Unbiased metagenomics for determining etiologies of idiopathic meningitis

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Meningitis Research Foundation Conference 2019
London, UK
06 Nov 2019

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CHRF
Prevent Infections, Save Lives

To Proma of Bangladesh

- 13-year old Proma was admitted with fever, vomiting, and headache
- She was eventually diagnosed with meningitis by *Mycobacterium tuberculosis*
- After almost 2 years, Proma remains bedridden with persistent neurocognitive impairment.

Child Health Research Foundation, Bangladesh

Child Health Research Foundation

CHRF *Prevent Infections, Save Lives*

"to improve child health in Bangladesh and around the world by facilitating appropriate policy decisions through research and advocacy"

Hib vaccine prevents deaths of 3,100 infants every year in Bangladesh

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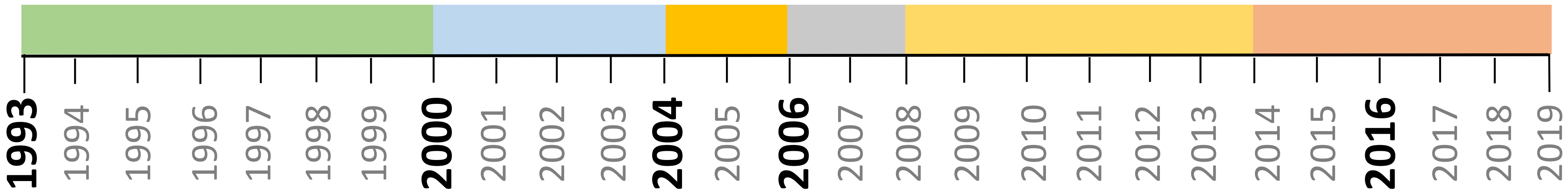
Meningitis surveillance in Bangladesh, 1993 - present

The objectives of our semi-national surveillance are:

- to describe epidemiology and estimate burden of disease
- to establish a platform to measure vaccine needs and impact
- to characterize circulating bacterial types – AMR, serotypes, etc
- to determine long-term outcome of meningitis patients

World Health Organization IB-VPD sentinel sites

Addition of diagnostic/surveillance methods to detect etiology



Culture

Latex
Nmen
Spn
Hib
GBS (<2 m)

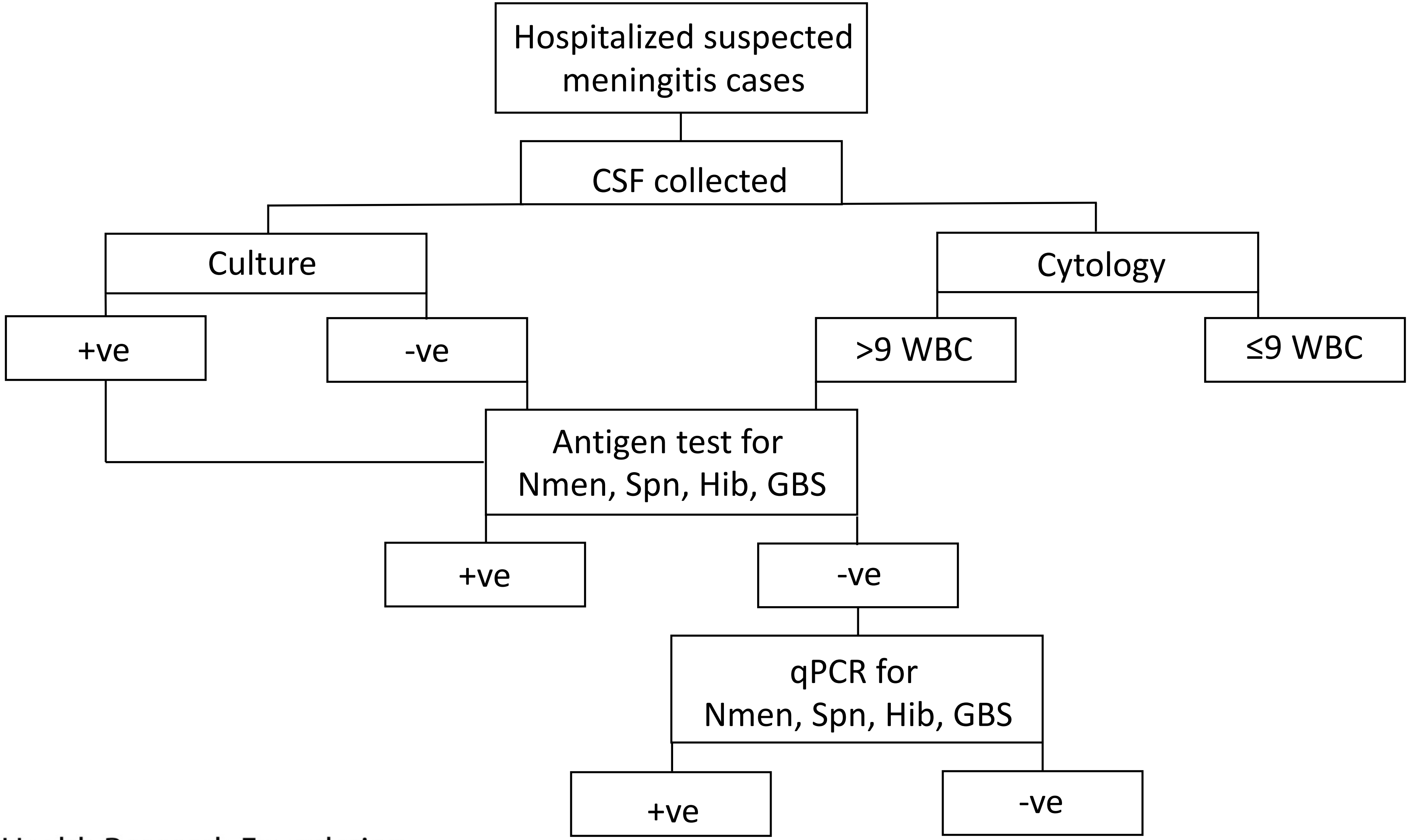
ICT/ BinaxNow
Spn

cPCR
Nmen
Spn
Hib
GBS (<2 m)

qPCR
Nmen
Spn
Hib
GBS (<2 m)

Spn: *Streptococcus pneumoniae*; Nmen: *Neisseria meningitidis*;
Hib: *Haemophilus influenzae* type b; GBS: Group B streptococcus

Meningitis surveillance in Bangladesh



Hospitalized suspected meningitis cases

CSF collected

Culture

+ve

-ve

Cytology

>9 WBC

≤9 WBC

Antigen test for Nmen, Spn, Hib, GBS

+ve

-ve

qPCR for Nmen, Spn, Hib, GBS

+ve

-ve

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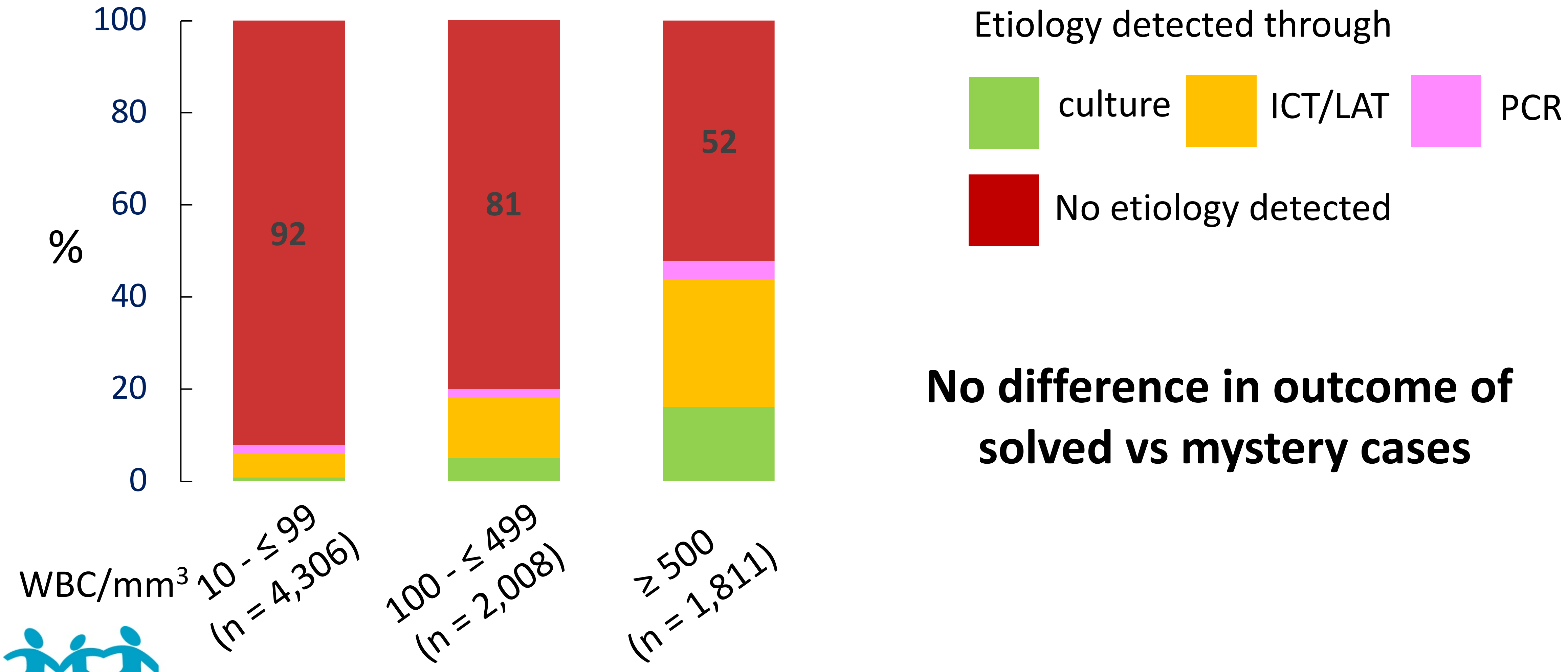
Etiology remains unknown for >50% of cases with ≥ 10 WBC/cmm (8,125 of 24,000, 2003 - 2016)



No difference in outcome of solved vs mystery cases

Lack of evidence hinders policy decisions

We hypothesized that **RNA metagenomics** could solve the mystery, but we had no evidence to show we can conduct sequencing on site.

Breaking the vicious cycle…

- The Gates Foundation introduced us to the Chan-Zuckerberg Biohub, who had the precise expertise on solving cases of mysterious meningitis and we had the samples!

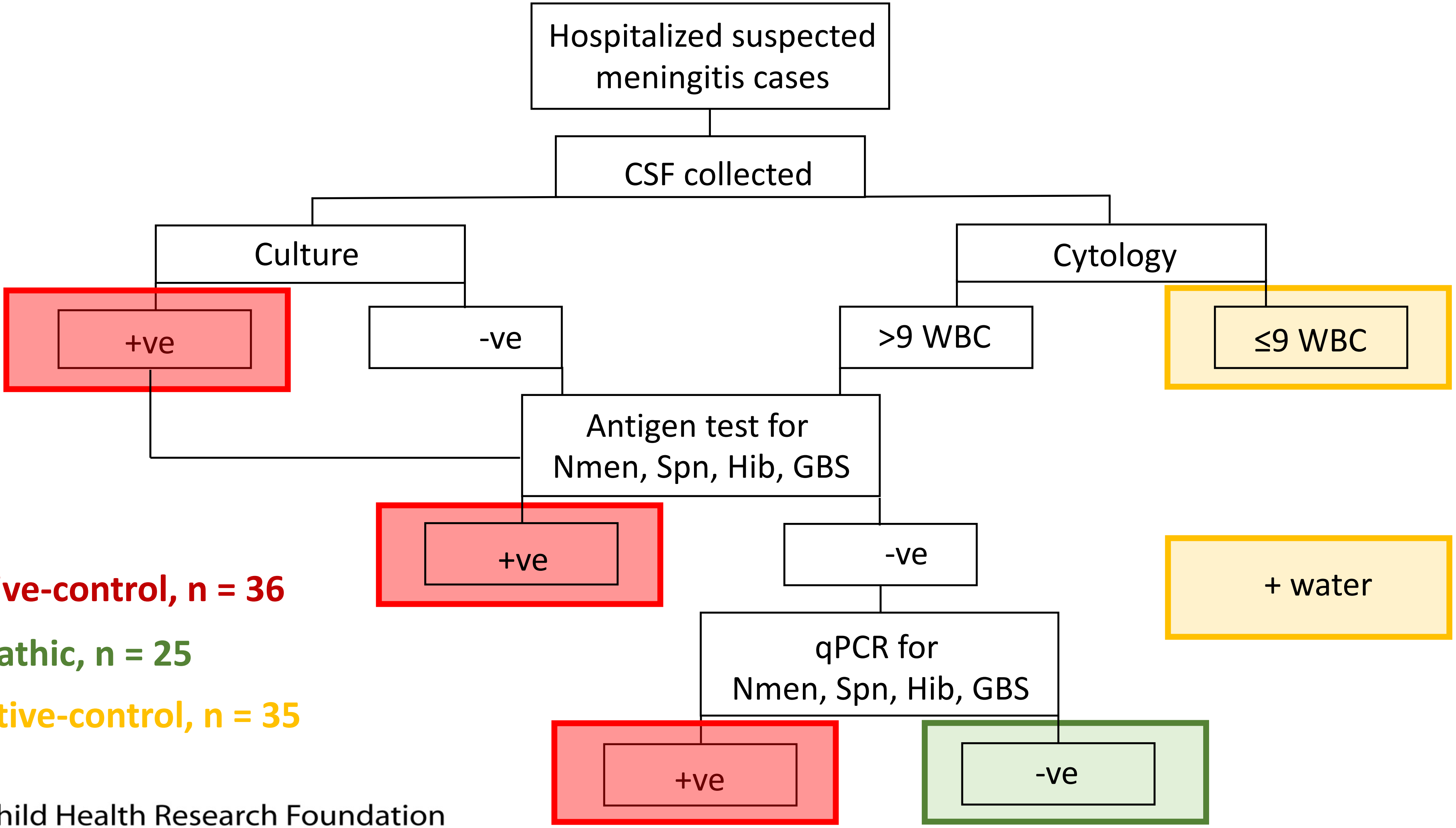
- Four important considerations:
  - **All SOPs need to be open access**
  - **Local scientist needs to be trained**
  - **All technologies/pipelines need to be transferrable and sustainable**
  - **We will own the data**

Dr. Farhad Imam, Gates Foundation
CHRF, Bangladesh; Feb 15, 2018

Elucidating the causative agents of meningitis using metagenomics: Phase I

96 samples and a Senjuti were shipped to the Biohub

Sample selection for Phase I



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Antigen test for Nmen, Spn, Hib, GBS

+ve

-ve

+ water

qPCR for Nmen, Spn, Hib, GBS

+ve

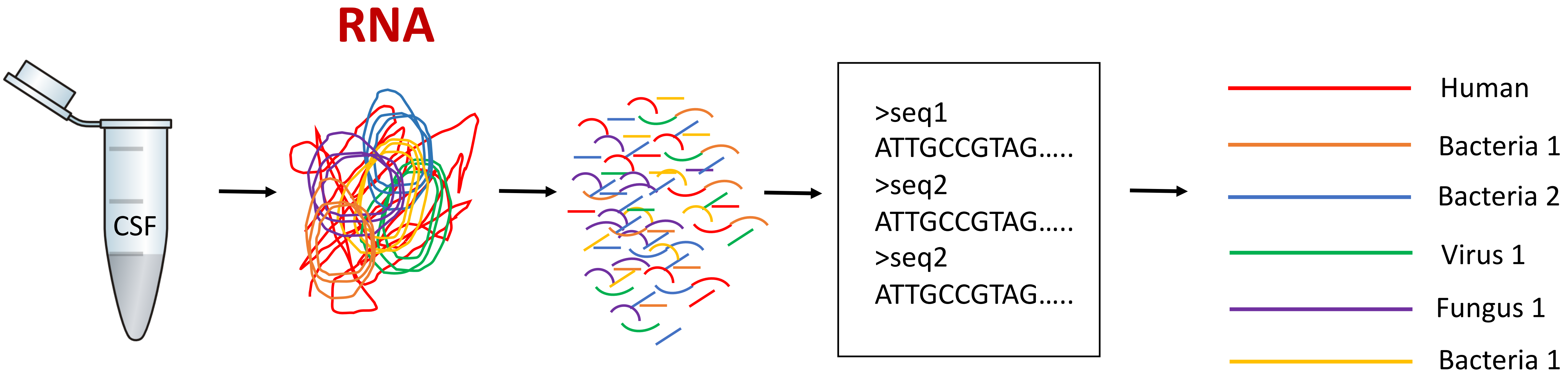
-ve

Positive-control, n = 36

Idiopathic, n = 25

Negative-control, n = 35

RNA metagenomics of CSF specimens



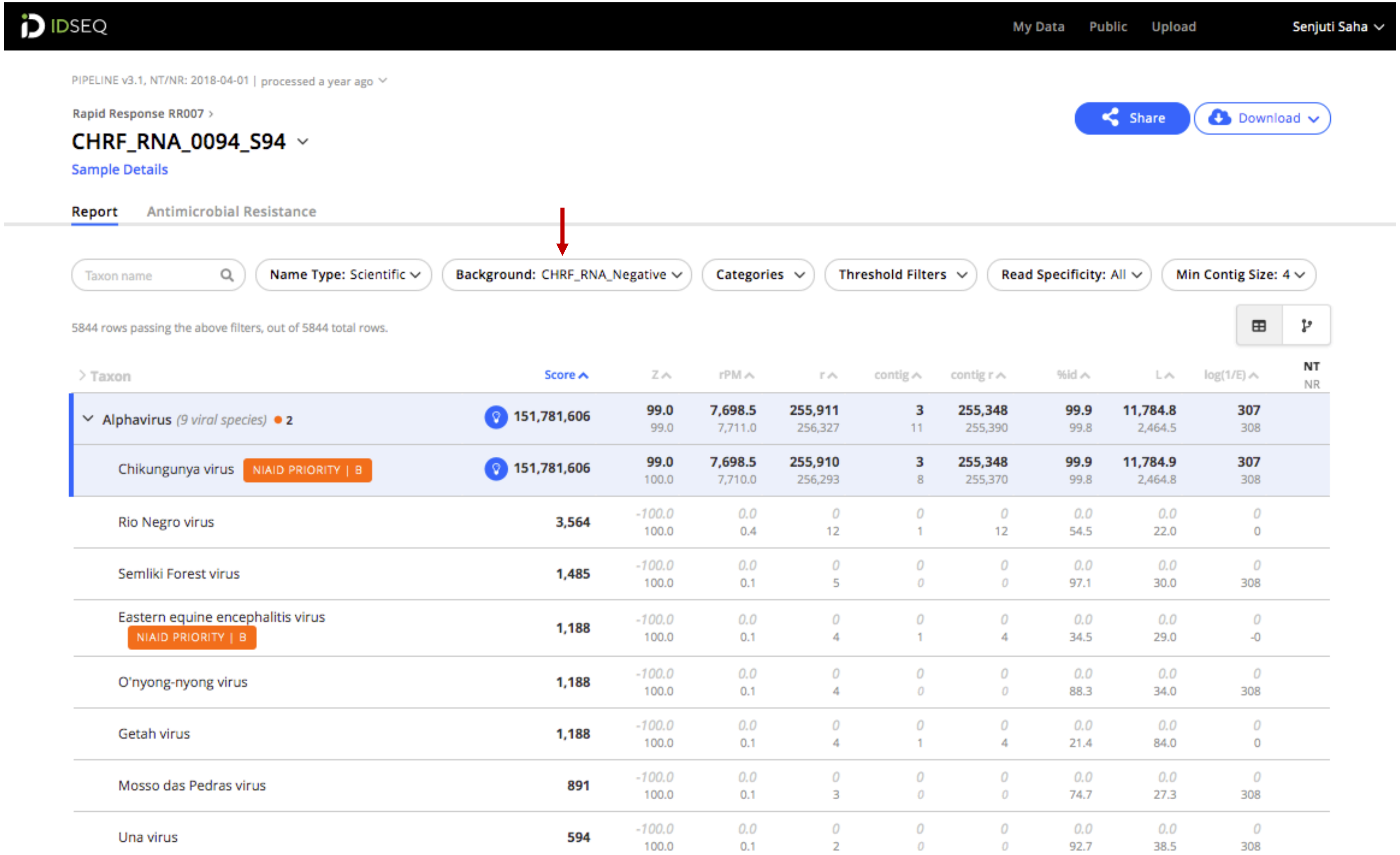
```
>seq1
ATTGCCGTAG.....
>seq2
ATTGCCGTAG.....
>seq2
ATTGCCGTAG.....
```

🟥	Human
🟧	Bacteria 1
🟦	Bacteria 2
🟩	Virus 1
🟪	Fungus 1
🟨	Bacteria 1

Extraction → Library preparation → Sequencing → Analysis

IDSEQ

Analysis: IDSeq



PIPELINE v3.1, NT/NR: 2018-04-01 | processed a year ago

Rapid Response RR007 ›

CHRF_RNA_0094_S94

Sample Details

Name Type: Scientific

Background: CHRF_RNA_Negative

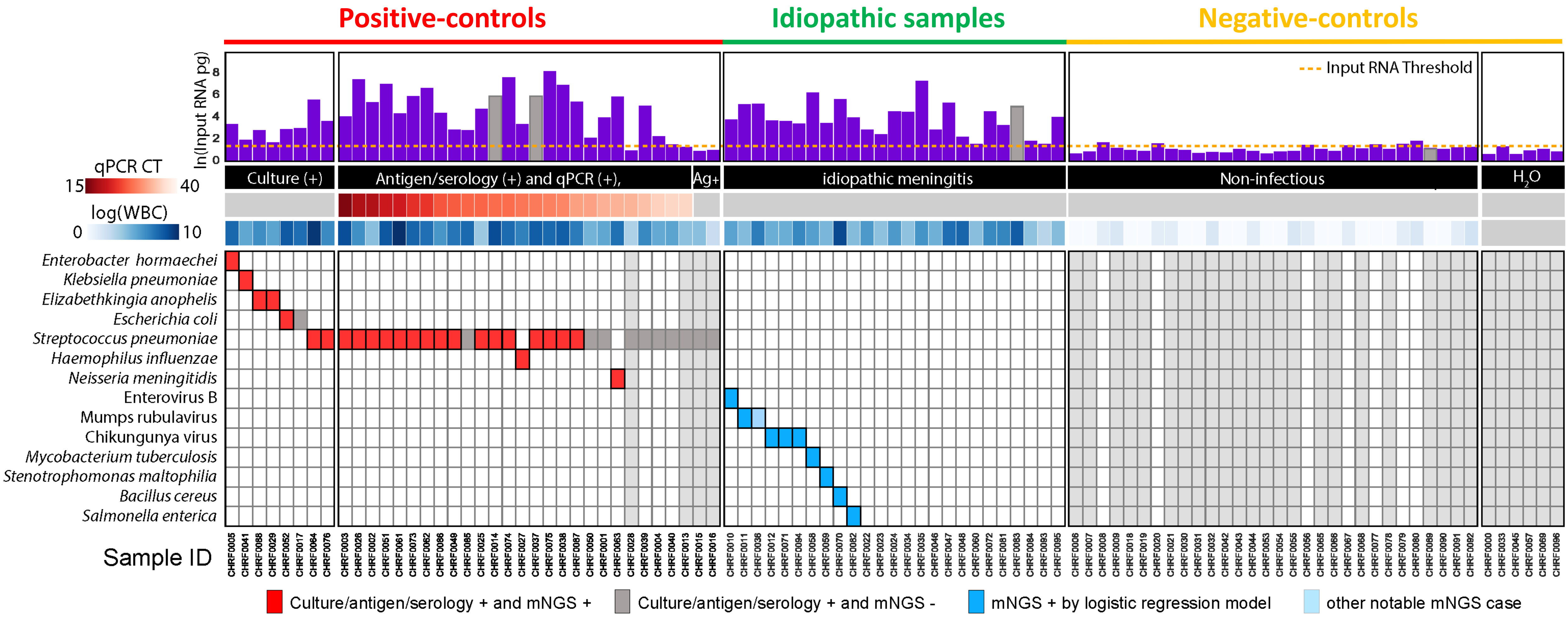
Read Specificity: All

Min Contig Size: 4

5844 rows passing the above filters, out of 5844 total rows.

Taxon	Score	Z	rPM	r	contig	contig r	%id	L	log(1/E)	NT NR
Alphavirus *(9 viral species)* ● 2	151,781,606	99.0 99.0	7,698.5 7,711.0	255,911 256,327	3 11	255,348 255,390	99.9 99.8	11,784.8 2,464.5	307 308	
Chikungunya virus NIAID PRIORITY | B	151,781,606	99.0 100.0	7,698.5 7,710.0	255,910 256,293	3 8	255,348 255,370	99.9 99.8	11,784.9 2,464.8	307 308	
Rio Negro virus	3,564	-100.0 100.0	0.0 0.4	0 12	0 1	0 12	0.0 54.5	0.0 22.0	0 0	
Semliki Forest virus	1,485	-100.0 100.0	0.0 0.1	0 5	0 0	0 0	0.0 97.1	0.0 30.0	0 308	
Eastern equine encephalitis virus NIAID PRIORITY | B	1,188	-100.0 100.0	0.0 0.1	0 4	0 1	0 4	0.0 34.5	0.0 29.0	0 -0	
O'nyong-nyong virus	1,188	-100.0 100.0	0.0 0.1	0 4	0 0	0 0	0.0 88.3	0.0 34.0	0 308	
Getah virus	1,188	-100.0 100.0	0.0 0.1	0 4	0 1	0 4	0.0 21.4	0.0 84.0	0 0	
Mosso das Pedras virus	891	-100.0 100.0	0.0 0.1	0 3	0 0	0 0	0.0 74.7	0.0 27.3	0 308	
Una virus	594	-100.0 100.0	0.0 0.1	0 2	0 0	0 0	0.0 92.7	0.0 38.5	0 308	

We found a diverse range of pathogens



Saha et al, 2019, Biorxiv

Clinical follow-up of 10 resolved mystery cases

Suspect etiology	Age of child (m) at diagnosis / follow-up	Hospital duration (days)	Treatment	Clinical outcome
Mumps	156 / 168	10	Ceftriaxone	Parents complain of lower IQ
Mumps	18 / 30	9	Ceftriaxone	Healthy
Enterovirus B	7 / 12	6	Ceftriaxone	Cannot eat solid food
Bacillus cereus	0 / 5	28	Ceftriaxone+	Needed vp shunt
M. tuberculosis	160 / 164	15	Ceftriaxone+	Bed-ridden; unresponsive to all stimuli
S. maltophilia	4 / NA	28	Ceftriaxone+	Not known
Salmonella enterica	4 / 8	23	Ceftriaxone+	Cannot sit yet
Chikungunya	0 / 5	10	Ceftriaxone+	Healthy
Chikungunya	1 / 13	6	Ceftriaxone+	Healthy
Chikungunya	86 / 86	45	Ceftriaxone+	Died

Saha et al, 2019, Biorxiv

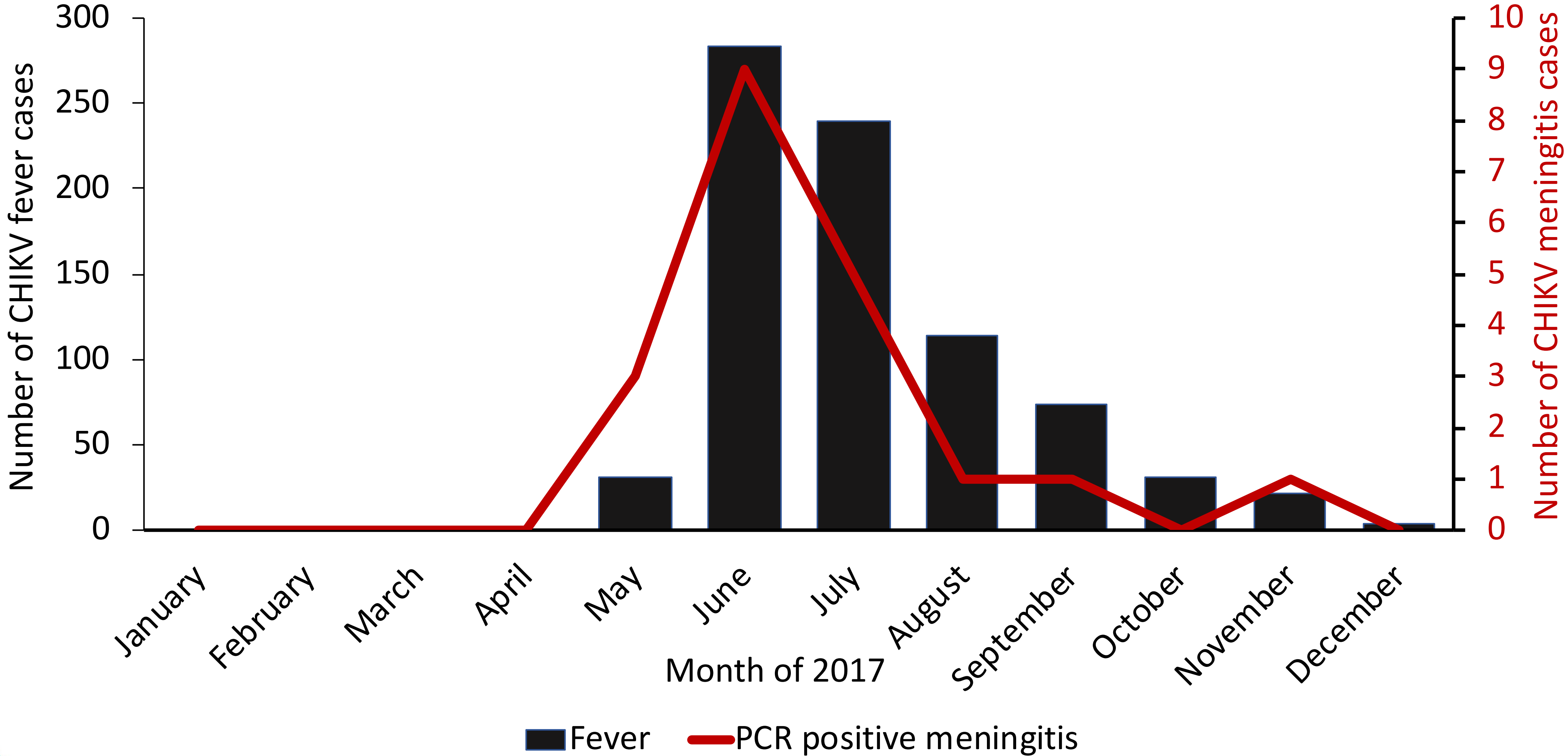
We optimized a quick qPCR method to test CSF for CHIKV

Month 2017	Total CSF tested	CHIKV positive	% CHIKV positive
January	31	0	0
February	23	0	0
March	41	0	0
April	36	0	0
May	56	3	5
June	50	9	18
July	42	5	12
August	48	1	2
September	40	1	3
October	38	0	0
November	42	1	2
December	31	0	0
Total	**478**	**20**	**4**

12%
CHIKV-positive

Saha et al, 2019, Biorxiv

CHIKV meningitis cases overlapped with CHIKV fever outbreak



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Saha et al, 2019, Biorxiv

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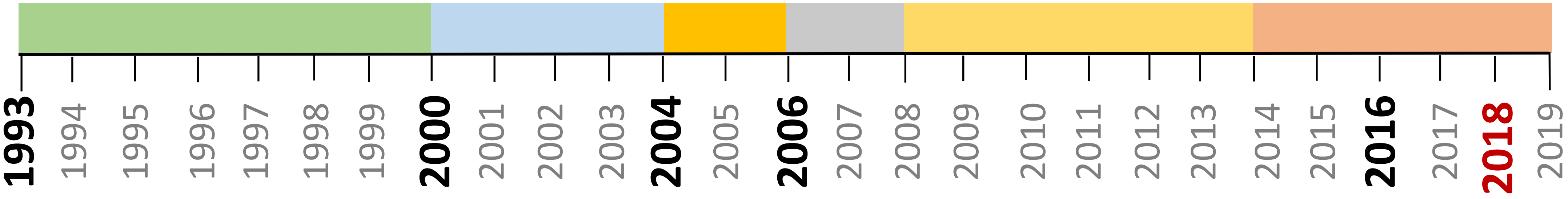

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↑
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CHIKV

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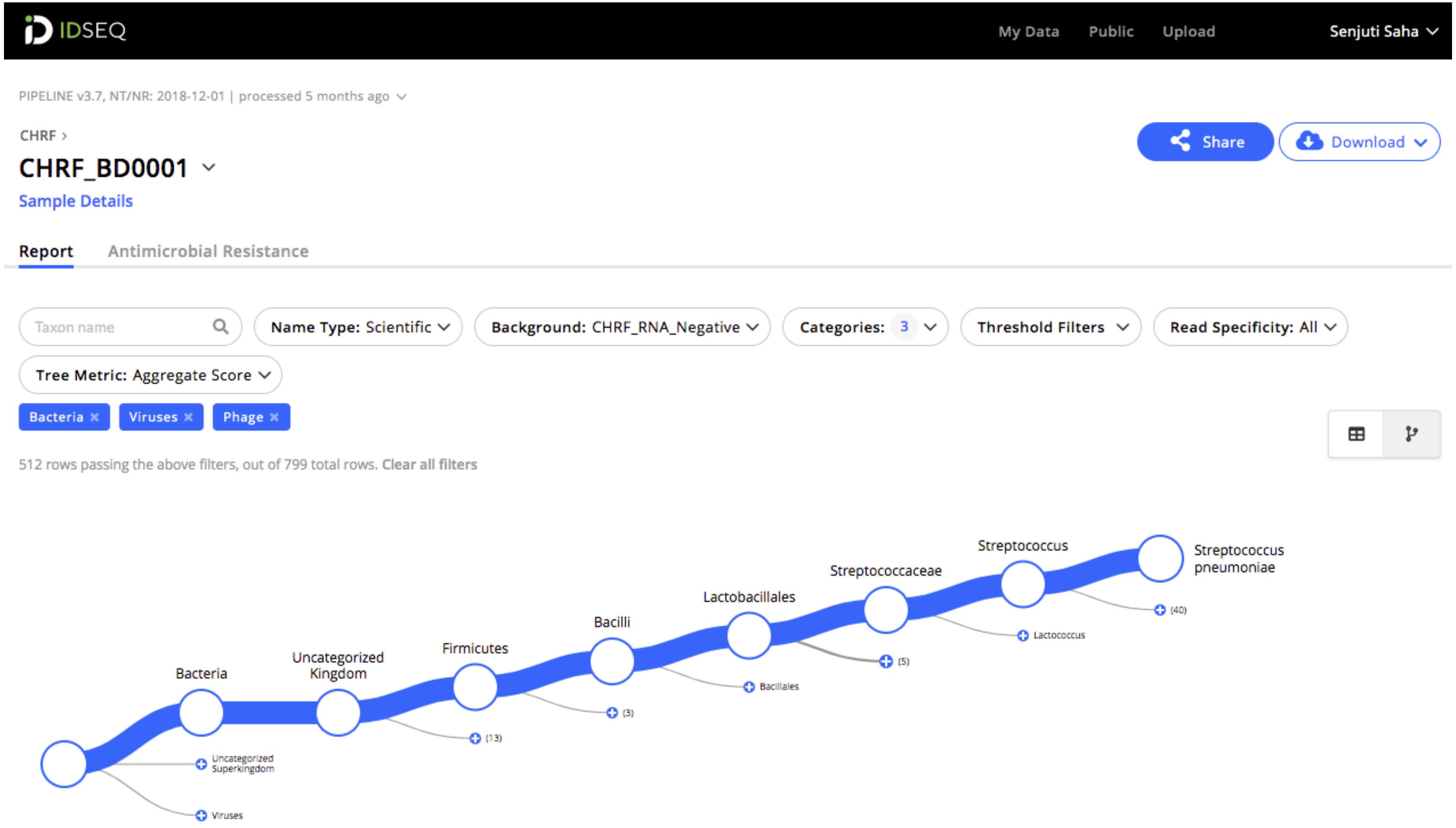
Elucidating the causative agents of meningitis using metagenomics: Phase II

We were funded to get our own sequencer!

Phase II: Received a grant for an iSeq100 Global Sequencer



PIPELINE v3.7, NT/NR: 2018-12-01 | processed 5 months ago

CHRF >

CHRF_BD0001

Name Type: Scientific

Background: CHRF_RNA_Negative

Categories: 3

Threshold Filters

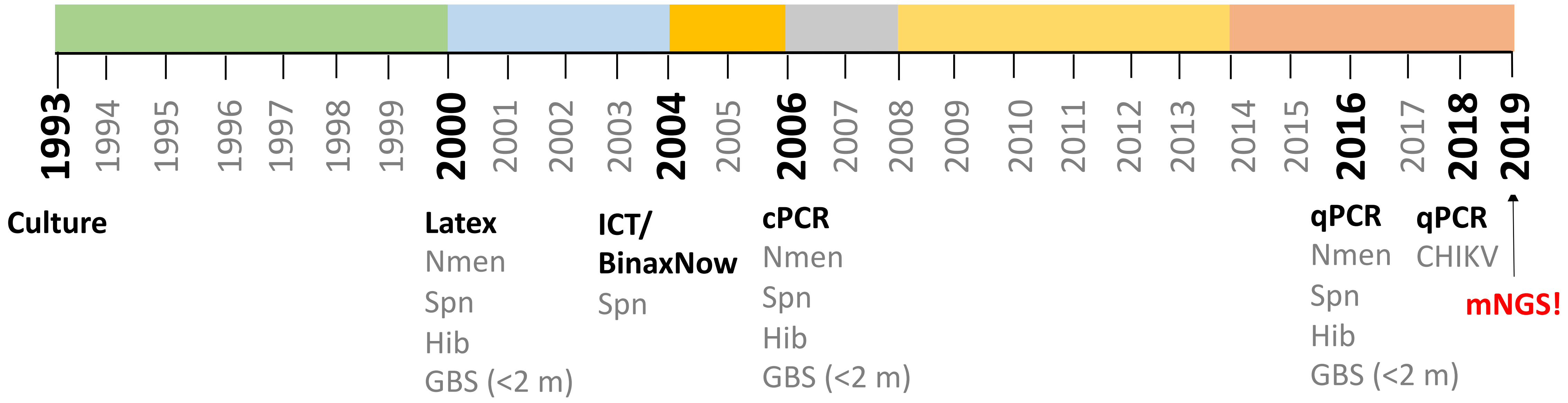
Read Specificity: All

Tree Metric: Aggregate Score

512 rows passing the above filters, out of 799 total rows.

Phase II: Received a grant for an iSeq100 Global Sequencer

Addition of diagnostic/surveillance methods to detect etiology



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CHIKV

mNGS!

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Empowering, educating, engaging local scientists at the frontlines of public health

Thank you

CHAN ZUCKERBERG BIOHUB

Chan Zuckerberg Initiative

BILL & MELINDA GATES foundation

CHRF