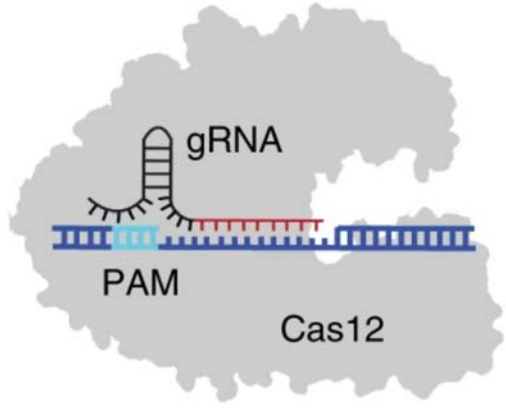
University of California
San Francisco



The Future of Diagnostic Tools in AFM

Charles Chiu, MD / PhD

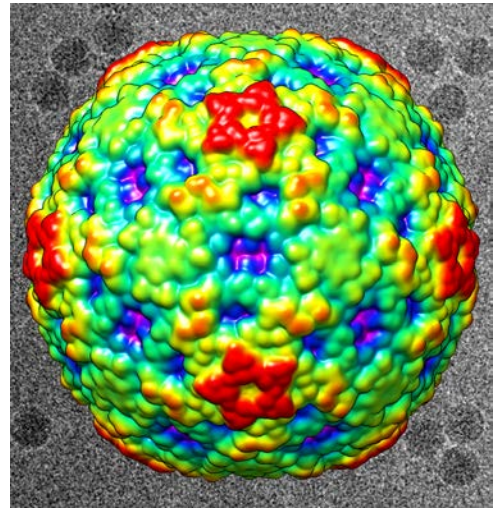
Professor, Department of Laboratory Medicine and Medicine / Infectious Diseases

Director, UCSF-Abbott Viral Diagnostics and Discovery Center

Associate Director, UCSF Clinical Microbiology Laboratory

University of California, San Francisco

AFM Virtual Symposium, June 19th, 2020

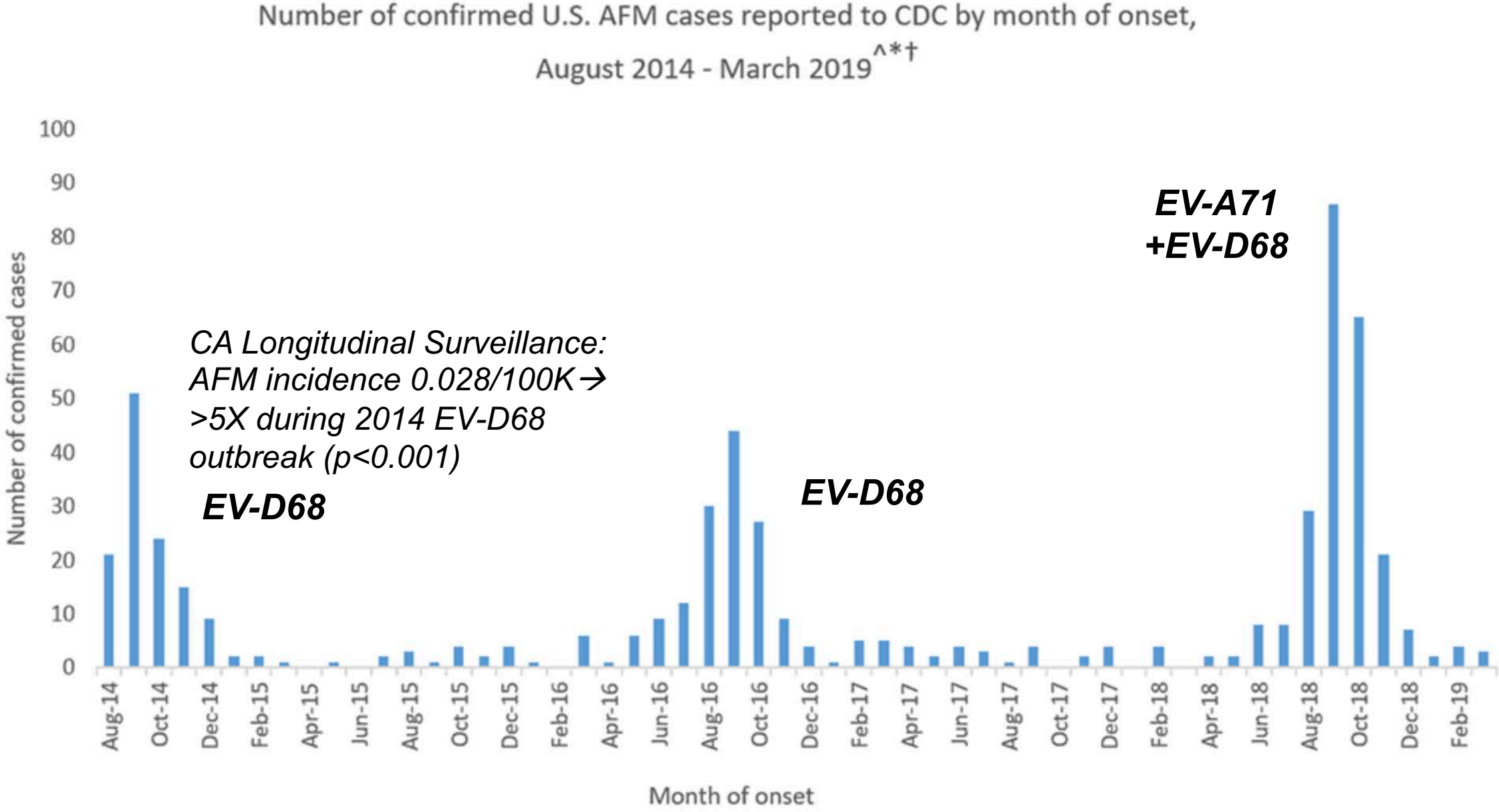


AFM Etiology?

- No infectious agents of clinical significance identified in CSF
  - *including metagenomic next-generation sequencing*
- No poliovirus in CSF, stool, rectal samples
- No WNV or other arboviruses detected
- In 2014 and 2016, EV-D68 most common pathogen detected from non-sterile sites (primarily respiratory)

Sejvar, J. J., et al. (2016). "Acute Flaccid Myelitis in the United States-August - December 2014: Results of Nation-Wide Surveillance." Clin Infect Dis.

Is EV-D68 a Cause of AFM?



https://www.cdc.gov/acute-flaccid-myelitis/afm-cases.html

Diagnostic Tools in Microbiology

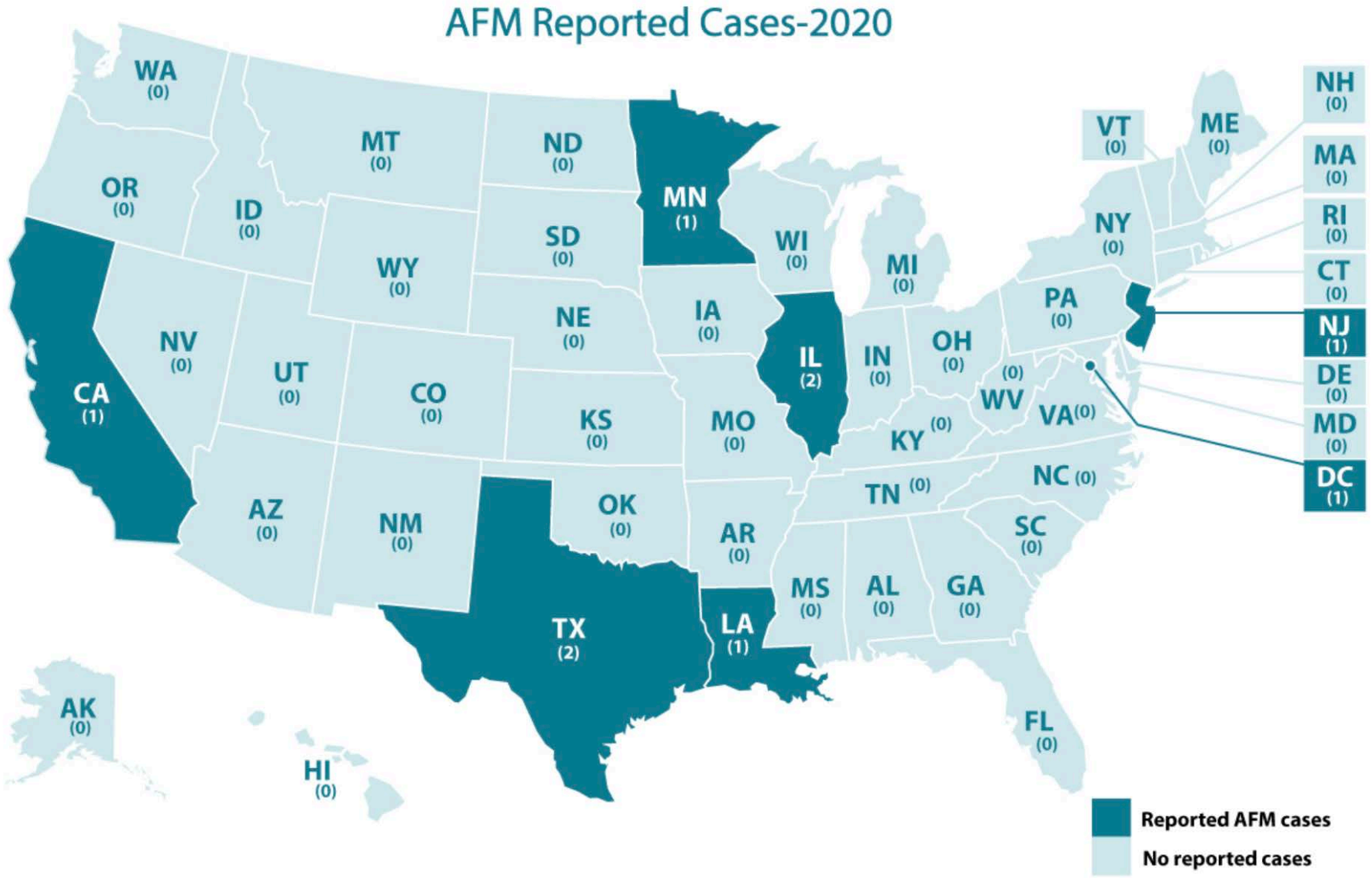
- Culture
- Nucleic Acid Testing (e.g. PCR)
- MALDI (matrix-assisted laser desorption ionization) spectrometry
- Electron Microscopy (rarely used)
- Antigen Testing
- Metagenomic Sequencing
- Serology (Antibody Testing)
- Host Response Biomarkers

Centers for Disease Control and Prevention (2015). Poliomyelitis. Epidemiology and Prevention of Vaccine-Preventable Diseases 13th Edition. J. Hamborsky, A. Kroger and S. Wolfe. Washington D. C., Public Health Foundation.

Diagnostic Tools in Microbiology (Infectious Causes of AFM)

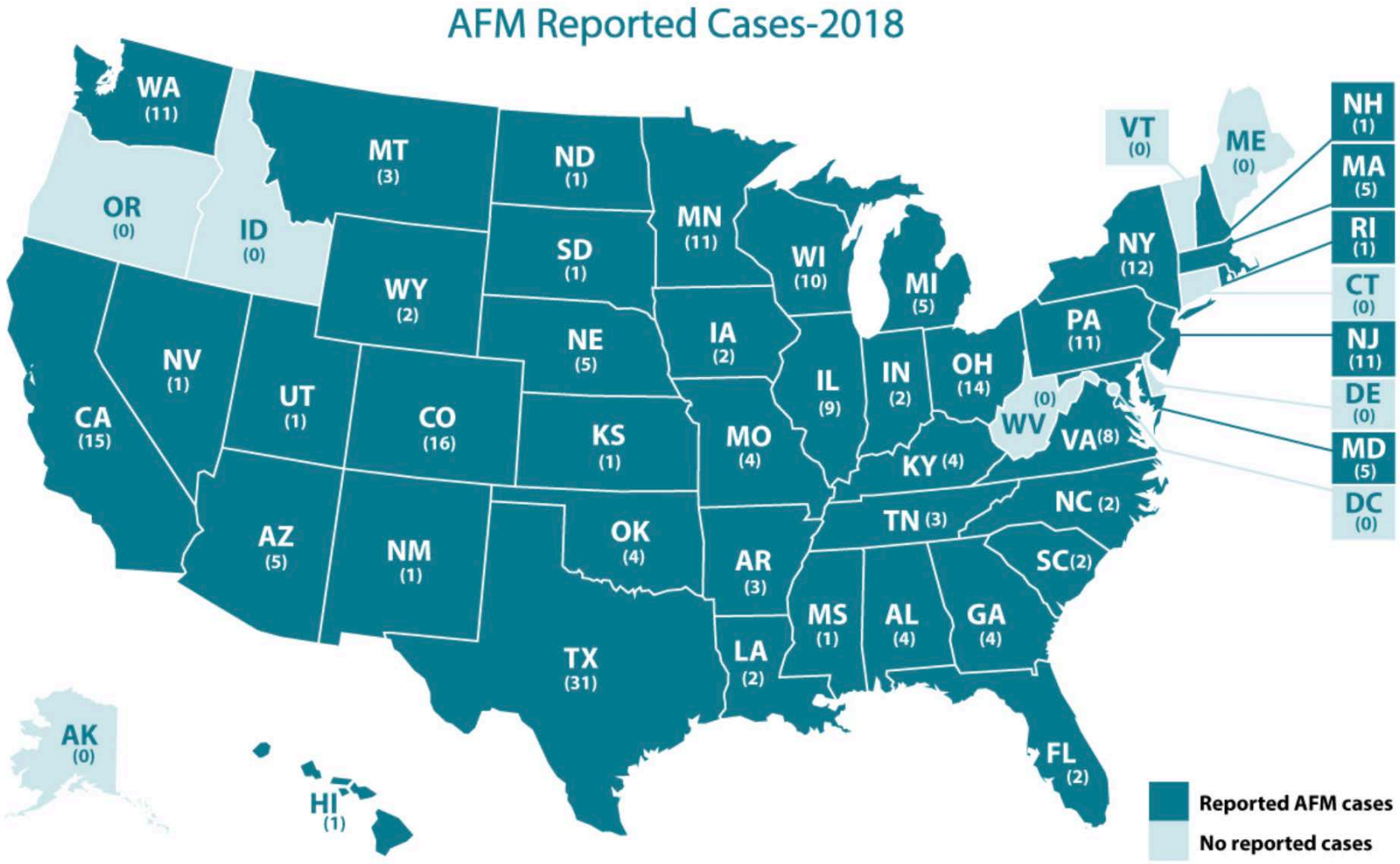
- Culture – *not done for routine diagnostics*
- Nucleic Acid Testing (e.g. PCR) / CRISPR-based detection – *diagnosis in acute phase (>7 days); respiratory secretions and stool, not CSF*
- MALDI – *bacterial/fungal detection only*
- Electron Microscopy – *not used for routine diagnostics*
- Antigen Testing – *unexplored area, includes metabolomics*
- Metagenomic Sequencing – *rule out other causes?*
- Serology (Antibody Testing) – *utility for CSF or serum (?)*
- Host Response Biomarkers – *may allow detection in "window period"*

Centers for Disease Control and Prevention (2015). Poliomyelitis. Epidemiology and Prevention of Vaccine-Preventable Diseases 13th Edition. J. Hamborsky, A. Kroger and S. Wolfe. Washington D. C., Public Health Foundation.



Thus far in 2020, there have been 9 confirmed cases in 6 states and the District of Columbia.

https://www.cdc.gov/acute-flaccid-myelitis/cases-in-us.html



In 2018, there were 238 total confirmed cases in 42 states. One of the confirmed cases is a foreign resident (based on the country of usual residence) and therefore not included in the state map.

https://www.cdc.gov/acute-flaccid-myelitis/cases-in-us.html

Enterovirus Detection in CSF, Respiratory, Stool samples

Vital Signs: Surveillance for Acute Flaccid Myelitis — United States, 2018

Weekly / July 12, 2019 / 68(27);608–614

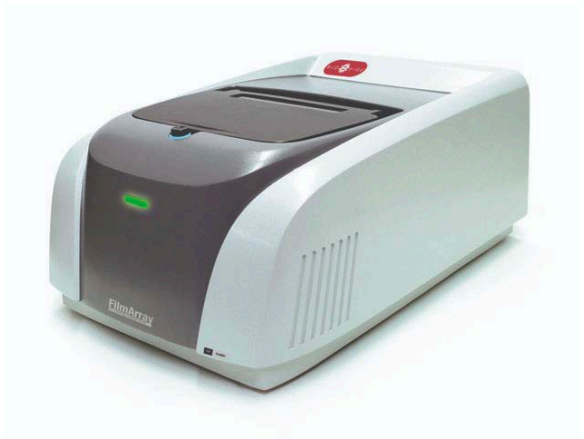
TABLE 2. Laboratory results from cerebrospinal fluid (CSF), respiratory, and stool specimens collected from patients with confirmed acute flaccid myelitis (N = 233) — United States, 2018

Specimen source	No. with specimens available (% of 233)	No. (%) positive	Positive test results (No.)
CSF	74 (32)	2/74 (3)	EV-A71 (1) EV-D68 (1)
Respiratory	123 (53)	54/123 (44)	EV-D68 (30) EV-A71 (10) Other/Untyped EV/RV (14)
Stool	100 (43)	13/100 (13)	EV-D68 (1) EV-A71 (2) Echovirus 11 (1) Coxsackievirus (3) Parechovirus (4) Other/Untyped EV/RV (2)

Abbreviations: EV = enterovirus; RV = rhinovirus.

Weekly / July 12, 2019 / 68(27);608–614

Multiplexed Viral PCR Testing Cannot Discriminate Rhinoviruses from Enteroviruses



FilmArray target	No. (%) of specimens		
	With target	Confirmed EV-D68 positive	EV-D68 negative
Human rhinovirus 1,4	22	20 (41)	2 (5)
Human rhinovirus 1,2,4	19	17 (34)	2 (5)
Human rhinovirus 4	13	7 (15)	6 (15)
Human rhinovirus 1,2,3,4	27	5 (10)	22 (55)
Human rhinovirus 3,4	4	0 (0)	4 (10)
Human rhinovirus 1,2	2	0 (0)	2 (5)
Human rhinovirus 1	1	0 (0)	1 (2.5)
Human rhinovirus 2,4	1	0 (0)	1 (2.5)
Enterovirus 1	0	0 (0)	0 (0)
Enterovirus 2	0	0 (0)	0 (0)
Total	89	49 (100)	40 (100)

Shibib, et al., (2016), JCM, 54(2):457-459

Organism (abbreviation)	Classification (Genome type)	Season of Highest Incidence[a]	Most Commonly Infected Demographic
Adenovirus (AdV)	Adenovirus (DNA)	Late winter to early summer[1]	All ages, immunocompromised[1]
Coronavirus (CoV) 229E, HKU1, NL63, OC43	Coronavirus (RNA)	Winter, spring[2-3]	Children, adults[2-3]
Enterovirus (EV)	Picornavirus(RNA)	Summer, early fall [4]	All ages[5]
Human Rhinovirus (HRV)	Picornavirus (RNA)	Fall, spring[6]	All ages[6]
Human Metapneumovirus (hMPV)	Paramyxovirus (RNA)	Winter, early spring[7]	Children[7]
Influenza A (Flu A) (subtypes H1, H1-2009, and H3)	Orthomyxovirus (RNA)	Winter [8]	All ages [8], 5-20 % of US population[9]
Influenza B (Flu B)	Orthomyxovirus (RNA)	Winter [8]	All ages [8], 5-20 % of US population[9]
Parainfluenza Virus 1 (PIV1)	Paramyxovirus (RNA)	Fall, periodicity of 1-2 years[10]	Infants, young children, immunocompromised[10]
Parainfluenza Virus 2 (PIV2)	Paramyxovirus (RNA)	Fall, periodicity of 1-2 years[10]	Infants, young children, immunocompromised[10]
Parainfluenza Virus 3 (PIV3)	Paramyxovirus (RNA)	Spring, summer[10]	Infants, young children, immunocompromised [10]
Parainfluenza Virus 4 (PIV4)	Paramyxovirus (RNA)	Unknown	All ages[11]
Respiratory Syncytial Virus (RSV)	Paramyxovirus (RNA)	Winter, varies by location[12-13]	Children, older adults[12-13]
Bordetella pertussis	Bacterium (DNA)	No peak season	All ages[14]
Chlamydophila pneumoniae	Bacterium (DNA)	No peak season	Older children, young adults, immunocompromised[15]
Mycoplasma pneumoniae	Bacterium (DNA)	Outbreaks most common in summer, outbreak periodicity 4 – 7 years	Older children, young adults[16-17]

[a] based on North American seasons

April 14th, 2020

nature biotechnology

LETTERS

https://doi.org/10.1038/s41587-020-0513-4

Check for updates

CRISPR–Cas12-based detection of SARS-CoV-2

James P. Broughton[1,7], Xianding Deng[2,3,7], Guixia Yu[2,3], Clare L. Fasching[1], Venice Servellita[2,3], Jasmeet Singh[1], Xin Miao[1], Jessica A. Streithorst[2], Andrea Granados[2,3], Alicia Sotomayor-Gonzalez[2,3], Kelsey Zorn[4], Allan Gopez[2], Elaine Hsu[2], Wei Gu[2], Steve Miller[2], Chao-Yang Pan[5], Hugo Guevara[5], Debra A. Wadford[5], Janice S. Chen[1]✉ and Charles Y. Chiu[2,3,6]✉

An outbreak of betacoronavirus severe acute respiratory syndrome (SARS)-CoV-2 began in Wuhan, China in December 2019. COVID-19, the disease associated with SARS-CoV-2 infection, rapidly spread to produce a global pandemic. We report development of a rapid (<40 min), easy-to-implement and accurate CRISPR–Cas12-based lateral flow assay for detection of SARS-CoV-2 from respiratory swab RNA extracts. We validated our method using contrived reference samples and clinical samples from patients in the United States, including 36 patients with COVID-19 infection and 42 patients with other viral respiratory infections. Our CRISPR-based DETECTR assay provides a visual and faster alternative to the US Centers for Disease Control and Prevention SARS-CoV-2 real-time RT–PCR assay, with 95% positive predictive agreement and 100% negative predictive agreement.

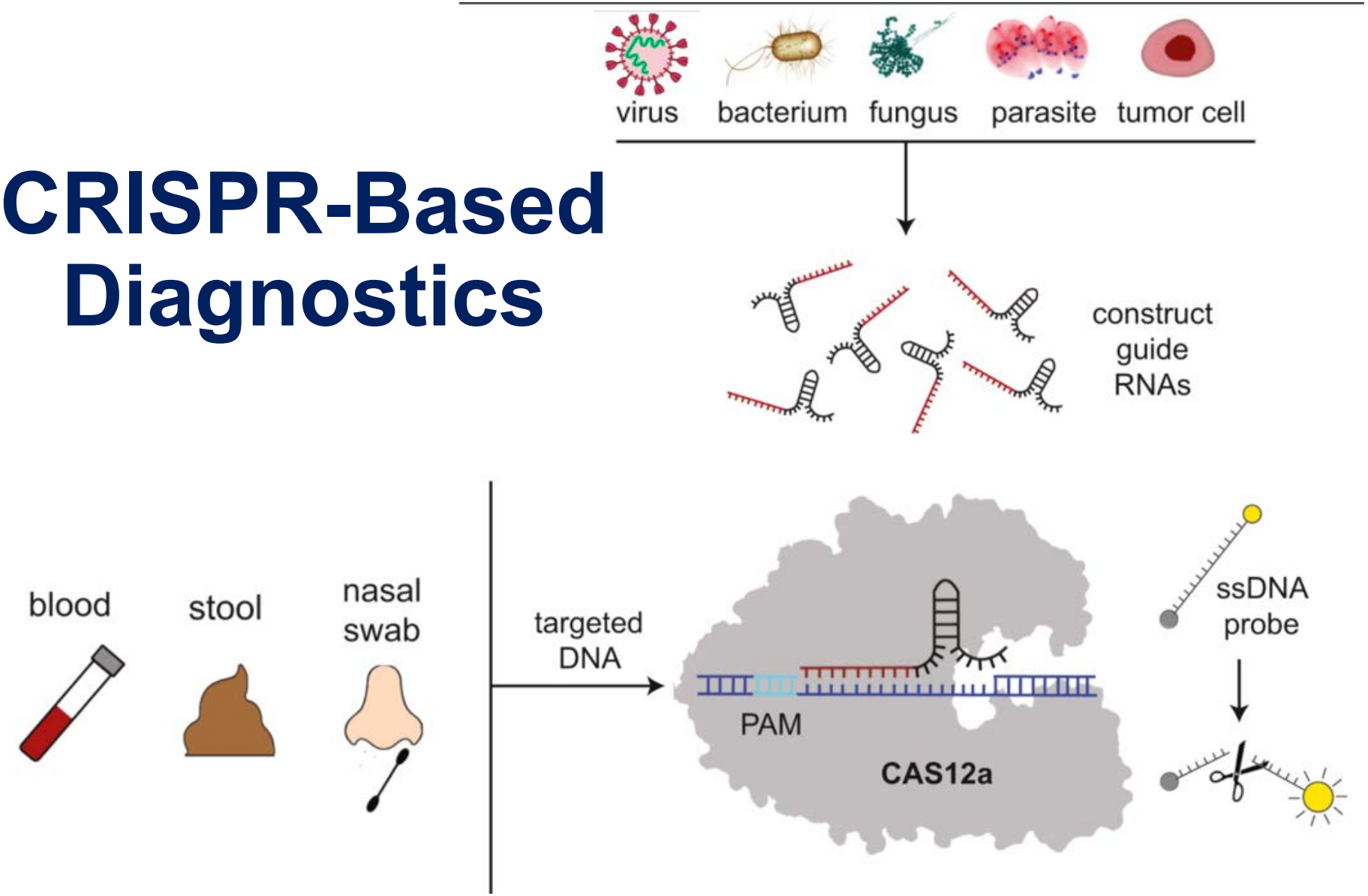
Over the past 40 years, there have been recurrent large scale

COVID-19 in the United States, on 28 February 2020, the US Food and Drug Administration (FDA) permitted individual clinically licensed laboratories to report the results of in-house-developed SARS-CoV-2 diagnostic assays while awaiting results of an EUA submission for approval[8].

Here we report the development and initial validation of a CRISPR–Cas12-based assay[9–13] for detection of SARS-CoV-2 from extracted patient sample RNA, called SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR). This assay performs simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT–LAMP)[14] for RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which cleavage of a reporter molecule confirms detection of the virus. We first designed primers targeting the E (envelope) and N (nucleoprotein) genes of

(collaboration with Mammoth Biosciences, Inc.)

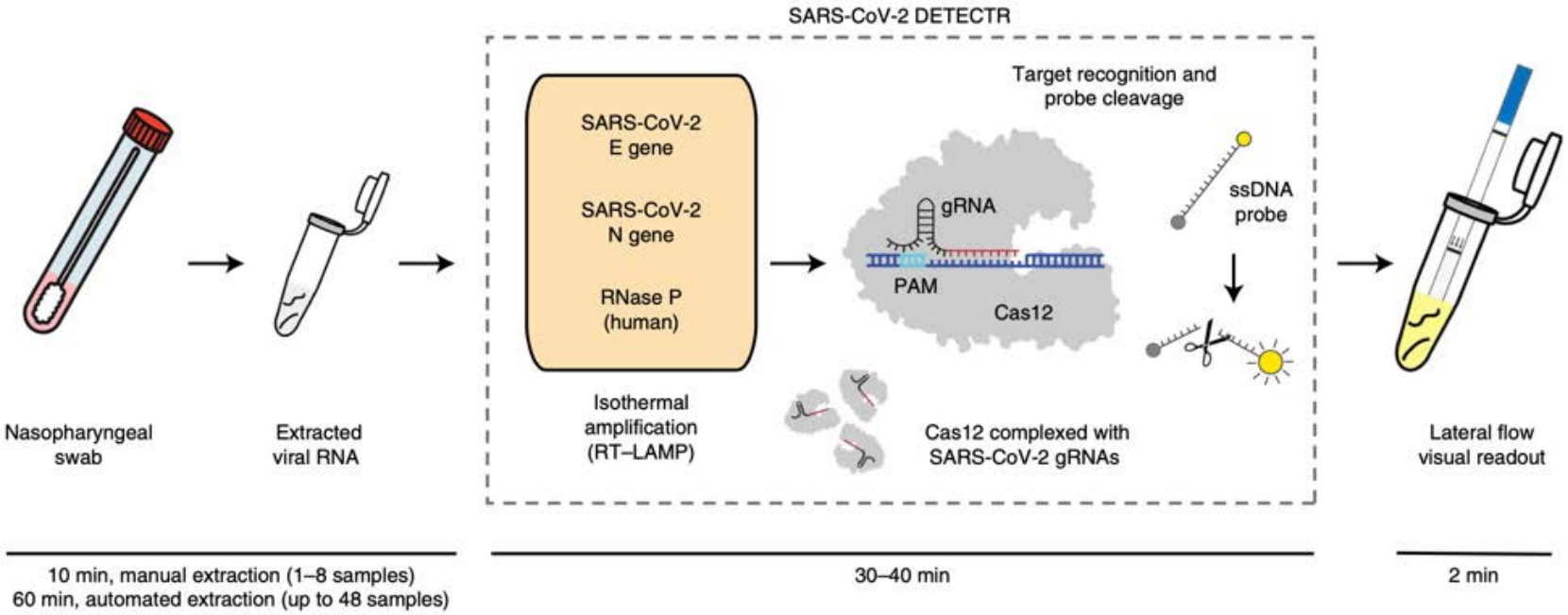
CRISPR-Based Diagnostics



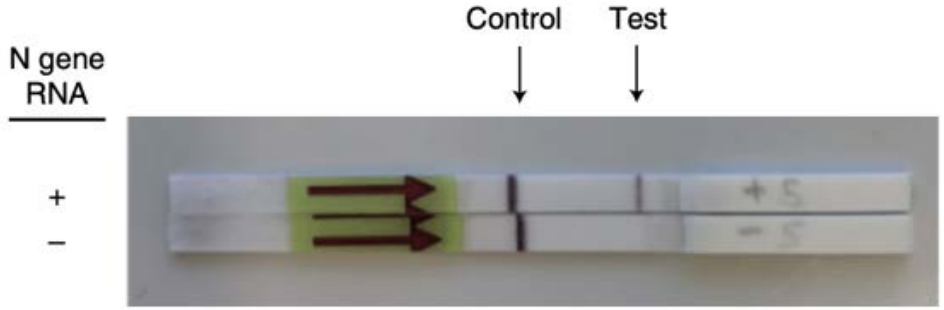
- diagnosis of infectious disease
- emerging pathogen surveillance
- viral genotyping
- antibiotic or antiviral resistance
- SNP identification
- companion diagnostic (+ treatment)
- cancer screening

(modified from Chiu, 2018, Cell Host & Microbe, 23(6):702-704)

A CRISPR Based Test for SARS-CoV-2



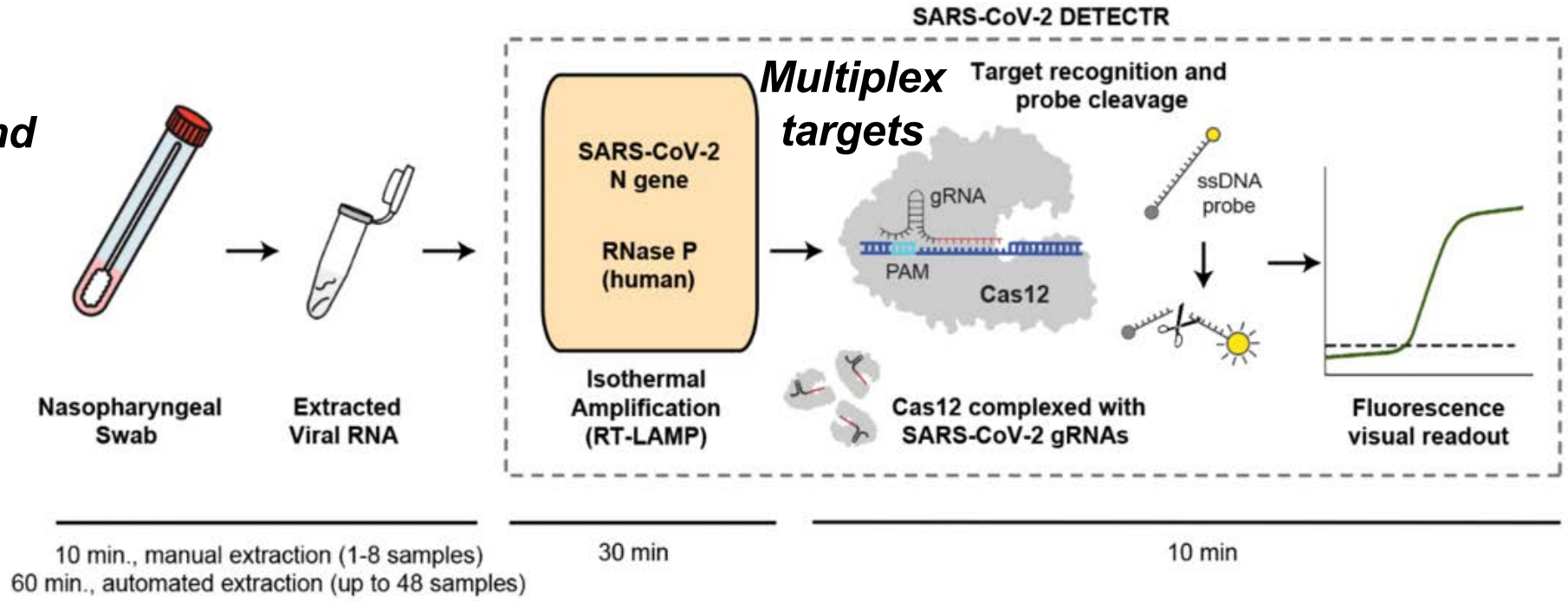
	CDC qRT–PCR positive	CDC qRT–PCR negative
DETECTR positive or presumptive positive	38	0
DETECTR negative	2	42
	PPA: (38 of 40 = 95%)	NPA: (42 of 42 = 100%)



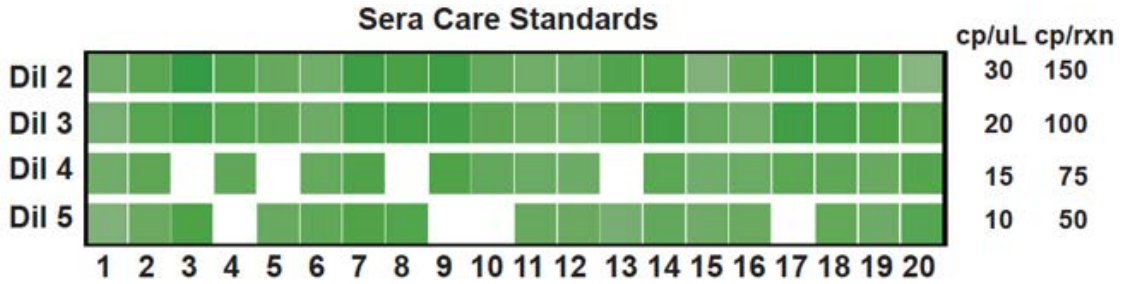
(Broughton, et al., 2020, Nature Biotechnology, DOI: 10.1038/s41587-020-0513-4)

Clinical Validation and Next Steps

5-min lysis and 95C heat inactivation



384-well plates



LoD: 20 copies/mL

Sensitivity: 27/29 = 93%

Specificity: 100%

(Servellita, et al., 2020, manuscript in preparation)

Metagenomic Sequencing

Clinical Laboratory

Metagenomic Next-Generation Sequencing

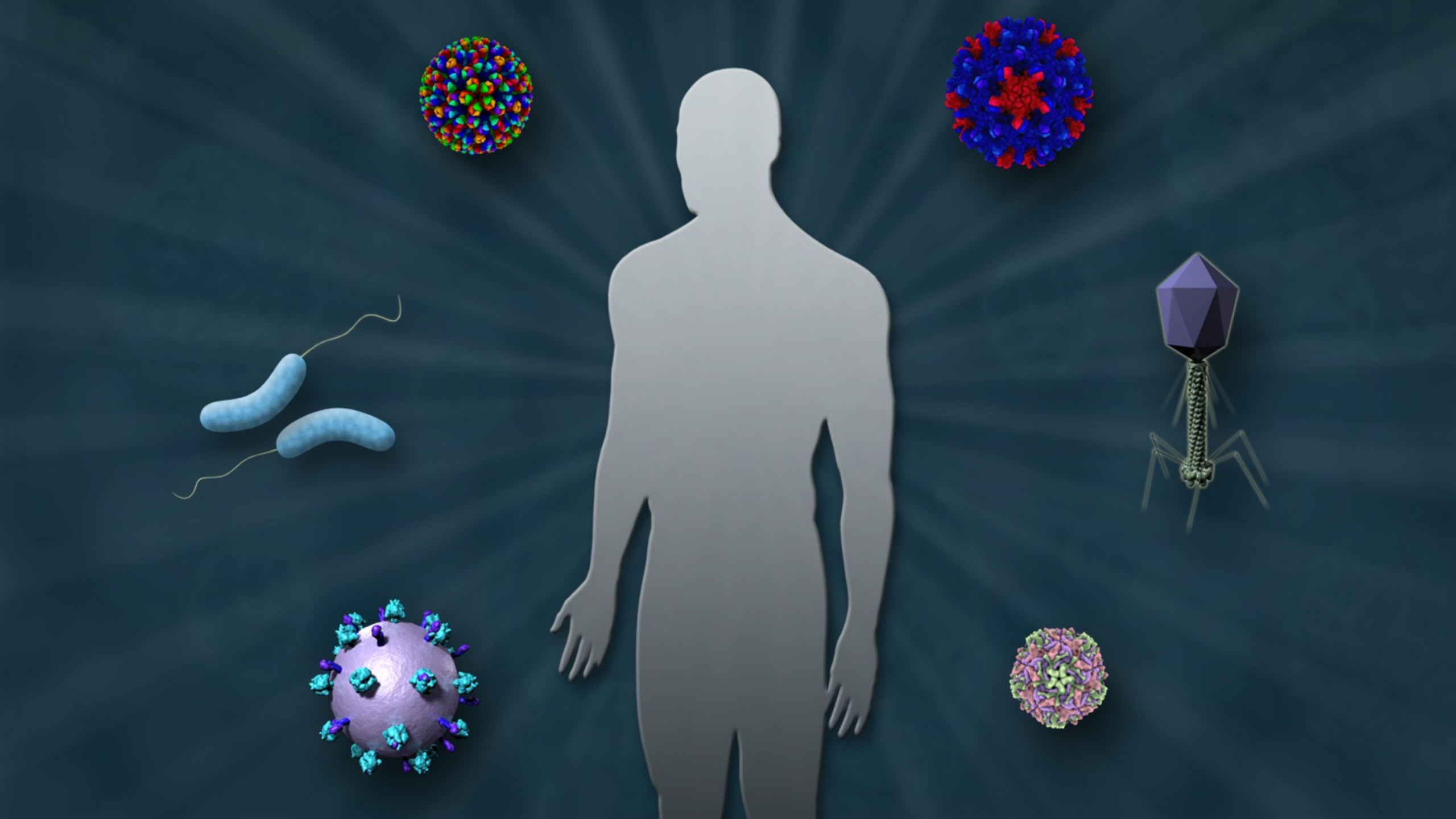
Bioinformatics

Clinical Dx

Cost-effective and actionable information for early treatment

Lower healthcare costs
Improved patient outcomes

Turnaround time: hours – days (versus days – weeks)



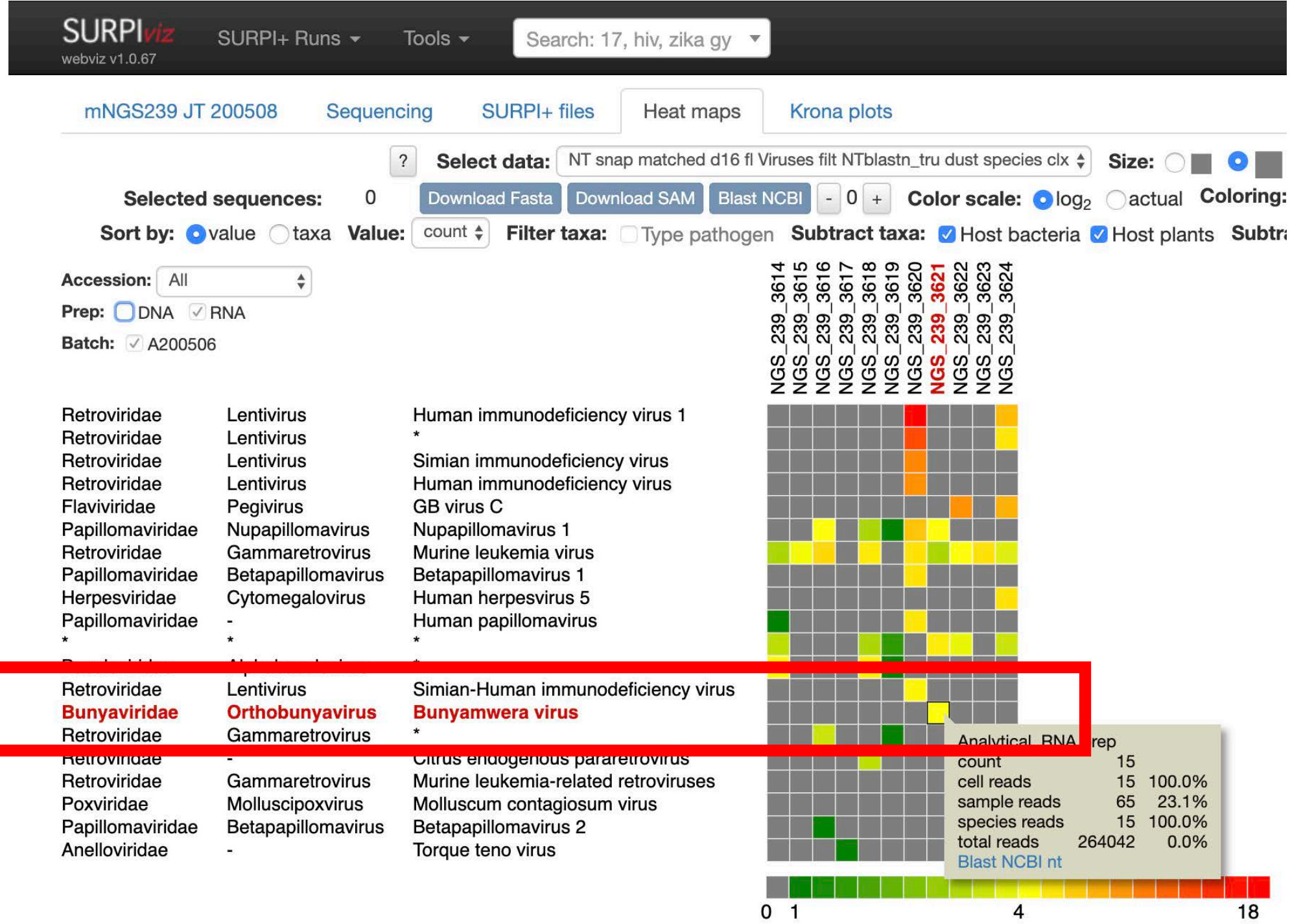
CLIA-Validated mNGS Assay at UCSF

http://nextgendiagnostics.ucsf.ed

Case Report from Cleveland Clinic

- 70 y/o male with mild dementia x 1 year
- Over past month, rapid decline until he became akinetic and mute
- No inflammatory changes on MRI or in CSF but fever x 3 weeks
- Treated with IV solumedrol and plasmapheresis
- Negative autoimmune encephalitis workup
- History of non-Hodgkin's lymphoma s/p chemotherapy, last cycle 2-3 years ago, also treated with Rituxumab
- From a rural area but not very active

With Dr. Carlos Isada, MD


SURPIviz
webviz v1.0.67
SURPI+ Runs
Tools
Search: 17, hiv, zika gy
mNGS239 JT 200508
Sequencing
SURPI+ files
Heat maps
Krona plots
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Selected sequences: 0
Download Fasta
Download SAM
Blast NCBI
Color scale: log2 actual
Coloring:
Sort by: value taxa
Value: count
Filter taxa: Type pathogen
Subtract taxa: Host bacteria Host plants
Accession: All
Prep: DNA RNA
Batch: A200506
NGS_239_3614
NGS_239_3615
NGS_239_3616
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NGS_239_3619
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NGS_239_3623
NGS_239_3624
Retroviridae Lentivirus Human immunodeficiency virus 1
Retroviridae Lentivirus *
Retroviridae Lentivirus Simian immunodeficiency virus
Retroviridae Lentivirus Human immunodeficiency virus
Flaviviridae Pegivirus GB virus C
Papillomaviridae Nupapillomavirus Nupapillomavirus 1
Retroviridae Gammaretrovirus Murine leukemia virus
Papillomaviridae Betapapillomavirus Betapapillomavirus 1
Herpesviridae Cytomegalovirus Human herpesvirus 5
Papillomaviridae - Human papillomavirus
* * *
Retroviridae Lentivirus Simian-Human immunodeficiency virus
Bunyaviridae Orthobunyavirus Bunyamwera virus
Retroviridae Gammaretrovirus *
Retroviridae Gammaretrovirus Murine leukemia-related retroviruses
Poxviridae Molluscipoxvirus Molluscum contagiosum virus
Papillomaviridae Betapapillomavirus Betapapillomavirus 2
Anelloviridae - Torque teno virus
Analytical_RNA_rep
count 15
cell reads 15 100.0%
sample reads 65 23.1%
species reads 15 100.0%
total reads 264042 0.0%
Blast NCBI nt
0 1 4 18

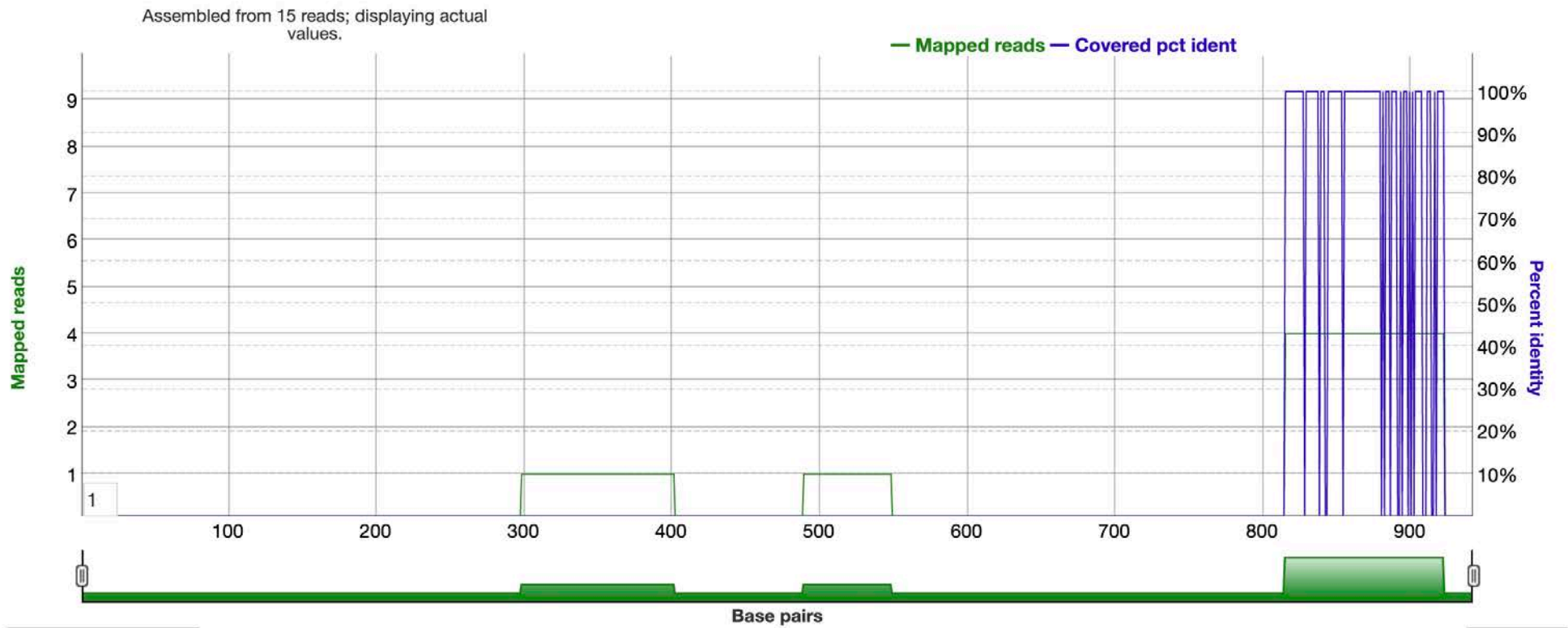
SURPIviz webviz v1.0.67 | SURPI+ Runs | Tools | Search: 17, hiv, zika gy | Charles.Chiu | Sign out

mNGS239 JT 200508 | Sequencing | SURPI+ files | Heat maps | Krona plots

? **Select file:** Orthobunyavirus Bunyamwera_virus 17225332 species **Series:** Coverage Percent identity **Shading:** min/max

Bunyamwera virus (gi|17225332|, 943 bp)

Bunyamwera virus segment S, complete sequence



Toggle Log/Linear Y-axis | Reset Zoom

Download Consensus | Save PDF

	Reference length bp	*Coverage in bp*	*Percent coverage*	*Avg coverage depth*	*Covered pct ident*
Overall	943	272	28.84	0.63	32.35
Displayed	943	272	28.84	0.63	32.35

Job Title	**15 sequences (SNL170:596:HFWTGBCX3:1:1103:14783:76991#A**
RID	BH133W2C014 *Search expires on 05-12 06:46 am* Download All
Results for	1:lcl|Query_39805 SNL170:596:HFWTGBCX3:1:1103:14783:76991#ATGCGCA
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_39805
Description	SNL170:596:HFWTGBCX3:1:1103:14783:76991#ATGCGCAG+CT ...
Molecule type	dna
Query Length	106
Other reports	Distance tree of results

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to **E value** to **Query Coverage** to

Filter Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments

Download | Manage Columns | Show 100

select all *16 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Potosi virus strain 89-3380 segment S, complete sequence	185	185	100%	2e-43	98.11%	MH484323.1
[illegible]	185	185	100%	2e-43	98.11%	NC_043647.1
Potosi virus nucleocapsid (N) and small non-structural protein (NSs) genes, complete cds	185	185	100%	2e-43	98.11%	AY729652.1
Maguari virus strain CoAr 3363 segment S, complete sequence	132	132	89%	3e-27	91.58%	KX100106.1
Cache Valley virus isolate CVV-078 nucleoprotein and NSs protein genes, complete cds	132	132	89%	3e-27	91.58%	GU018034.1
Cache Valley virus isolate CVV-002 nucleoprotein and NSs protein genes, complete cds	132	132	89%	3e-27	91.58%	GU018033.1
Cache Valley virus isolate R103016b segment S, complete sequence	126	126	89%	2e-25	90.53%	MK861965.1
Cache Valley virus strain MNZ-92011 nucleoprotein and NSs protein genes, complete cds	126	126	89%	2e-25	90.53%	KC436108.1
Cache Valley virus isolate CVV-390 nucleoprotein and NSs protein genes, complete cds	126	126	89%	2e-25	90.53%	GU018036.1

Potosi virus

- Orthobunyavirus first isolated from *Aedes albopictus* mosquitoes in Potosi, MO, in 1989
- Subsequent isolations in Illinois, Michigan, Ohio, and the Carolinas, northeastern United States (Armstrong, et al., *J Med Entol*, 2005, 42(5): 875-881)
- Never described in human illness
- Close relative, Cache Valley virus recently described in case reports of chronic meningoencephalitis in patients with SCID and on Rituxamab ((https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5546801/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6037180/)

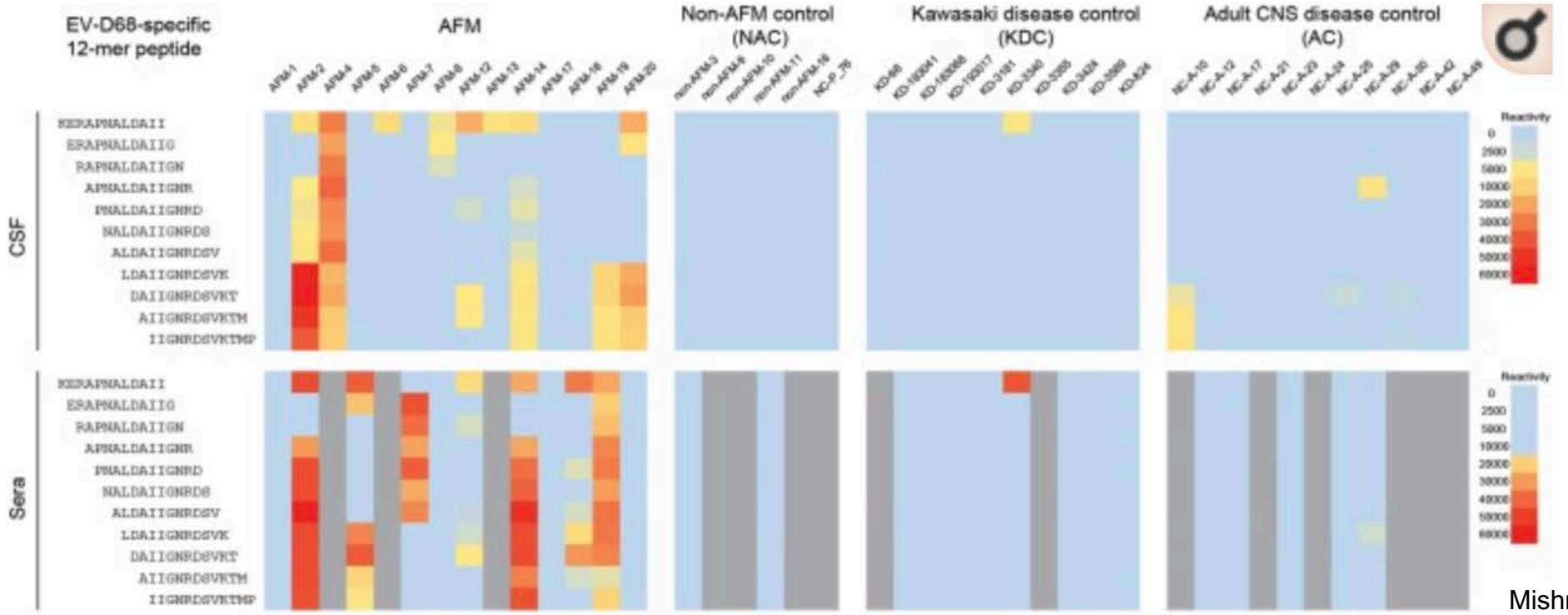
With Dr. Carlos Isada, MD

Serology (Antibody Testing)

mBio. 2019 Jul-Aug; 10(4): e01903-19.
Published online 2019 Aug 13. doi: 10.1128/mBio.01903-19
PMCID: PMC6692520
PMID: 31409689

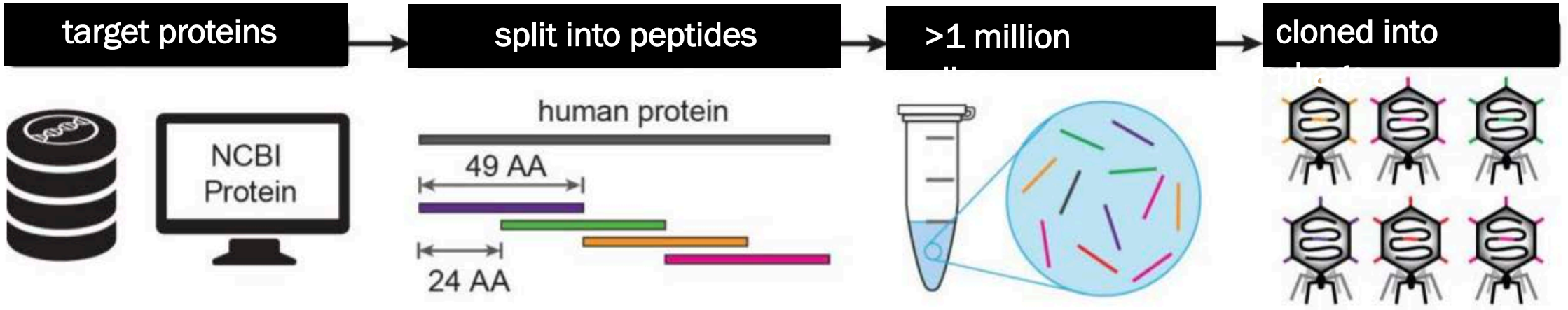
Antibodies to Enteroviruses in Cerebrospinal Fluid of Patients with Acute Flaccid Myelitis

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



Mishra, et al., mBio, 2019, 10(4): e01903-19.

Pan-Serological Approaches for Diagnosis of Infections using Phage Display Immunoprecipitation and Sequencing



Michael Wilson, MD

Joseph DeRisi, PhD

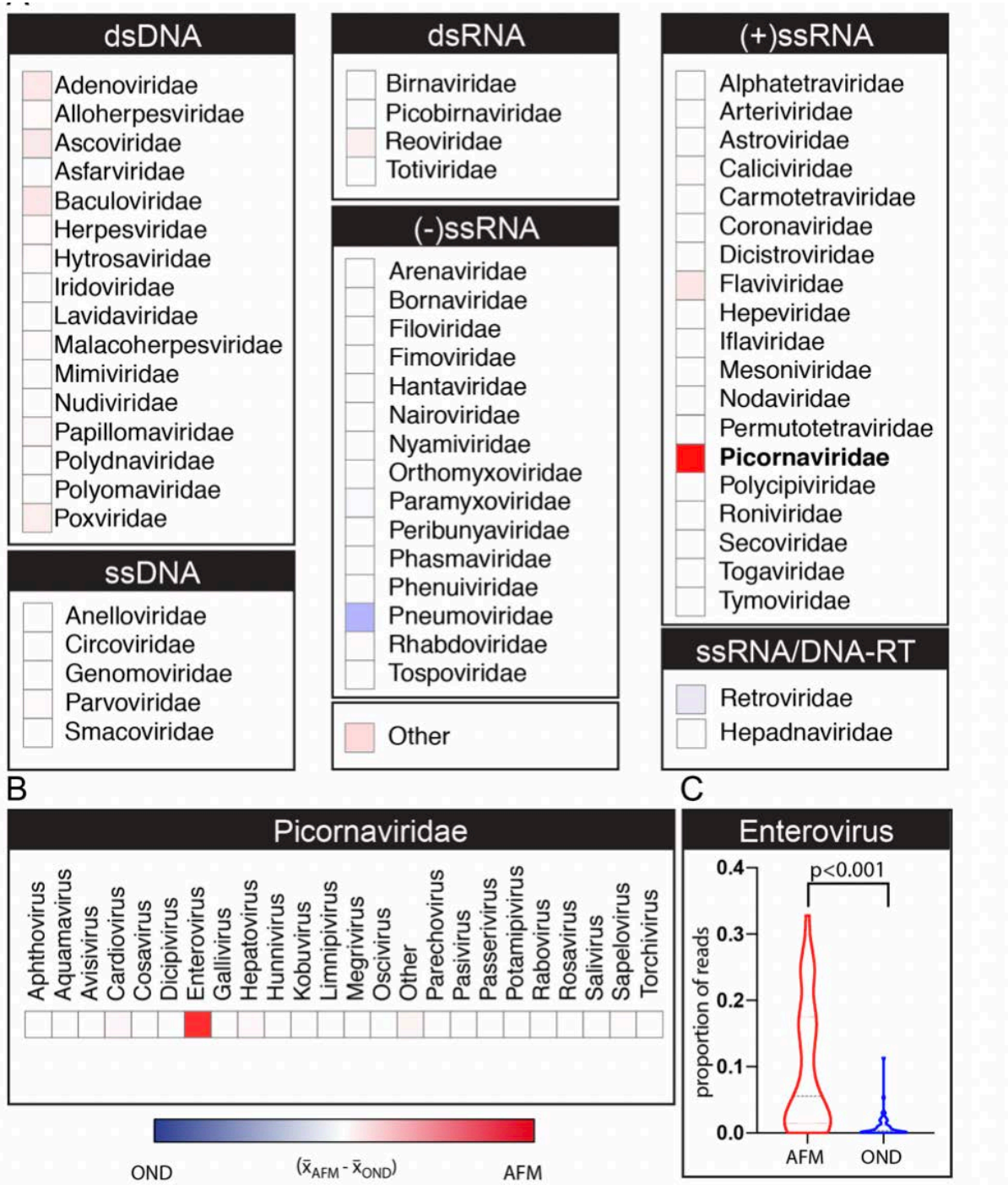
Schubert, et al., 2019, *Nature Medicine,* 25(11):1748-1752.

Enterovirus Immunoreactivity in Acute Flaccid Myelitis

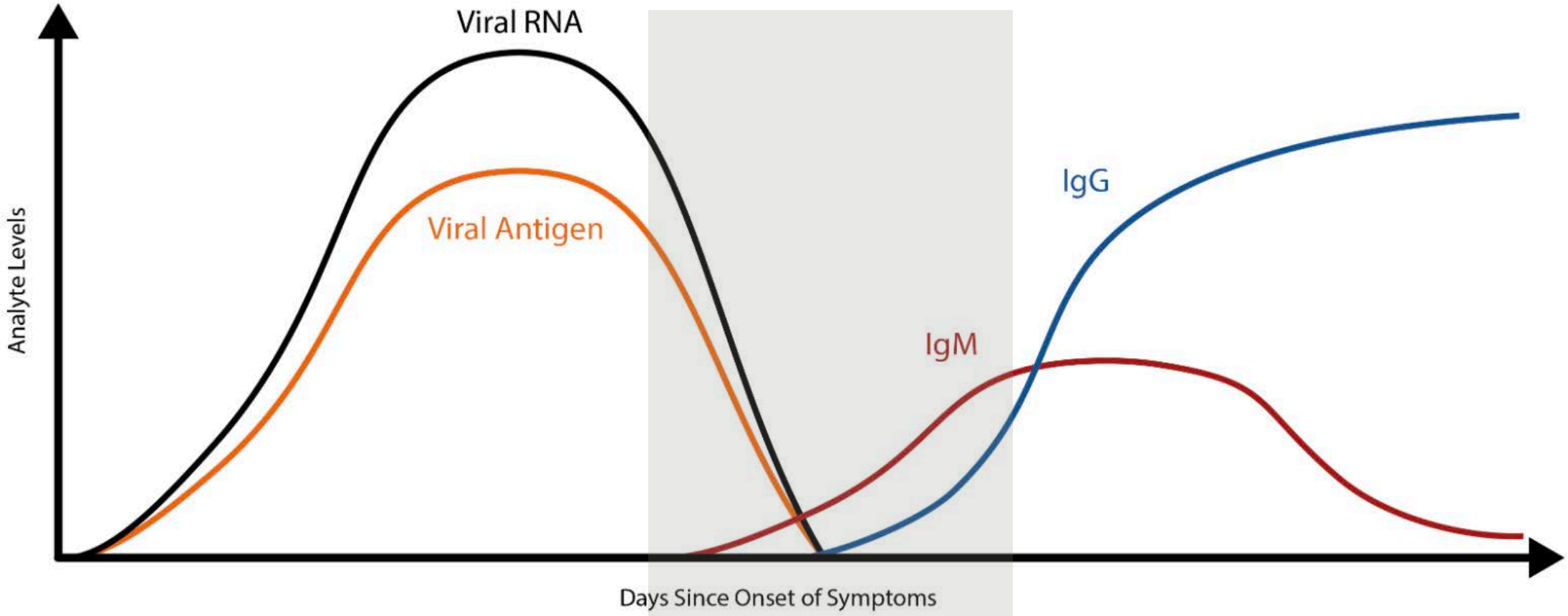
Ryan Schubert, MD

Michael Wilson, MD

Schubert, et al., 2019, *Nature Medicine,* 25(11):1748-1752.



The “Window Period” of Infection



Direct detection (e.g. PCR)
Usually cannot detect in CSF
Cannot discriminate enteroviruses/rhinoriuses
Difficult to routinely test

“window period”

Indirect detection (e.g. serology)
May not be EV-D68-specific,
Not as useful in acute infection

Liu, et al., 2020, *Microbes and Infection,* doi: 10.1016/j.micinf.2020.05.008

Host-Based RNA-Seq Classifier

(Viral Meningitis Versus Enterovirus-Associated AFM)

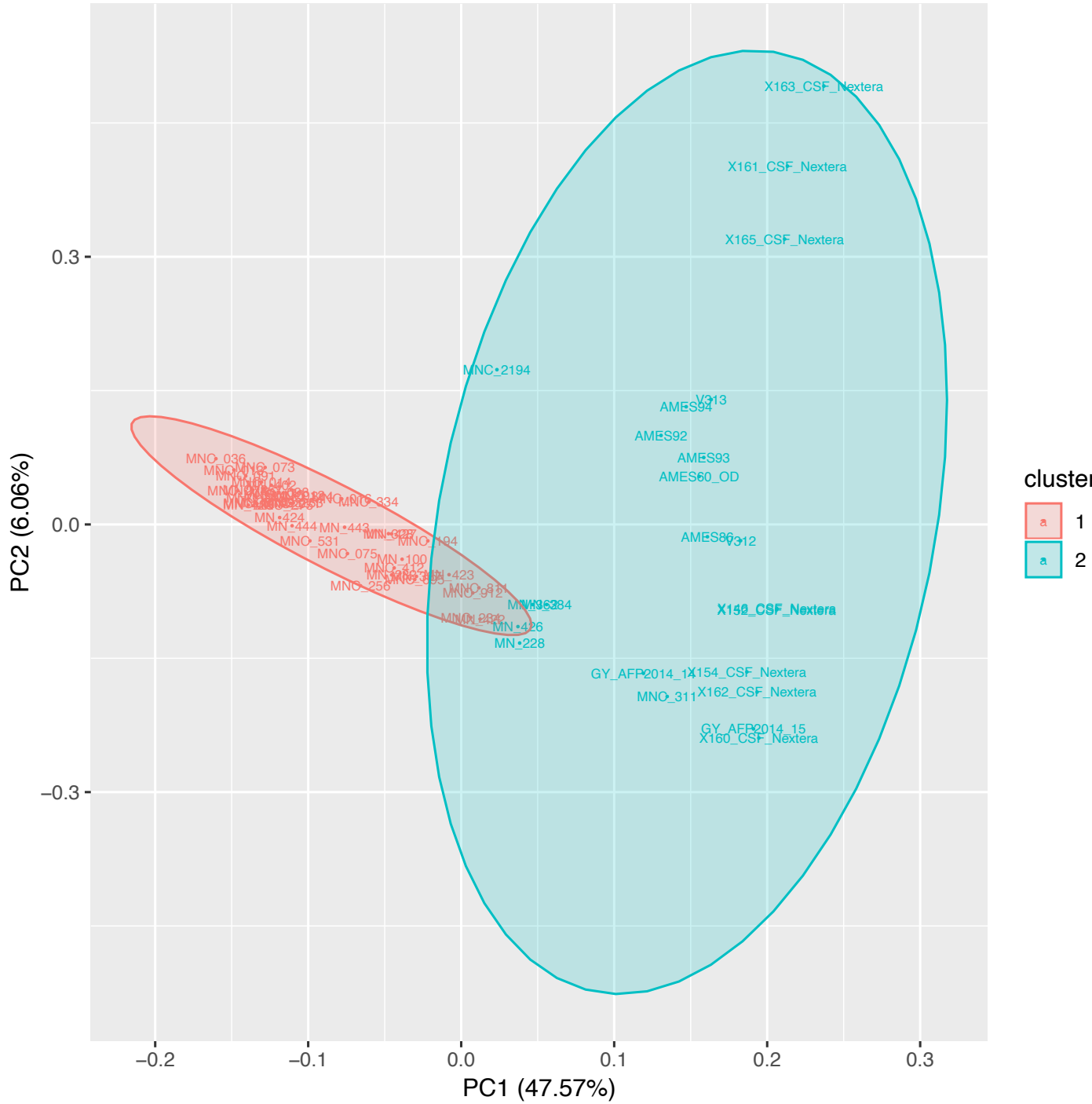
Based on 185 differentially expressed genes
Only includes AFM for which a virus was found

Misclassified samples:

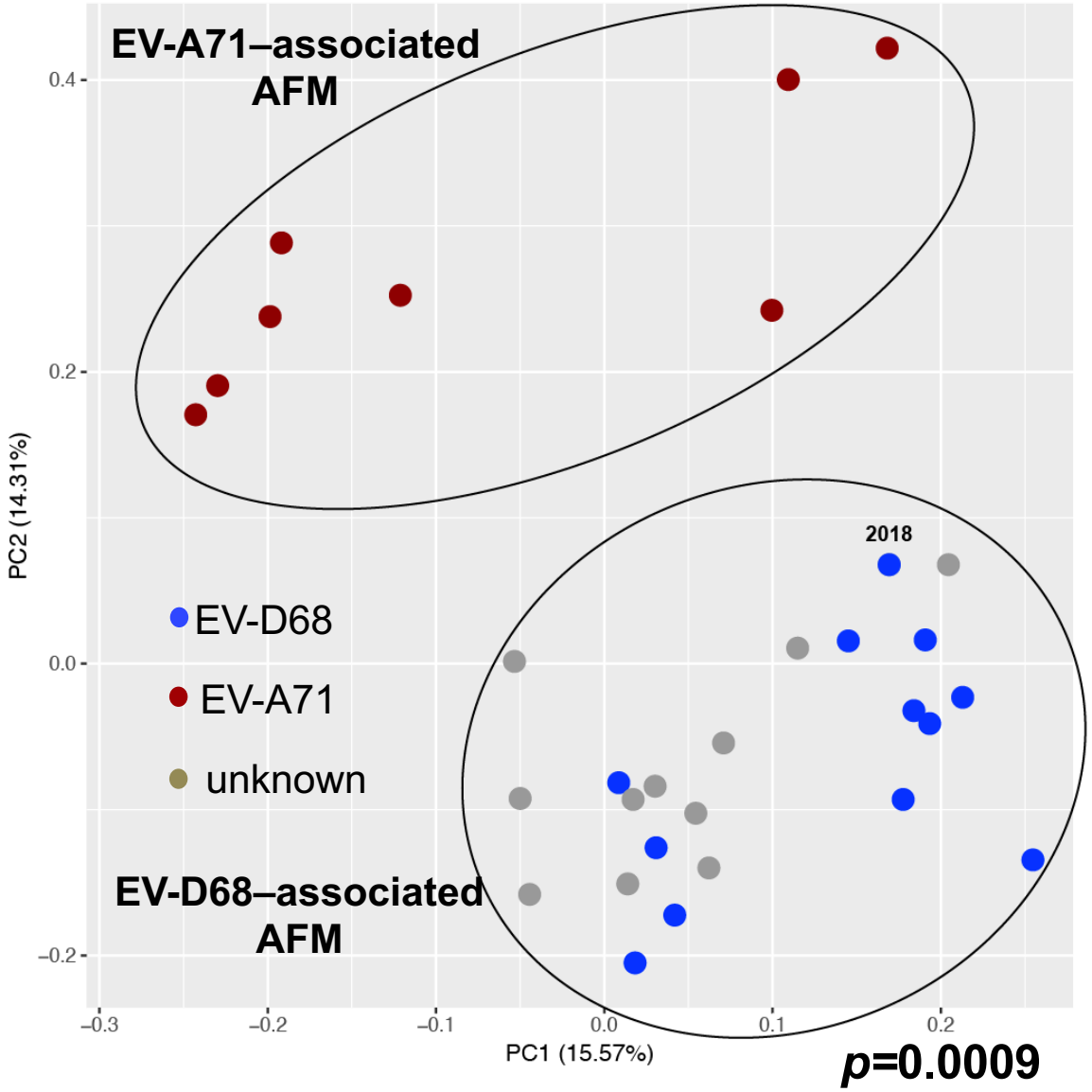
MNA_362:	Enterovirus B
MNA_384:	CMV
MNA_426:	CMV
MNC_311:	WNV
MNA_228:	WNV

Out of 45 viral samples, 18 AFM samples:

Sensitivity = 100%
Specificity = 88.8%



CSF Host Response Profiling Discriminates Enterovirus Signatures in Acute Flaccid Myelitis

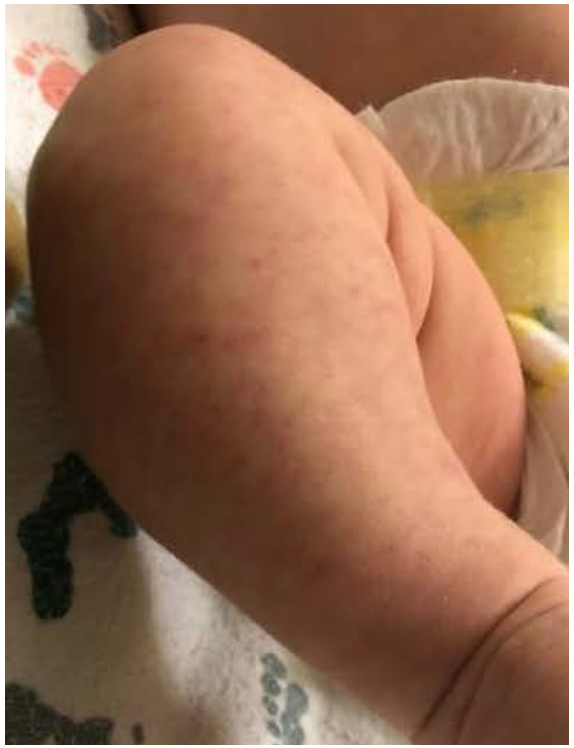


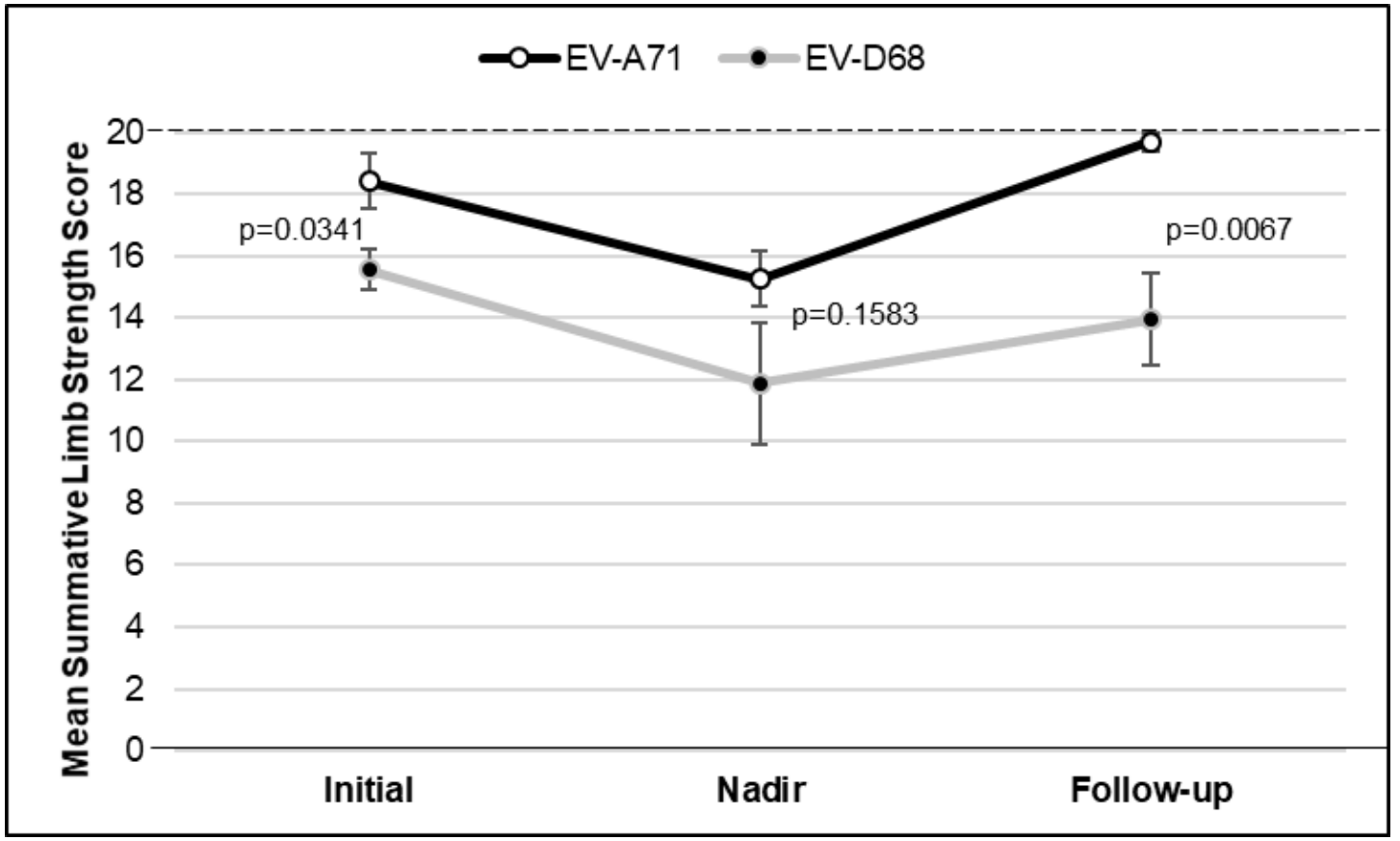
Top Differentially Expressed Pathways in EV-D68-associated AFM

- negative regulation of cell fate commitment
- spinal cord association neuron differentiation
- negative regulation of cell differentiation
- negative regulation of cell fate specification
- negative regulation of neurogenesis
- negative regulation of gastrulation
- negative regulation of nervous system development
- dorsal spinal cord development
- negative regulation of multicellular organismal process
- negative regulation of developmental process
- negative regulation of cell development
- positive regulation of endoplasmic reticulum calcium ion concentration
- regulation of transcription involved in primary germ layer cell fate commitment
- negative regulation of neuron differentiation
- regulation of gene expression
- regulation of cell fate commitment
- regulation of cell fate specification
- positive regulation of neural precursor cell proliferation
- olfactory pit development
- dorsal/ventral pattern formation
- epithelial cell differentiation
- neurogenesis
- negative regulation of necrotic cell death
- central nervous system neuron differentiation

Briggs, Santos, et al., 2019 (manuscript in preparation)

EV-A71-associated AFM





- ~50% returned to neurologic baseline by discharge
- All but one child with AFM had complete recovery of strength with normal neurologic exam at most recent follow-up

Initially reported in Messacar, K., et al. (2018). "Notes from the Field: Enterovirus A71 Neurologic Disease in Children - Colorado, 2018." MMWR Morb Mortal Wkly Rep 67(36): 1017-1018.
Updated with final case counts (unpublished, under review)

(slide courtesy of Dr. Kevin Messacar)

Differentially Expressed Genes Between EV-D68 and EV-A71 Infection

Initial pathway analysis:

Negative Regulation of Viral Entry into host cell

Response to Interferon-alpha

Type I interferon signaling pathway

Primary neural tube formation

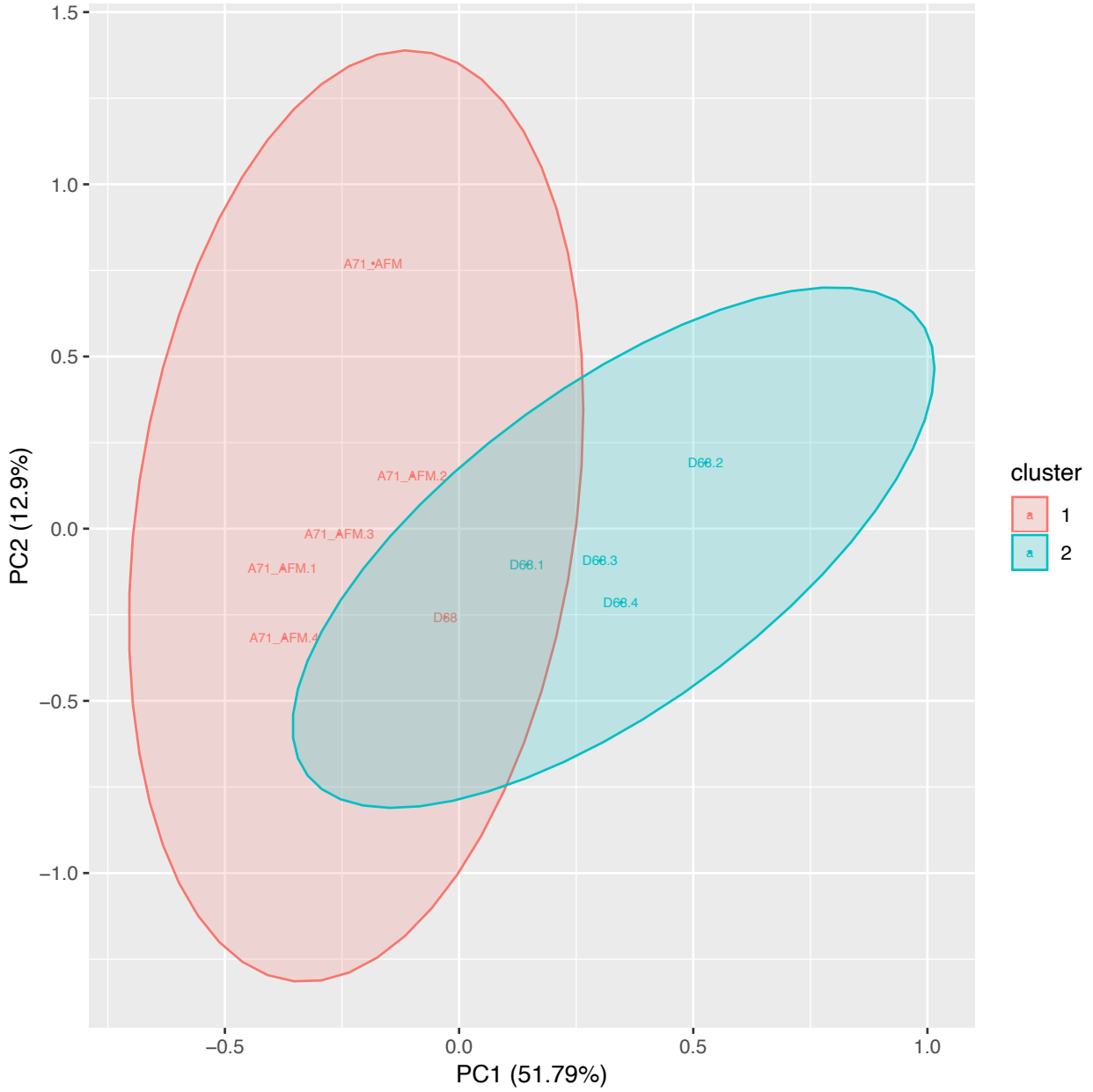
Innate immune response- activating signal transduction

A71 – D68 Classifier (training set)

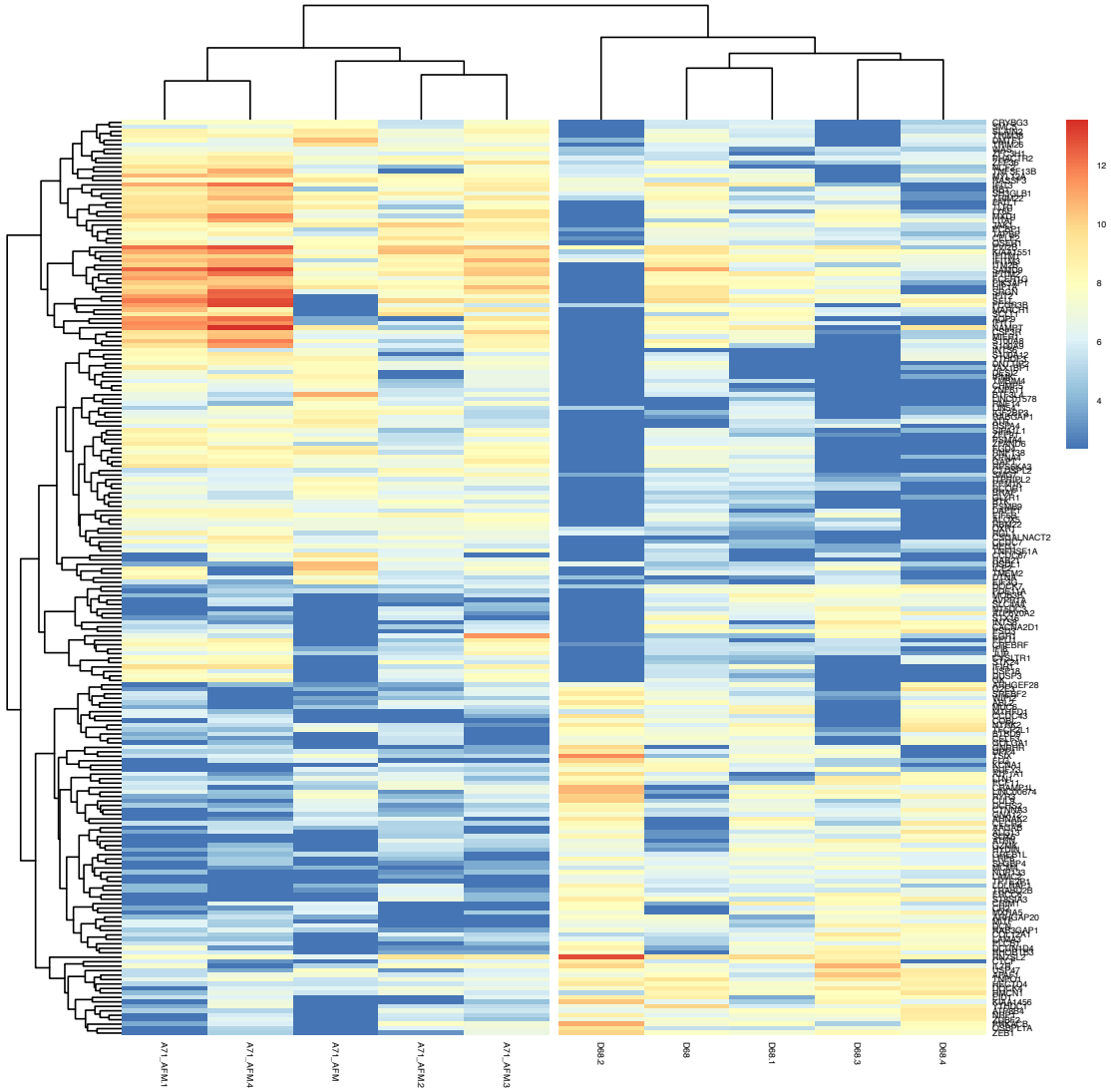
1 Missed call:

GY_AFP2014_15 (D68) -> A71

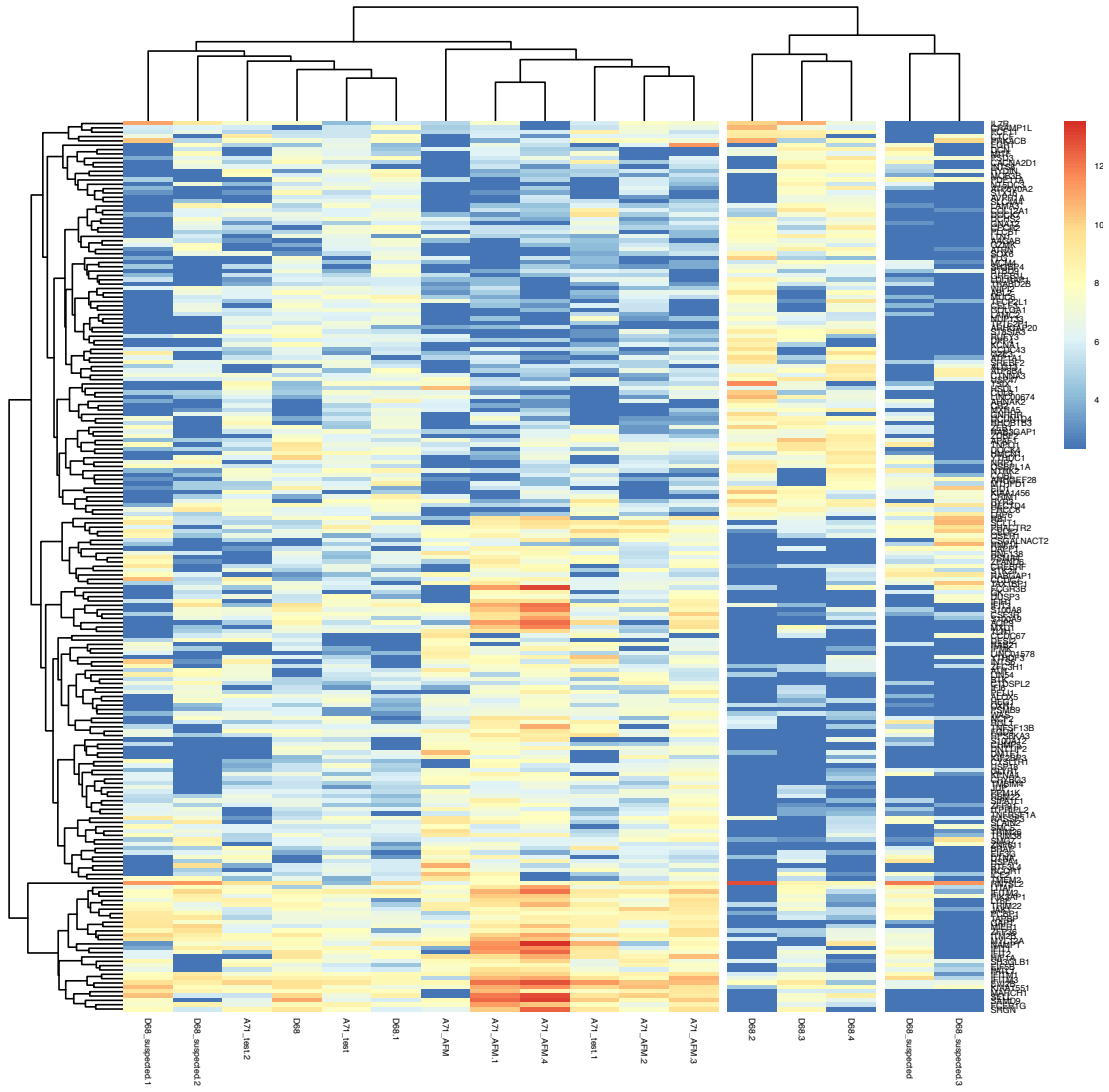
(90% accuracy)



A71 – D68 Classifier (training set)



A71 – D68 Classifier (test set)



A71 – D68 Classifier (test set)

Neural Net Model

D68: 3/4 = 75%

A71 AFM: 3/3

A71 Meningitis: 7/9 = 77.7%

Overall Accuracy: 13/16 = 81.25%

D68:	Predicted	Prob. D68	Prob. A71
GY_AFP2014_12	D68	0.8114	0.1886
GY_AFP2014_10	A71	0.2651	0.7349
GY_AFP2014_13	D68	0.5183	0.4817
GY_AFP2014_16	D68	0.8114	0.1886

A71:	Predicted	Prob. D68	Prob. A71
MNC_2194	A71	0.2651	0.7349
AMES162	A71	0.2651	0.7349
AMES152	A71	0.338	0.662

A71_meningitis:	Predicted	Prob. D68	Prob. A71
MNC-2846	D68	0.5221	0.4779
MNC-2899	A71	0.2651	0.7349
AMES145	A71	0.2651	0.7349
AMES149	A71	0.2651	0.7349
AMES150	D68	0.5192	0.4808
AMES151	A71	0.2651	0.7349
AMES155	A71	0.2651	0.7349
AMES156	A71	0.2651	0.7349
AMES157	A71	0.2651	0.7349

Conclusions

- **Diagnostic testing for AFM is challenging**
  - *diagnosis is currently based on a clinical case definition*
- **PCR testing alone is likely insufficient**
  - *Negative testing from CSF*
  - *Cannot discriminate rhinoviruses from enteroviruses*
  - *Difficult to routinely test early in the disease course*
- **Indirect testing methods are needed**
  - *CSF antibody testing and "host response" testing during the window period of infection*
- **Timely diagnosis is critically important**
  - *more aggressive interventions may be indicated if diagnosis of EV-D68 can be made earlier*

Looking at the host genes in AFM

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