	Analyte 21 Ba pX01	Analyte 22 Ba pX02	Analyte 29 <i>Y.pestis</i>
NTC	77	78	78	77
	76	81	83	76
<i>B.anthraxis</i> Sterne 1.0 ng DNA	11,841	16,257	94	92
<i>B.anthraxis</i> Ames 1.0ng DNA	7,795	12,081	12,214	82
<i>Y.pestis</i> 1.0 ng DNA	5,530	10,955	10,529	88
<i>Y.pestis</i>	77	79	84	12,606
1.0 ng DNA	89	82	87	13,486

Existing MultiFLEX™ Panels for Endpoint Assays (Real-time available for all targets)

- Biothreat pathogen panel (17 targets)
- Fever-causing pathogen panel (20 targets)
- Mosquito-borne pathogen panel (23 targets)
- Tick-borne pathogen panel (22 targets)

Luminex MagPIX Data

- Each row is a PCR reaction with one template and all assay primer pairs present
- Product of the PCR is hybridized with all beads sets
- Each bead set is conjugated with a different capture oligo
- Each column displays the hybridization to a specific bead set
- Data are presented as Mean Fluorescence Intensity (MFI)
- High numbers indicate that the target sequence was present in the sample, amplified and hybridized specifically to the capture oligo
- NTC = no template control
- Specificity studies were conducted with genomic material when available; if RNA was not available, cDNA was used

GenArraytion MultiFLEX™ Bioassay Mosquito Panel

Mosquito-Borne MultiFLEX™ Panel, Luminex MAGPIX® Instrument															
Sample	K12 Int Control	MS2 Int Control	Chikungunya Virus	P falciparum	West Nile Virus	Yellow Fever	Dengue1	Dengue2	Dengue3	Dengue4	Zika1	Zika2	Zika3	Zika4	Zika1234
No Template Control	8,108	12,980	66	71	64	70	66	75	66	72	63	63	59	63	73
	9,324	14,814	80	73	78	77	85	80	81	69	72	73	79	91	81
Chikungunya Virus	9,304	14,141	9,031	82	85	83	87	99	82	73	76	83	93	90	77
	9,553	15,017	8,632	87	73	448	78	76	81	68	74	82	73	87	72
Plasmodium falciparum	6,064	12,383	78	10,439	83	75	78	75	69	82	58	66	78	88	69
	5,589	11,579	78	9,847	79	75	70	75	80	79	57	74	77	71	77
West Nile Virus	9,104	13,769	73	82	12,713	72	66	79	68	72	64	73	66	80	69
	9,744	14,119	62	69	13,512	62	62	64	61	59	56	62	66	56	67
Yellow Fever Virus	9,219	15,000	63	89	68	7,781	96	74	78	87	82	79	86	83	73
	8,116	13,338	76	67	75	6,395	77	78	76	80	81	63	71	74	74
Dengue Type 1	8,956	14,543	78	82	85	74	10,042	83	82	69	73	81	82	74	80
	9,127	14,754	77	91	81	90	10,452	88	88	86	76	98	83	97	93
Dengue Type 2	7,222	13,441	79	106	86	83	90	7,442	87	91	87	82	94	90	88
	7,222	13,136	85	92	88	91	88	7,102	89	85	79	88	97	97	86
Dengue Type 3	9,947	15,463	93	92	89	89	89	89	4,239	73	103	85	96	97	95
	9,610	14,873	87	94	70	80	89	77	3,772	80	69	69	78	84	86
Dengue Type 4	9,524	15,028	83	95	87	86	86	89	92	5,998	86	85	112	91	101
	6,561	12,682	93	83	95	81	88	87	83	4,437	82	83	84	84	76
Zika Virus	7,915	11,986	141	95	88	93	87	81	93	77	6,295	16,050	10,448	14,955	12,356
	7,141	10,545	99	76	83	77	78	86	93	69	5,795	15,506	9,682	13,467	11,740

Mosquito-borne Illness Panel

- Zika virus (six targets distinguish between African and Brazilian lineages)
- chikungunya virus
- Dengue-1
- Dengue-2
- Dengue-3
- Dengue-4
- *Plasmodium falciparum*
- *Plasmodium knowlesi*
- *Plasmodium malariae*
- *Plasmodium ovale*
- *Plasmodium vivax*
- *Leptospira interrogans*
- Western equine encephalitis virus
- Eastern equine encephalitis virus
- Venezuelan equine encephalitis virus
- Japanese encephalitis virus
- West Nile virus
- Yellow fever virus

Tick-borne Illness Panel

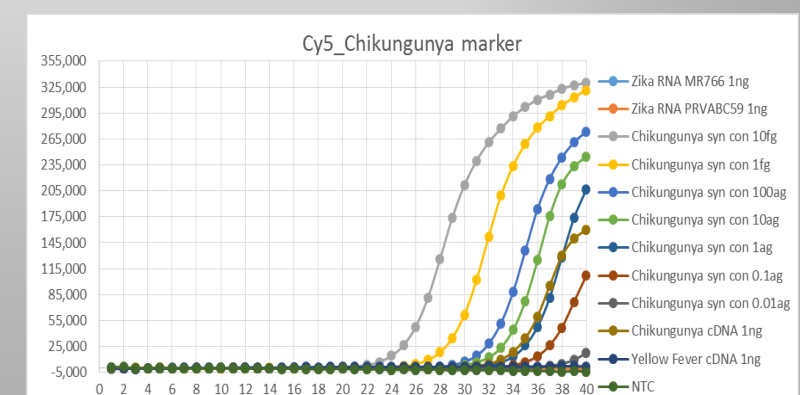
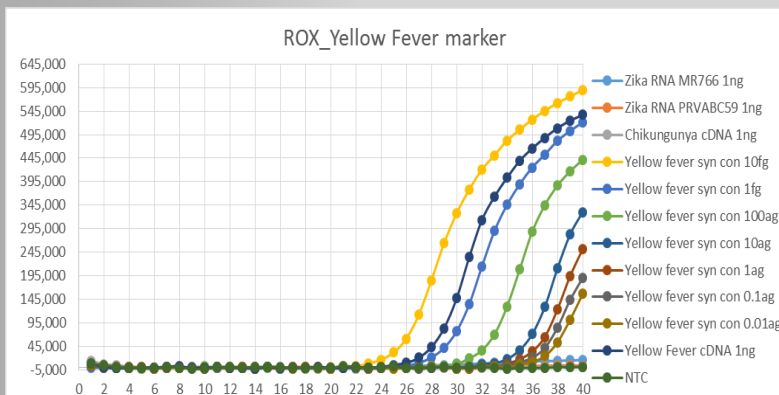
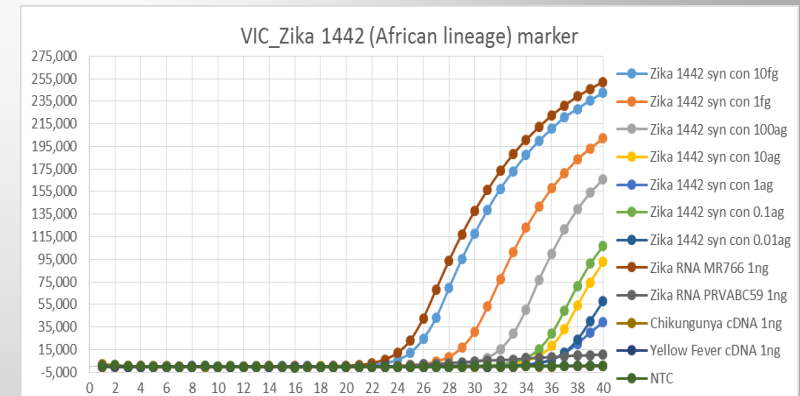
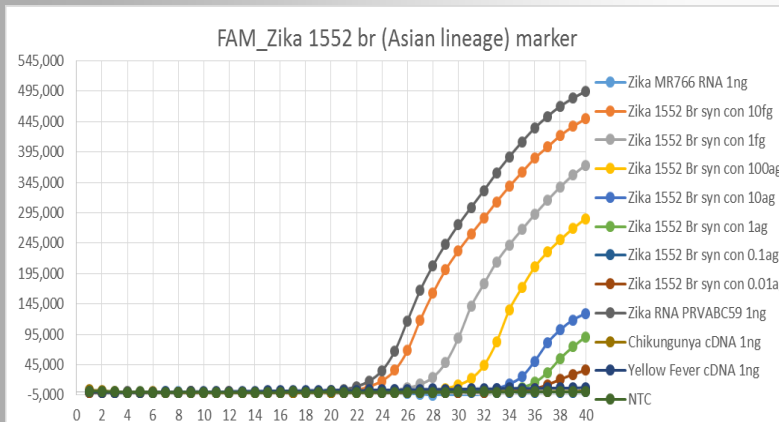
- *Anaplasma phagocytophilum*
- *Anaplasma* spp. (*A. centrale*, *A. marginale*, and *A. phagocytophilum*)
- *Babesia microti*
- *Babesia* spp. (*B. bigemina*, *B. bovis*, *B. divergens*, *B. microti* and *B. orentalis*)
- *Bartonella* spp. (*B. bacilliformis*, *B. henselae*, *B. quintana* and *B. vinsonii*)
- *Borrelia afzelli*
- *Borrelia burgdorferi*
- *Borrelia garinii*
- *Borrelia miyamotoi*
- *Borrelia* spp. (*B. crocidurae*, *B. duttonii* and *B. recurrentis*),
- *Coxiella burnetii*
- *Ehrlichia canis*
- *Ehrlichia chaffeensis*
- *Ehrlichia* spp. (*E. canis*, *E. chaffeensis* and *E. muris*)
- Powassan virus
- *Rickettsia helvetica*
- *Rickettsia japonica*
- *Rickettsia parkeri*
- *Rickettsia rickettsii*
- *Rickettsia* spp. (*R. conorii*, *R. japonica*, *R. massiliae*, *R. parkeri*, *R. phillipi*, *R. peacockii*, *R. raoultii*, *R. rhipicephali*, *R. rickettsii* and *R. slovaca*)
- Tick borne encephalitis virus

Real-Time PCR Assays

- Demonstrated compatibility with multiple platforms
 - Applied Biosystems® 7500
 - QIAGEN Rotor Gene 2
 - Cepheid SmartCyclers® 1&2
 - PSID™ Firefly
 - Tetracore® T-COR 8™
 - BD MAX™
 - Focus Diagnostics 3M Integrated Cyclor
- Over ninety targets available, more in development

Sensitivity and Specificity

Zika (Brazilian & African), Chikungunya, and Yellow Fever virus assays



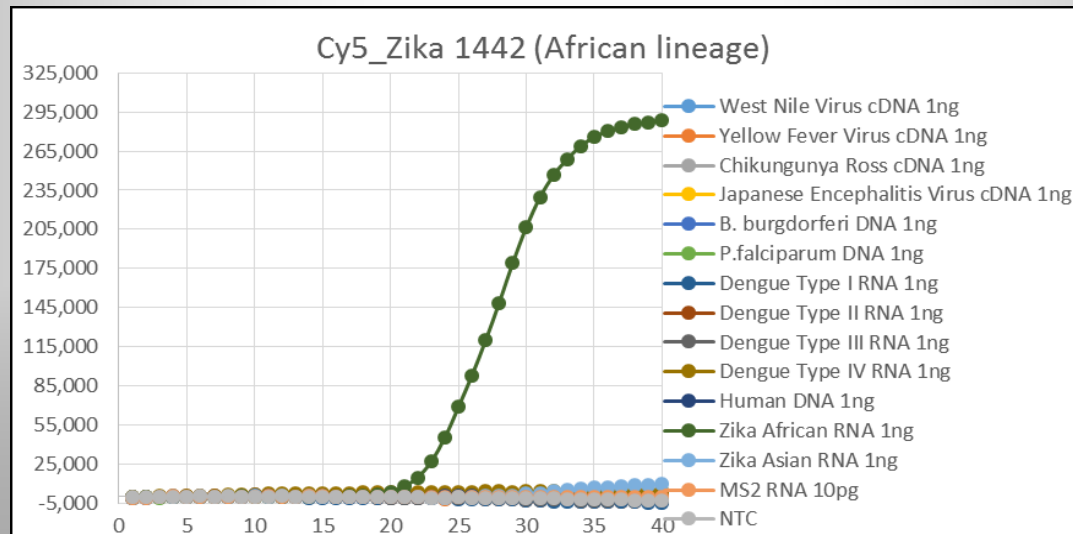
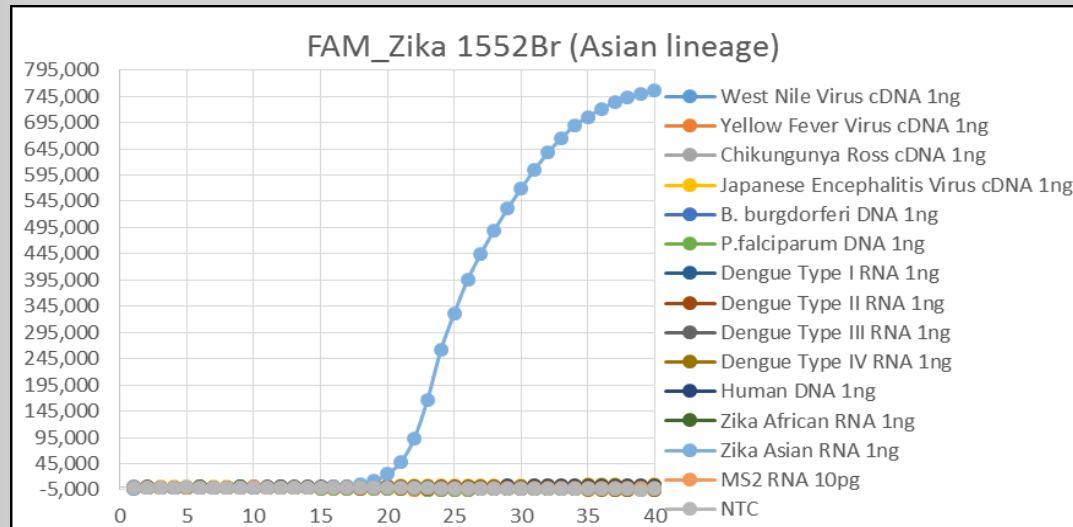
RNA templates are 1.0 ng to demonstrate specificity

Synthetic dsDNA constructs are used to determine sensitivity, 1.0 ag = 3 copies

Exclusivity Testing Results

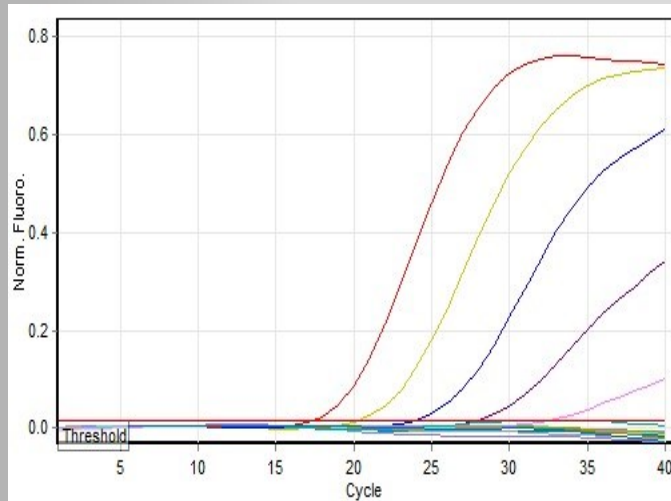
Near neighbor cross-reactivity	Amount of template	Zika 1552br marker (Brazilian lineage)	Zika 1442 marker (African lineage)
West Nile virus cDNA	1 ng	No cross-reactivity	No cross-reactivity
Yellow Fever virus cDNA	1 ng	No cross-reactivity	No cross-reactivity
Chikungunya Ross cDNA	1 ng	No cross-reactivity	No cross-reactivity
Dengue type I RNA	1 ng	No cross-reactivity	No cross-reactivity
Dengue type II RNA	1 ng	No cross-reactivity	No cross-reactivity
Dengue type III RNA	1 ng	No cross-reactivity	No cross-reactivity
Dengue type IV RNA	1 ng	No cross-reactivity	No cross-reactivity
Japanese encephalitis virus	1 ng	No cross-reactivity	No cross-reactivity
<i>Borrelia burgdorferi</i> Ross cDNA	1 ng	No cross-reactivity	No cross-reactivity
<i>Plasmodium falciparum</i> DNA	1 ng	No cross-reactivity	No cross-reactivity
Human DNA	1 ng	No cross-reactivity	No cross-reactivity

Exclusivity Testing Data



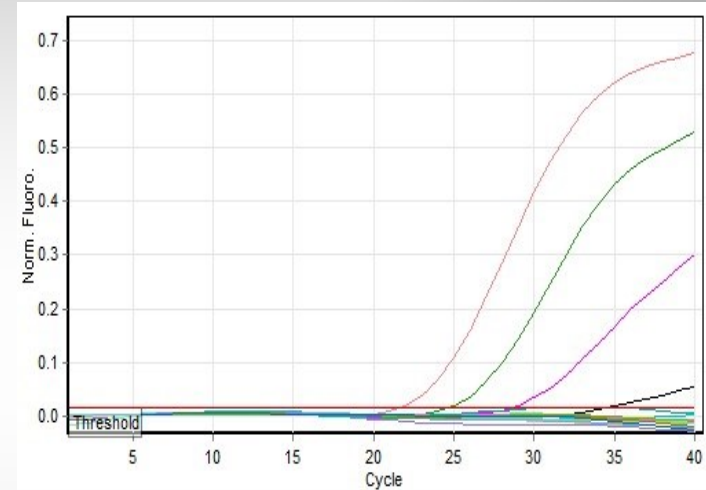
Chikungunya Sensitivity and Specificity

Chikungunya dsDNA Synthetic Construct



Color	Name	Ct	Dilution
Red	Chik89 synthetic construct	17.59	100fg
Yellow	Chik89 synthetic construct	20.45	10fg
Blue	Chik89 synthetic construct	24.07	1fg
Purple	Chik89 synthetic construct	28.01	100ag
Pink	Chik89 synthetic construct	32.68	10ag
Light Blue	Chik89 synthetic construct		1ag
Teal	Chik89 synthetic construct		0.1ag
Light Cyan	Dengue Virus 1		
Light Blue	Dengue Virus 2		
Light Purple	Dengue Virus 3		
Light Purple	Dengue Virus 4		
Brown	Zika Virus African Lineage		
Light Green	Zika Virus Asian Lineage (Brazil)		
Light Green	MS2 RNA		
Blue	NTC		

Chikungunya Synthetic RNA (ATCC)



Color	Name	Ct	Dilution
Red	Chikungunya synthetic RNA (ATCC)	21.84	100fg
Green	Chikungunya synthetic RNA (ATCC)	24.83	10fg
Magenta	Chikungunya synthetic RNA (ATCC)	28.77	1fg
Black	Chikungunya synthetic RNA (ATCC)	34.91	100ag
Cyan	Chikungunya synthetic RNA (ATCC)		10ag
Tan	Chikungunya synthetic RNA (ATCC)		1ag
Light Green	Chikungunya synthetic RNA (ATCC)		0.1ag
Light Cyan	Dengue Virus 1		
Light Blue	Dengue Virus 2		
Light Purple	Dengue Virus 3		
Light Purple	Dengue Virus 4		
Brown	Zika Virus African Lineage		
Light Green	Zika Virus Asian Lineage (Brazil)		
Light Green	MS2 RNA		
Blue	NTC		

Summary

***GenArraytion's* molecular assay approach is transforming infectious disease diagnostics**

- MultiFLEX™ panels for real-time & endpoint platforms
- Rapid development and commercialization
- Compatible with Luminex LX-200 and MagPix platforms
- Compatible with all tested real-time PCR platforms
- Zika virus assay developed in three weeks for both bead-based and real-time assays
- Panels can be customized to meet user requirements

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Browse our vector-borne disease research materials at www.atcc.org/vectorborne

Learn more about GenArraytion's bioassays and services at www.genarraytion.com



PCR Assays by Category

Respiratory Infections

Influenza

SARS (human coronavirus)

HCoV 229E

HCoV OC43

HCoV NL63

HCoV HKU1

Porcine Respiratory Coronavirus

ISU-1

MERS

Mid-East Respiratory Coronavirus

Gastrointestinal Infections

Clostridium difficile, chromosome

Clostridium difficile, Toxin A

Clostridium difficile, Toxin B

Norovirus Genogroup 1 (GI)

Norovirus Genogroup 2 (GII)

Porcine Trans Gastro Virus

Purdue P115

PCR Assays by Category

Hospital Associated Infections

Staphylococcus aureus (chrom)

S. aureus mecA gene (MRSA)

Acinetobacter baumannii

Enterococcus faecalis

Enterococcus faecium

Enterococcus Van A

Enterococcus VanB

Enterococcus Van R

Enterococcus TetM

Clostridium difficile, chromosome

Clostridium difficile, Toxin A

Clostridium difficile, Toxin B

Food Pathogens

Salmonella enterica

Cryptosporidium parvum

Cryptosporidium hominis

PCR Assays by Category

Vector-borne Mosquito

Chikungunya Virus

Dengue Virus

Serotype 1

Serotype 2

Serotype 3

Serotype 4

Japanese Encephalitis Virus

West Nile Virus

Yellow Fever Virus

Zika Virus (4 targets)

Equine Encephalitis Virus

Eastern

Venezuelan

Western

Rift Valley Fever Virus

Malaria

Plasmodium falciparum

Plasmodium knowlesi

Plasmodium malariae

Plasmodium ovale

Plasmodium vivax

PCR Assays by Category

Vector-borne Tick

Anaplasma spp.

A. phagocytophilum

Babesia spp.

B. microti

Bartonella spp.

Borrelia spp.

B. afzelii

B. burgdorferi

B. garinii

B. miyamotoi

Coxiella burnetii

Ehrlichia spp.

E. canis

Rickettsia spp.

R. helvetica

R. japonica

R. parkeri

R. rickettsii

Tick-borne Encephalitis Virus

Powassan Virus

PCR Assays by Category

Fever-Causing

Crimean Congo Hem Fever

Ebola Virus

Strain Bundibugyo

Strain Ivory Coast

Strain Sudan

Strain Reston

Strain Zaire

Lassa Virus

Strain Josiah

Strain Pinneo

Leishmania spp.

Leptospira spp.

Marburg Virus

Orthopoxvirus

Veterinary

Bacillus anthracis (chromosome)

B. anthracis (pXO1)

B. anthracis (pXO2)

Burkholderia spp.

B. mallei

B. pseudomallei

Francisella tularensis

Brucella spp.

B. abortus

B. canis

B. melitensis

B. neotomae

B. suis