Molecular Strategies for Pathogen Discovery and Surveillance in Outbreak Response

Capture sequencing
rapid, sensitive, inexpensive platform for discovery, surveillance, and differential diagnosis

Microarrays/Phage Display
assays for exposure
early evidence of cross species transmission
enable development of inexpensive ELISA and lateral flow assays
find correlates of protection

454 Pyrosequencing: Dandenong Virus (2007)

***Three weeks** to identification of causative agent (Australia)*

nature **Rapid sequencer puts virus in the frame for deaths**

Heidi Ledford | 02 May 2007 | doi: 10.1038/447012b

Although this sort of sequencing has been used to identify viruses in the past, the 454 technology cuts down on time and effort.

Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases

nature biotechnology **The development and impact of 454 sequencing**

Jonathan M Rothberg & John H Leamon | 09 October 2008 | doi: 10.1038/nbt1485

The 454 Sequencer has dramatically increased the volume of sequencing conducted by the scientific community and expanded the range of problems that can be addressed by the direct readouts of DNA sequence. Key breakthroughs in the development of the 454 sequencing platform included higher throughput, simplified all *in vitro* sample preparation and the miniaturization of sequencing chemistries, enabling massively parallel sequencing reactions to be carried out at a scale and cost not previously possible. Together with other recently released next-generation technologies, the 454 platform has started to democratize sequencing, providing individual laboratories with access to capacities that rival those previously found only at a handful of large sequencing centers.

The NEW ENGLAND JOURNAL of MEDICINE **A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases**

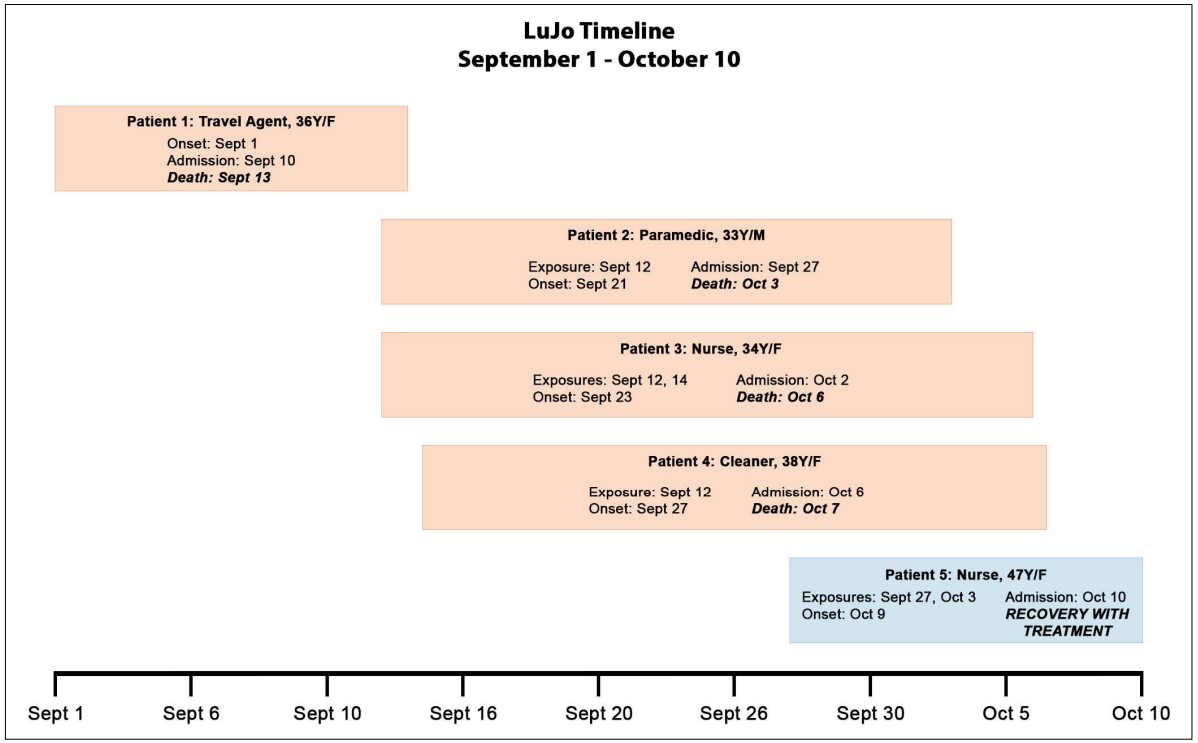
ORIGINAL ARTICLE | March 6, 2008 | DOI: 10.1056/NEJMoa073785

Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, Conlan S, Quan PL, Hui J, Marshall J, Simons JF, Egholm M, Paddock CD, Shieh WJ, Goldsmith CS, Zaki SR, Catton M, Lipkin WI

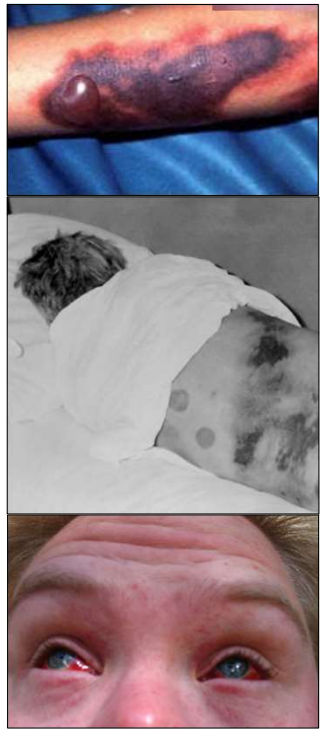
In first use of sequencing in outbreak investigation, the discovery enabled by Google.org led to diagnostic tests that prevented further outbreaks.

454 Pyrosequencing: LuJo Virus (2008)

***One week** to identification; 80% morality rate (Lusaka/Johannesburg)*



Identification required shipment to a specialized laboratory in New York City, staffing 5 people working 24/7 for 7 straight days after testing at the Special Pathogens Unit, National Institute for Communicable Diseases of the South Africa National Health Laboratory Service was unable to identify the pathogen.



Capture Sequencing: VirCapSeq-VERT

Faster, cheaper, 1,000-fold more sensitive than standard next-gen sequencing (NGS)

2015 WORLD CHANGING IDEAS

H1N1 INFLUENZA is one of many viruses snared by a single new test.

Trawling for Viruses

A new method identifies every virus in a given sample with near-perfect accuracy

When doctors want to identify the virus behind an infection, they usually turn to the polymerase chain reaction (PCR), a method for "amplifying" scattered bits of DNA into a sample large enough to study. To use PCR, however, a physician must know what kind of virus to look for, and that involves guesswork.

This past September a team of Columbia University researchers described a new method that could eliminate that guesswork. The technique, which has the unfortunate name of "virome capture sequencing platform for vertebrate viruses," or VirCapSeq-VERT, can find every virus in a given drop of saliva, tissue or spinal fluid with near-perfect accuracy. The method makes it possible to simultaneously analyze 21 samples in less than 48 hours at an estimated cost of just $200 per sample. It can also detect novel or mutated viruses, so long as they are at least 40 percent identical to known ones. "When someone goes into an emergency room and winds up having all kinds of tests run, it costs thousands of dollars," says W. Ian Lipkin, John Snow Professor of Epidemiology at Columbia University's Mailman School of Public Health. "This method is very inexpensive and allows us to personalize medicine by telling you exactly what you have."

To develop the technique, Lipkin and his colleagues first created a database of more than 1,000 vertebrate viruses. Then they synthesized genetic probes to match every strain of every virus—two million of them, each a strand of DNA 25 to 50 nanometers long. When a probe encounters a matching virus, it binds to it. To extract those viruses, laboratory workers add magnetic beads measuring one to three microns in diameter to the mix; a chemical linker binds the beads to the genetic probes and the viruses they have captured. Re… tube containing t… stand, which pulls… walls. After resear… probe-bead-virus… sequence the viru… false positives. Lip… now looking to team up with a commercial provider that can distribute the technology to hospitals and clinics around the world. They are also planning on adding probes for all known infectious bacteria and fungi.

—R.N.

ASTRONOMY Big Telescopes, Big Battles

MEDICINE Genomics for the People

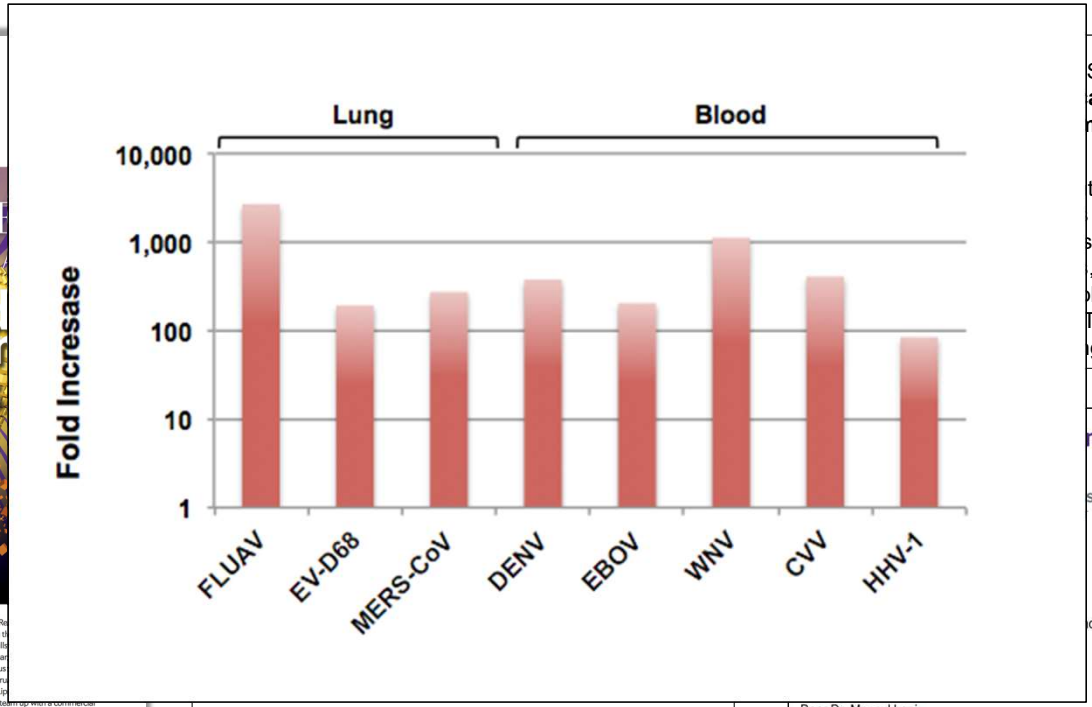
World Changing Ideas

10 big advances that will improve life, transform computing and maybe even save the planet.

PLUS 170 YEARS OF SCIENTIFIC AMERICAN

34 Scientific American, December 2015

© 2015 Scientific American



Columbia University freely provides the underlying intellectual property for public health.

September 19, 2016
ation Date: September 20, 2018
nent Identifier: US 20180265935 A1

tion provides novel methods, systems, tools,
detection, identification and/or
known or suspected to infect vertebrates.
, and kits described herein are based upon the
latform ("VirCapSeq-VERT"), a novel platform
The invention also provides methods and kits
g of the virome capture sequencing platform.

nt

SETT, M.D., M.P.H.

KRISTIN M. PROUD
Acting Executive Deputy Commissioner

omic Medicine

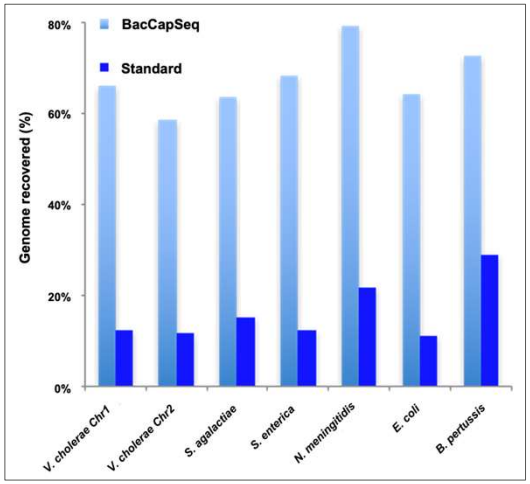
PFI: 7313
Project ID: 85920

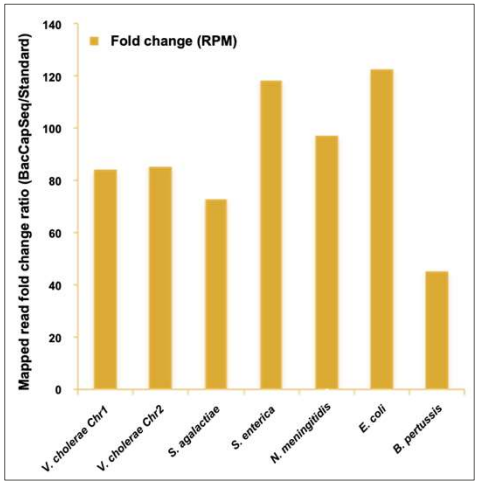
Dear Dr. Mansukhani:

Thank you for submitting portions of your standard operating procedure manual and validation data needed to evaluate your next generation sequencing-based method for Columbia VirCapSeq-VERT in plasma specimens. After carefully evaluating all the information provided, the Clinical Laboratory Reference System's reviewers have found your validation data for this testing acceptable. You may offer this testing under your current permit in the category of Virology.

Capture Sequencing: BacCapSeq

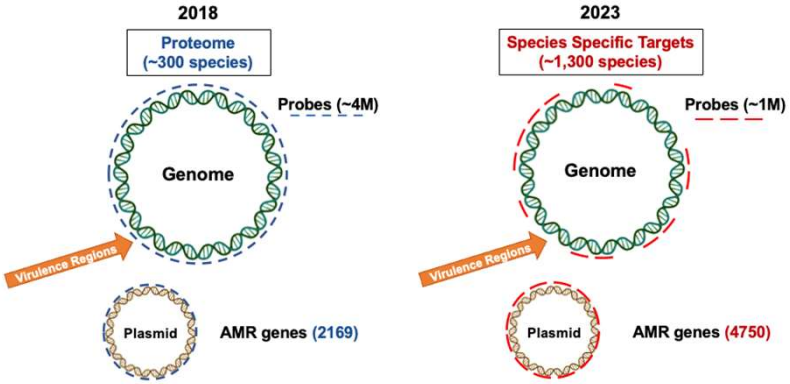
Rapid detection of bacterial and antimicrobial resistance elements





	Blood	Urine	CSF	Resp
A. baumannii	+	+/-	+/-	+
Campylobacter spp.	+/-	-	-	-
C. difficile	+/-	-	-	-
Enterobacter spp.	+	+	+/-	+
Enterococcus spp.	+	+	+/-	+
E. coli	+	+	+/-	+
K. pneumoniae	+	+	+/-	+
N. gonorrhoeae	+/-	+	+/-	-
Non-tuberculous mycobacteria (NTM)	+/-	-	+/-	+
Non-typhoidal Salmonella spp.	+	-	-	-
P. aeruginosa	+	+	+/-	+
S. enterica serovar Typhi	+	-	-	-
Shigella spp.	+/-	-	-	-
S. aureus	+	+	+/-	+
S. pneumoniae	+		+	+

Priority for regulatory validation by pathogen and sample type.
+, high priority; +/-, intermediate priority; -, lower priority; CSF, cerebrospinal fluid; Resp, respiratory.



BacCapSeq Targets

	Blood	Urine	CSF	Resp
A. baumannii	+	+/-	+/-	+
Campylobacter spp.	+/-	-	-	-
C. difficile	+/-	-	-	-
Enterobacter spp.	+	+	+/-	+
Enterococcus spp.	+	+	+/-	+
E. coli	+	+	+/-	+
K. pneumoniae	+	+	+/-	+
N. gonorrhoeae	+/-	+	+/-	-
Non-tuberculous mycobacteria (NTM)	+/-	-	+/-	+
Non-typhoidal Salmonella spp.	+	-	-	-
P. aeruginosa	+	+	+/-	+
S. enterica serovar Typhi	+	-	-	-
Shigella spp.	+/-	-	-	-
S. aureus	+	+	+/-	+
S. pneumoniae	+		+	+

Table 1. Priority for regulatory validation by pathogen and sample type. +, high priority; +/-, intermediate priority; -, lower priority; CSF, cerebrospinal fluid; Resp, respiratory.

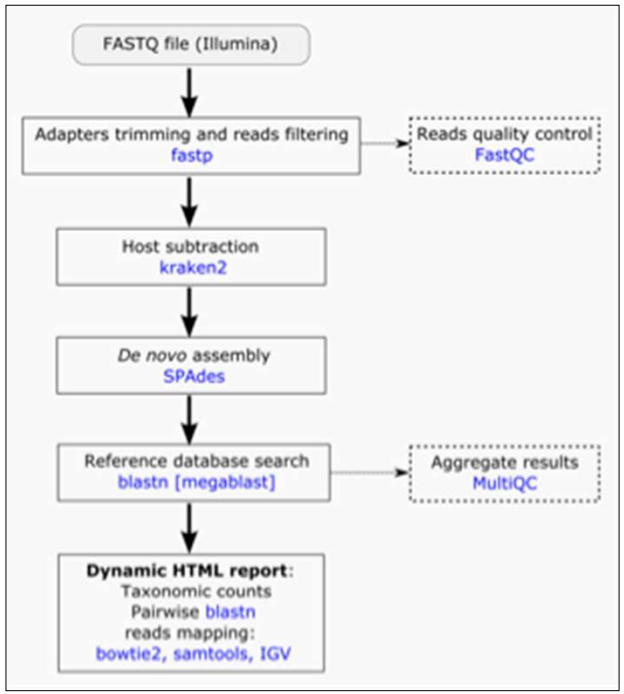
CETR agents	Susceptible examples	Resistant examples	Antibiotic resistance represented
Carbapenem-resistant Enterobacteriaceae (CRE)	ATCC 25922, ATCC 43816	ATCC BAA-2523, ATCC BAA-2524, ATCC BAA-2472, ATCC BAA-2452, ATCC BAA-1705, ATCC BAA-2340, ATCC BAA-2468, ATCC BAA-2341	Carbapenem (ertapenem, imipenem, meropenem, doripenem), glycylcycline (tigecycline)
Drug-resistant Neisseria gonorrhoeae	NCTC 13477	ATCC BAA-1846, ATCC 700825, BAA-3082, NCTC 13479, NCTC 13480, NCTC 13821, NCTC 13821	Fluoroquinolone (ciprofloxacin), cephalosporin (cefixime), tetracycline (tetracycline), aminoglycoside (streptomycin), macrolide (azithromycin)
Carbapenem-resistant Acinetobacter	ATCC 1709	ATCC BAA-1605, ATCC BAA-1794, ATCC BAA-1799, ATCC BAA-2800, NCTC 13303	Carbapenem (imipenem), cephalosporin (cefotaxime), fluoroquinolone (levofloxacin), aminoglycoside (gentamicin), colistin, glycylcycline (tigecycline), tetracycline (tetracycline), monobactam (aztreonam)
Extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs)	ATCC 25922, ATCC 43816	ATCC 51983, ATCC BAA-196, ATCC 700603, NCTC 13464 ATCC BAA-2777, NCTC 13353	Penicillin (ampicillin), cephalosporin (cefotaxime, ceftazidime), fluoroquinolone (ciprofloxacin), tetracycline (tetracycline), monobactam (aztreonam)
Vancomycin-resistant Enterococcus (VRE)	ATCC 29212, ATCC BAA-2127	ATCC 700221, ATCC BAA-2365, ATCC 2317, ATCC 51299, ATCC 51858	Glycopeptide (vancomycin, teicoplanin), penicillin (ampicillin), fluoroquinolone (ciprofloxacin), tetracycline (tetracycline), macrolide (erythromycin)
Multidrug-resistant Pseudomonas aeruginosa	ATCC 27853 ATCC 25668	ATCC BAA-2108, ATCC BAA-2795, ATCC BAA-2110, ATCC BAA-2114	Aminoglycoside (amikacin), penicillin (ampicillin), cephalosporin (cefotaxime, cefepime), fluoroquinolone (ciprofloxacin), tetracycline (tetracycline), monobactam (aztreonam), glycylcycline (tigecycline), carbapenem (imipenem)
Drug-resistant Shigella spp.	ATCC 700930 ATCC 13313 ATCC 25931	S. flexneri Wadsworth S. sonnei Wadsworth	Fluoroquinolone (ciprofloxacin), macrolide (azithromycin)
Drug-resistant Staphylococcus aureus [methillicin (MRSA) and vancomycin (VRSA)]	ATCC 25923	ATCC BAA-38, ATCC 43300, ATCC 33592, ATCC BAA-1556, ATCC BAA-2312, ATCC 700699	Penicillin (methicillin, oxacillin), cephalosporin (cefoxitin), tetracycline (tetracycline), glycopeptide (vancomycin, aminoglycoside (gentamicin)
Drug-resistant Streptococcus pneumoniae	ATCC BAA-255 ATCC 49619	ATCC 700677, ATCC 700670, ATCC BAA-612, ATCC 700671	Penicillin (ampicillin), macrolide (erythromycin), tetracycline (tetracycline),
Non-tuberculous Mycobacteria	ATCC 19420, ATCC BAA-1052, ATCC 19977, ATCC 19210, ATCC 700898, ATCC BAA-968	ATCC 700738, ATCC BAA-2683, ATCC 700869, ATCC 35746, ATCC 35746, ATCC 700897	Penicillin (penicillin), fluoroquinolone (ciprofloxacin), oxazolidinone (linezolid), aminoglycoside (streptomycin), cephalosporin (cefoxitin), tobramycin, penicillin, macrolide (azithromycin)

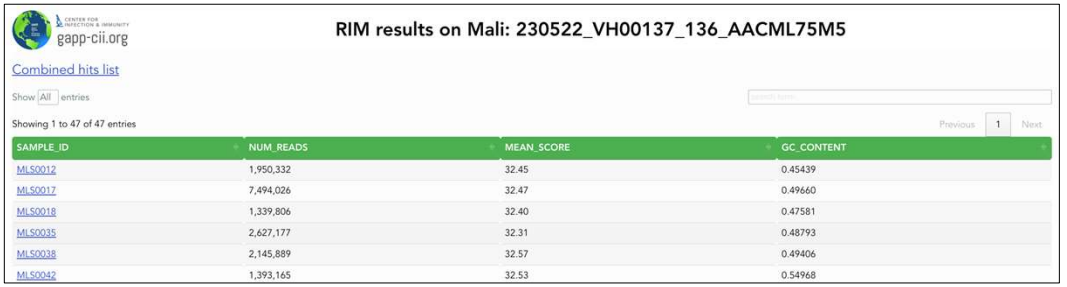
Table 2. List of bacteria to be included for BacCapSeq development and validation.

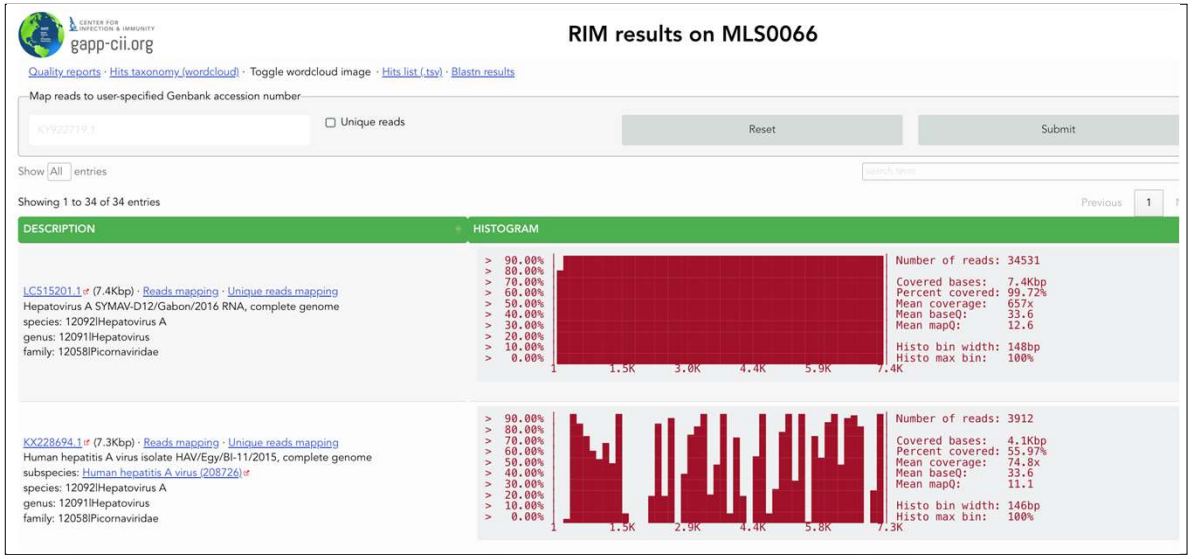
MicrobeCapSeq: Comprehensive Detection of Viruses, Bacteria and AMR.

	Specific Viral Read Counts			
Sample ↓/Capture Probes→	**Unbiased mNGS**	**VirCapSeq-VERT**	**MicrobeCapSeq**	**Fold increase over mNGS**
EV-D68 (50 copies/mL)	13	29,902	28,874	~2,500
Human herpesvirus 1 (500 copies/mL)	104	1,984,600	906,867	~10,000
Human herpesvirus 2 (500 copies/mL)	31	469,716	242,162	~10,000
Negative Control	0	0	0	0
ERCC (Positive Control)	0	0	0	0

Simplified Bioinformatics: Rapid Identification of Microbes (RIM)







Sample Receipt to Pathogen Identification in <12 hours

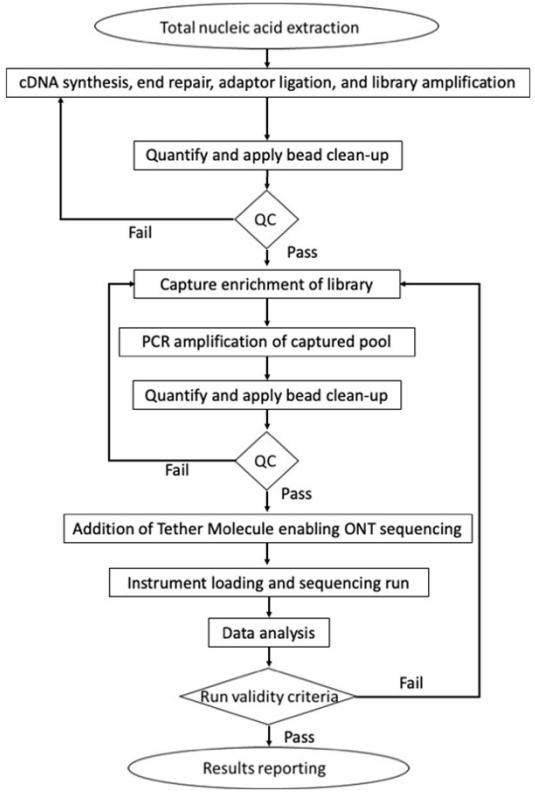
Instrument	# of Samples	# of Reads	Read Length	Runtime
Illumina NextSeq 1000/2000	100	1.2 billion	150 nt	11h
Illumina NextSeq 500	40	400 million	150 nt	12h
Illumina MiSeq	3	25 million	300 nt	5h
Illumina MiniSeq	3	25 million	150 nt	4h
Illumina iSeq	1	4 million	150 nt	9.5h
Oxford Nanopore MinION	1	5 million	1000 nt	8h

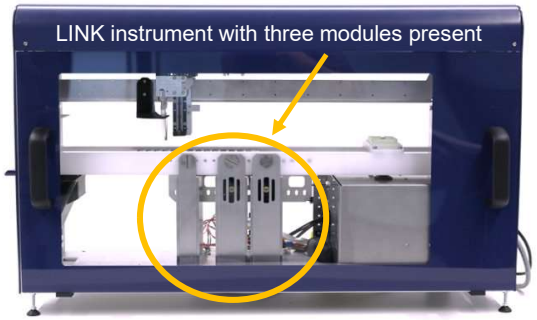
Workflow	Unbiased	Capture
Extraction	30 minutes	30 minutes
Library preparation	5 hours	5 hours
Hybridization	n/a	1 hours
Sequencing on MiniSeq	4h	4h
Bioinformatic analysis	8 hours	30 minutes
Total turn around time	17.5 hours	11 hours
Sensitivity	1x	100-1000x

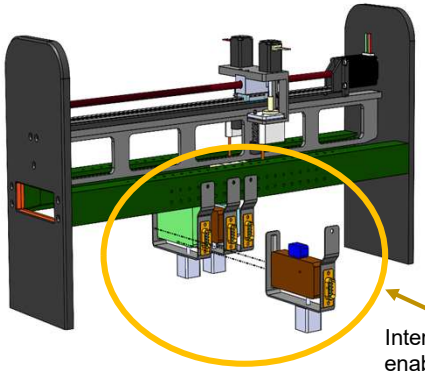
Fully Automated Sequencing Platform

Sample extraction to sequence analysis (collaboration with TTP Group; Cambridge, UK)

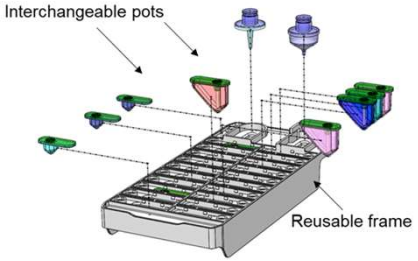
Automated Workflow of TTP Link System and Additional Modules





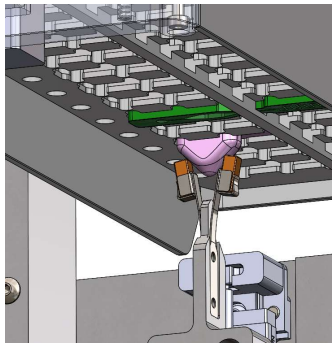


Interchangeable modules enabling additions of new functions and biology



Completely customizable with interchangeable pot geometries

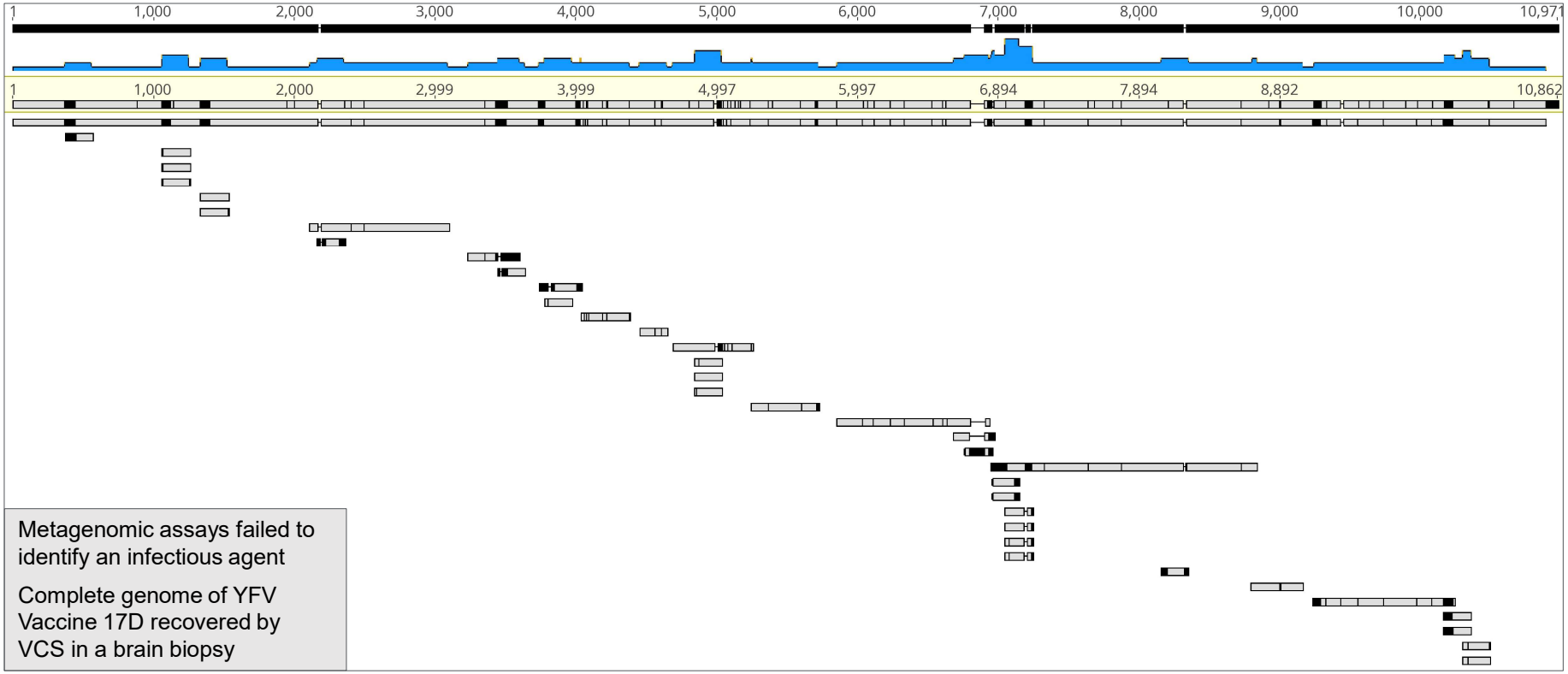
Magnetic module for magnetic bead capture (RNA-seq workflows)



Magnet module engaging with a custom pot

Rapid Detection of YFV Vaccine Sequences in Brain of Patient With Rapid Onset Dementia Using VirCapSeq-VERT

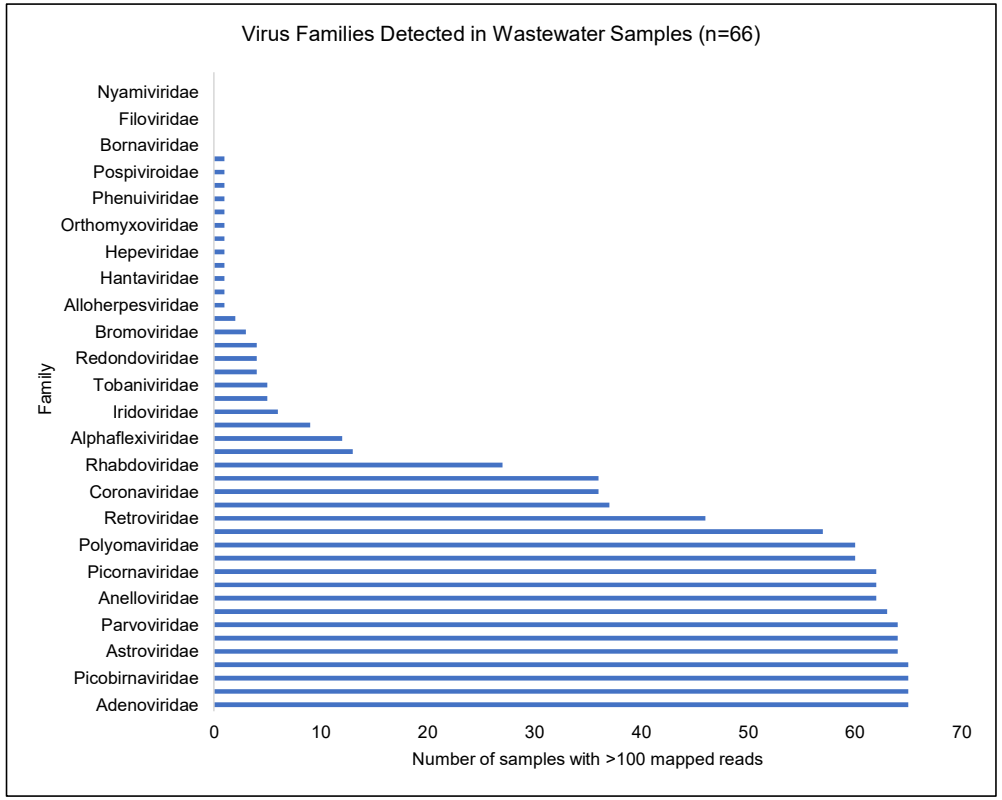
43YO man, progressive weakness, cognitive decline, hypogammaglobulinemia

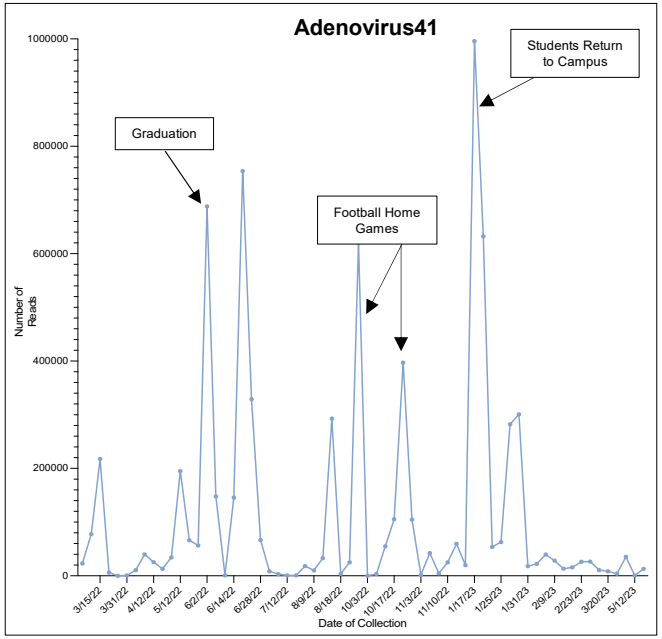


Metagenomic assays failed to identify an infectious agent

Complete genome of YFV Vaccine 17D recovered by VCS in a brain biopsy

Detection of Adenovirus in US Air Force Academy Wastewater Using VirCapSeq-VERT

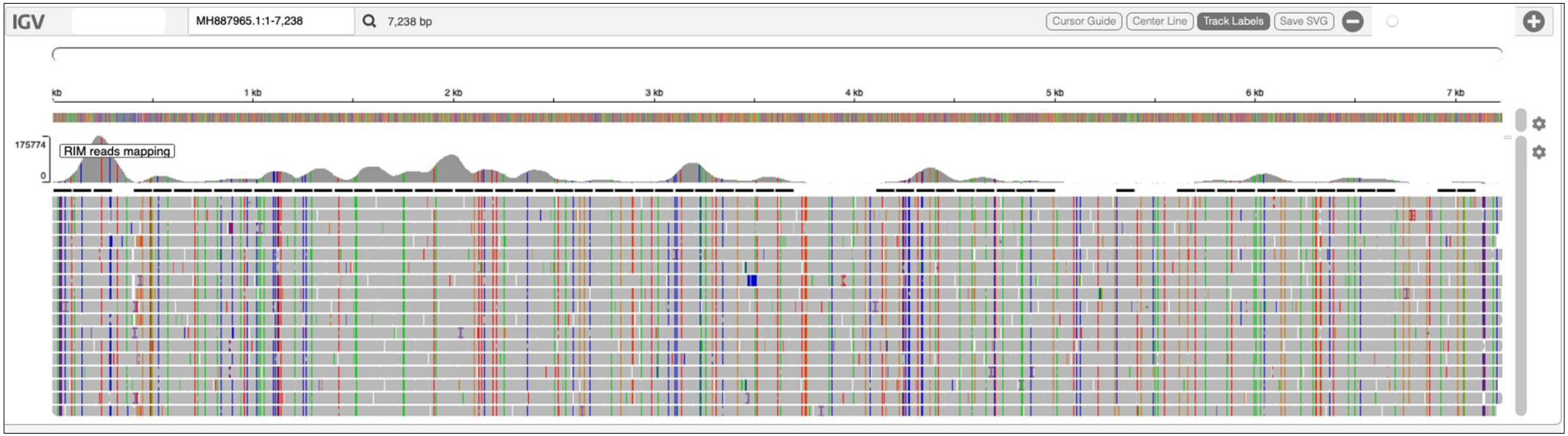




- Wastewater collected Feb 2022-May 2023
- Major events on campus with visitors illustrate spikes in Adenovirus 41

VirCapSeq-VERT Detection of Lassa Virus

58 complete genomes previously negative with Illumina capture sequencing system (mouse kidney)



Large segment: 6.7 kb, 23,722 mapped reads, 93% genome coverage
Small segment: 3.4 kb, 53,978 mapped reads, 99% genome coverage

Anise and Christian Happi, African Centre of Excellence for Genomics of Infectious Disease, Redeemer's University

Acute Encephalitis Syndrome (AES)

Pediatric epidemic in Uttar Pradesh, India (2017)


Uttar Pradesh


Musahar children


AES hospital under construction

The New York Times

By JEFFREY GETTLEMAN and HARI KUMAR
AUG. 17, 2017
The Night the Oxygen Ran Out in an Indian Hospital

By SAMAR HALARNKAR AUG. 24, 2017
Can India Stop Its Children From Dying?


Waiting room


Bed shortage

VirCapSeq-VERT and BacCapSeq

Detected infectious agents in cerebrospinal fluid, allowing for >33% of cases to be treatable with antivirals and antibiotics.

Cost to treat 5 children with doxycycline = 25 rupees ($0.30 USD)

डा. सौम्या स्वामीनाथन
सचिव, भारत सरकार
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
एवं
महानिदेशक, आई सी एम आर
Dr. Soumya Swaminathan
MD, FASc, FNASc, FAMS
Secretary to the Government of India
Department of Health Research
Ministry of Health & Family Welfare
&
Director-General, ICMR

भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
वी. रामलिंगस्वामी भवन, अंसारी नगर
नई दिल्ली-110 029 (भारत)
Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi-110 029 (INDIA)

No. Secy.(DHR) & DG,ICMR/ 2017
Dated, the 17th August, 2017

Dear Prof. Lipkin

I am writing to invite you to visit the ICMR Hqrs in New Delhi and the NIV field Unit at Gorakhpur, Uttar Pradesh to assist with the investigation of encephalitis outbreak.

As you are aware, AES has been claiming the lives of children in Eastern Uttar Pradesh for many years and the etiology remains unknown in 50% of the cases. Your inputs to improve the diagnosis using advanced sequencing techniques for pathogen discovery will be very useful. I look forward to meeting you in India next week.

With regards,

Yours sincerely,

(**Soumya Swaminathan**)

Chief Scientist, WHO 2019-22

Prof. W. Ian Lipkin, MD
John Snow Professor of Epidemiology and Director
Center for Infection and Immunity
Mailman School of Public Health
Professor of Pathology and Neurology
College of Physicians & Surgeons
Columbia University
722 West 168th Street, 17th Floor
New York, NY 10032

Acute Encephalitis Syndrome (AES)

Pediatric epidemic in Uttar Pradesh, India (2017); VirCapSeq-VERT/BacCapSeq

	Total	2015	2016	2017
Total AES Samples	543	153	148	242
Fatal	222	75	77	67
Non-Fatal	321	77	61	175

535 samples (CSF = 532; brain autopsies = 3) negative in standard NGS

Known Pathogenic Agents	n = Cases Positive	% Positivity
Japanese Encephalitis Virus	22/543	4.0%
Enteroviruses	14/543	2.5%
Herpesviruses	21/543	3.8%
Orientia tsutsuganmushi	73/543	13.5%
Rickettsia sp.	86/543	15.8%
Co-infection	42/543	7.7%
Other agents*	126/543	23.2%
Total	**342/543**	**63.0%**

**Other viruses, or low coverage, or plant, or insect origin viruses*

High Throughput Serology: Zika

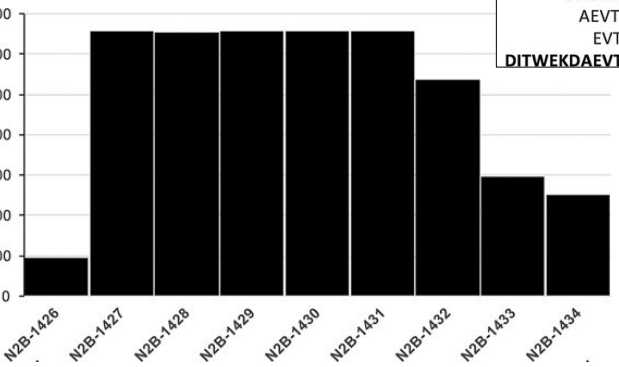
Discovery of NS2B ZIKV peptide that enabled development of an ELISA

Diagnosis of Zika Virus Infection by Peptide Array and Enzyme-Linked Immunosorbent Assay

Mishra N, Caciula A, Price A,…Lipkin WI

Research Article | Published 06 September 2018 | doi: 10.1128/mbio.00095-18

A high-density microarray comprising nonredundant 12-mer peptides that tile, with one-residue overlap, the proteomes of Zika, dengue, yellow fever, West Nile, Ilheus, Oropouche, and chikungunya viruses. Serological analysis enabled discovery of a ZIKV NS2B 20-residue peptide that had high sensitivity (96.0%) and specificity (95.9%) versus natural infection.



DITWEKDAEVTG	NS2b-1426
ITWEKDAEVTGN	NS2b-1427
TWEKDAEVTGNS	NS2b-1428
WEKDAEVTGNSP	NS2b-1429
EKDAEVTGNSPR	NS2b-1430
KDAEVTGNSPRL	NS2b-1431
DAEVTGNSPRLD	NS2b-1432
AEVTGNSPRLDV	NS2b-1433
EVTGNSPRLDVA	NS2b-1434
DITWEKDAEVTGNSPRLDVA	**NS2b-1426-1434**

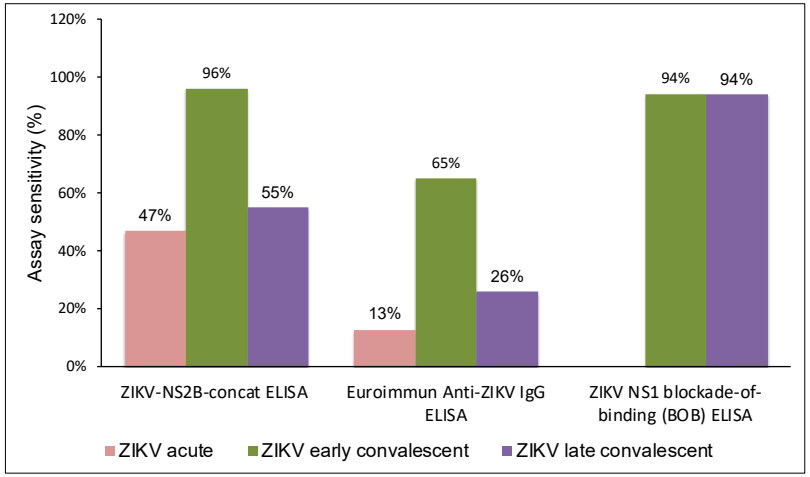
Transfer reactive peptides to simple peptide ELISA

Zika Virus Peptide ELISA (ZIKV-NS2B-Concat ELISA) for Detection of IgG Antibodies to Zika Virus Infection

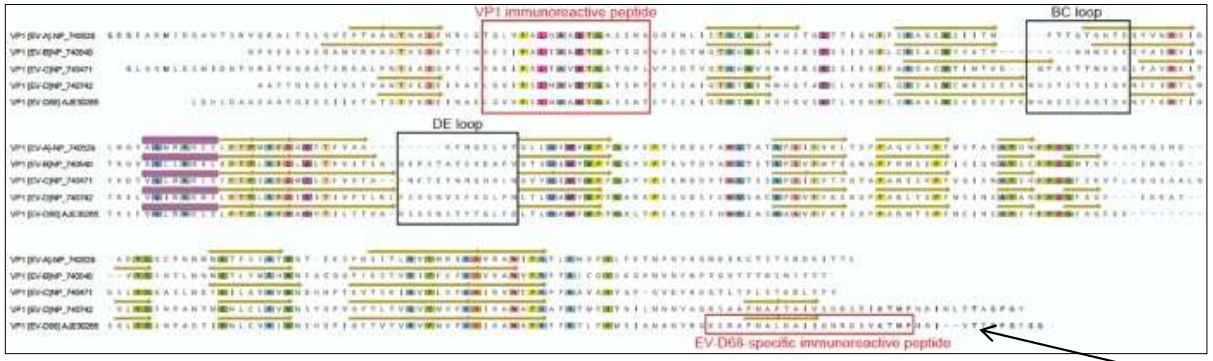
Mishra N, Thakkar R, Ng J, Lipkin WI

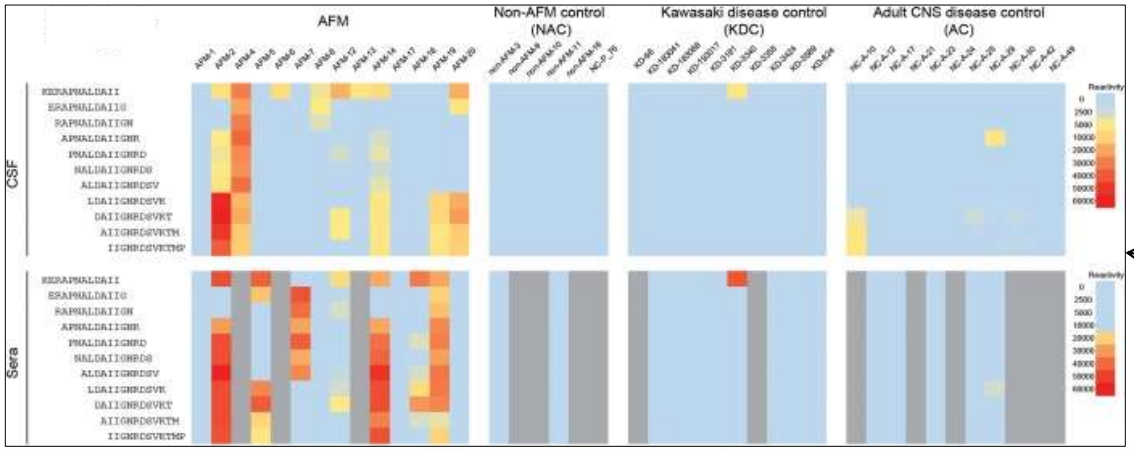
Protocol | Published 05 May 2020 | doi: 10.1007/978-1-0716-0581-3_10

An affordable ZIKV NS2B biotinylated peptide ELISA was built and compared with peptide array:
~47% sensitivity in ZIKV acute patients (2-3 weeks post-infection)
~96% sensitivity in ZIKV early convalescent (1-6 months post-infection)
~55% sensitivity in late convalescent (>6 months post-infection)



High Throughput Serology: EV D68 Antibodies in CSF in Acute Flaccid Myelitis





Antibodies to Enteroviruses in Cerebrospinal Fluid of Patients with Acute Flaccid Myelitis

Nischay Mishra, Terry Fei Fan Ng, Rachel L. Marine, Komal Jain, James Ng, Riddhi Thakkar, Adrian Caciula, Adam Price, Joel A. Garcia, Jane C. Burns, Kiran T. Thakur, Kimbell L. Hetzler, Janell A. Routh, Jennifer L. Konopka-Anstadt, W. Allan Nix, Rafal Tokarz, Thomas Briese, M. Steven Oberste, W. Ian Lipkin

Research Article | Published 13 August 2019 | doi: 10.1128/mbio.01903-19

Immunoreactivity against an EV-D68-specific 22-aa VP1 capsid peptide in patients with AFM, non-AFM controls (NAC), Kawasaki disease controls (KDC), and adult CNS disease controls (AC).

Identification of an immunoreactive peptide sequence region in VP1 protein of reference sequence entries for EV-A, EV-B, EV-C, and EV-D

Phage Display Panels

Hemorrhagic Fever Panel	
Filoviriade	Zika virus
Zaire EBOV Mayinga (VACCINE)	Rubella virus
Vesicular stomatitis Indiana virus (vector)	Mumps virus
Lyssavirus	Measles morbillivirus
Dengue viruses (1-4)	*Mycobacterium tuberculosis*
Yellow fever virus	*Salmonella Typhi*
West Nile virus	Plasmodium falciparum
Semliki Forest virus	SARS-CoV-2
Rift valley fever virus	Other human coronaviruses
Chikungunya virus	Human enteroviruses
O'nyong'nyong virus	Random scrambled non-specific peptides

Other short-peptide phage display panels:

Pox viruses
Pan arboviruses

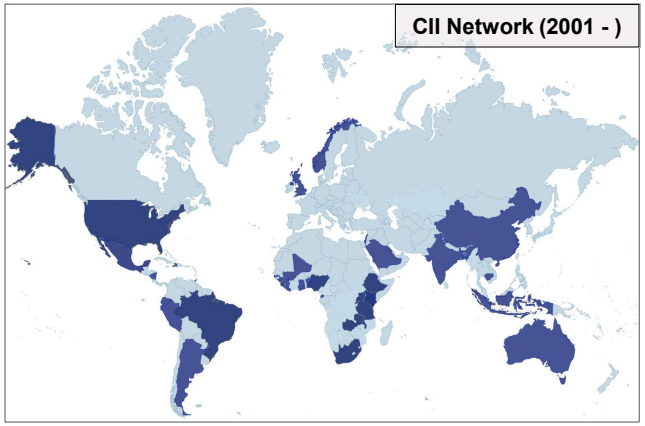
Human Respiratory and Latent Viruses

Family	Virus
Herpesviridae	Human herpesvirus 8 strain GK18, complete genome Human herpesvirus 4, complete genome Human herpesvirus 6B, complete genome Human herpesvirus 3, complete genome Human herpesvirus 7, complete genome Human herpesvirus 1 strain 17, complete genome Human herpesvirus 5 strain Merlin, complete genome Human betaherpesvirus 6A, variant A DNA, complete virion genome, isolate U1102 Human herpesvirus 2 strain HG52, complete genome Human gammaherpesvirus 4, complete genome
Parvoviridae	Human bocavirus 4 NI strain HBoV4-NI-385, complete genome Human bocavirus 4 NI strain HBoV4-NI-385, complete genome Human bocavirus 2b NI strain HBoV2B-NI-213, complete genome Human bocavirus 2a TU strain HBoV2A-TU-A-114-06, complete genome Human bocavirus 3 strain HBoV3B-TU-A-210-07, complete genome Human bocavirus 2 strain HBoV2B-NI-327, complete genome Human bocavirus isolate KU2, complete genome Human bocavirus 2 isolate KU1, complete genome Human parvovirus B19, complete genome Human parvovirus 4 G1, complete genome
Adenoviridae	Human adenovirus 2, complete genome Human adenovirus B2, complete genome Human adenovirus B1, complete genome Human adenovirus A, complete genome Human adenovirus type 35, complete genome Human adenovirus type 1, complete genome Human adenovirus 5, complete genome Human adenovirus D, complete genome Human adenovirus F, complete genome Human adenovirus type 7, complete genome Human adenovirus D, complete genome Human adenovirus C, complete genome Human adenovirus E, complete genome Human adenovirus 54, complete genome Human adenovirus 17 isolate D17, complete genome Human mastadenovirus A, complete genome
Polyomaviridae	Human polyomavirus 6, complete genome Human polyomavirus 7, complete genome Human polyomavirus 9, complete genome Human polyomavirus 12 strain hu1403, complete genome JC polyomavirus, complete genome Merkel cell polyomavirus isolate R17b, complete genome MW polyomavirus, complete genome KI polyomavirus Stockholm 60, complete genome BK polyomavirus, complete genome LI polyomavirus isolate LIPyV, complete genome Trichodysplasia spinulosa-associated polyomavirus, complete genome MW polyomavirus, complete genome KI polyomavirus Stockholm 60, complete genome BK polyomavirus, complete genome LI polyomavirus isolate LIPyV, complete genome Trichodysplasia spinulosa-associated polyomavirus, complete genome

Family	Virus
Papillomavirus	All Human papillomaviruses (L1, L2, E6, E7, and E2 proteins)
Picornaviridae	Human enterovirus A, complete genome Human enterovirus 68 strain Fermon, complete genome Human rhinovirus C, complete genome Human enterovirus D, complete genome Human enterovirus B, complete genome Human rhinovirus 3, complete genome Human coxsackievirus A2 strain Fleetwood, complete genome Human rhinovirus 1 strain ATCC VR-1559, complete genome Human rhinovirus 89, complete genome Rhinovirus B14, complete sequence Poliovirus, complete genome Enterovirus D68 isolate NY130 polyprotein gene, complete cds Human rhinovirus NAT001 polyprotein gene, complete cds.
Coronaviriade	Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 Human coronavirus HKU1, complete genome Human Coronavirus NL63, complete genome Human coronavirus 229E, complete genome Human coronavirus OC43 strain ATCC VR-759, complete genome Middle East respiratory syndrome-related coronavirus isolate HCoV-EMC/2012, complete genome SARS coronavirus Tor2, complete genome 43 SARS-CoV-2 variants of concern
Orthomyxoviridae	Influenza A virus (A/Korea/426/1968(H2N2)) Influenza A virus (A/Goose/Guangdong/1/96(H5N1)) Influenza A virus (A/Puerto Rico/8/1934(H1N1)) Influenza A virus (A/California/07/2009(H1N1)) Influenza A virus (A/Shanghai/02/2013(H7N9)) Influenza A virus (A/New York/392/2004(H3N2)) Influenza A virus (A/Hong Kong/1073/99(H9N2)) Influenza B virus (B/Lee/1940)
Paramyxoviridae	Human parainfluenza virus 3, complete genome Human parainfluenza virus 1, complete genome Human parainfluenza virus 3 strain ZHYMgz01, complete genome Human parainfluenza virus 4a viral cRNA, complete genome, strain: M-25 Human rubulavirus 2, complete genome
Pneumoviridae	Respiratory syncytial virus, complete genome Human orthopneumovirus Subgroup A, complete cds Human orthopneumovirus Subgroup B, complete genome Human metapneumovirus isolate 00-1, complete genome
Retroviridae	Human endogenous retrovirus K113 complete genome Human endogenous retrovirus HCML-ARV, complete genome Human endogenous retrovirus K115 complete genome Human endogenous retrovirus K113 complete genome
Scrambled Peptides	600 scrambled peptides for background correction and threshold

Global Alliance for Preventing Pandemics (GAPP)

supplies, computational tools, databases



Mexico and Germany Cohorts
Columbia University, February 2023

Training Cohorts:

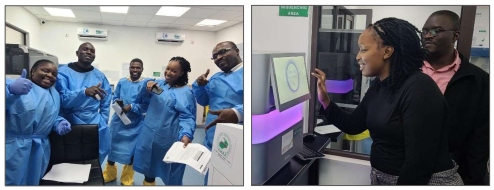
2021: Liberia

2022: Mali, Zambia

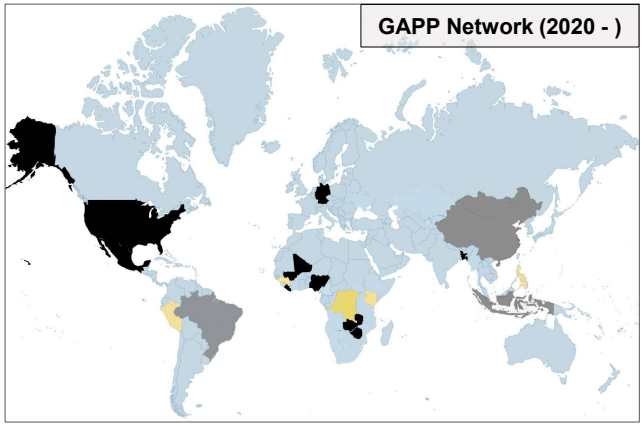
2023: Bangladesh, Germany, Mali, Mexico, Nigeria, US Air Force Academy, Zambia

2024: Angola, Ecuador, Gabon, Guinea, Kenya, Mexico, the Netherlands, the Philippines, South Africa, Sri Lanka, Taiwan, US Air Force, US Army, Zimbabwe

DRC?



In-country training
Churches Health Association of Zambia, August 2023



Developing agreements
(Lunda people and MPs)
Zambia, May 2024