Diagnostica di laboratorio avanzata nelle malattie neuro-infettive acute

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Controversie in neurologia d'emergenza e urgenza

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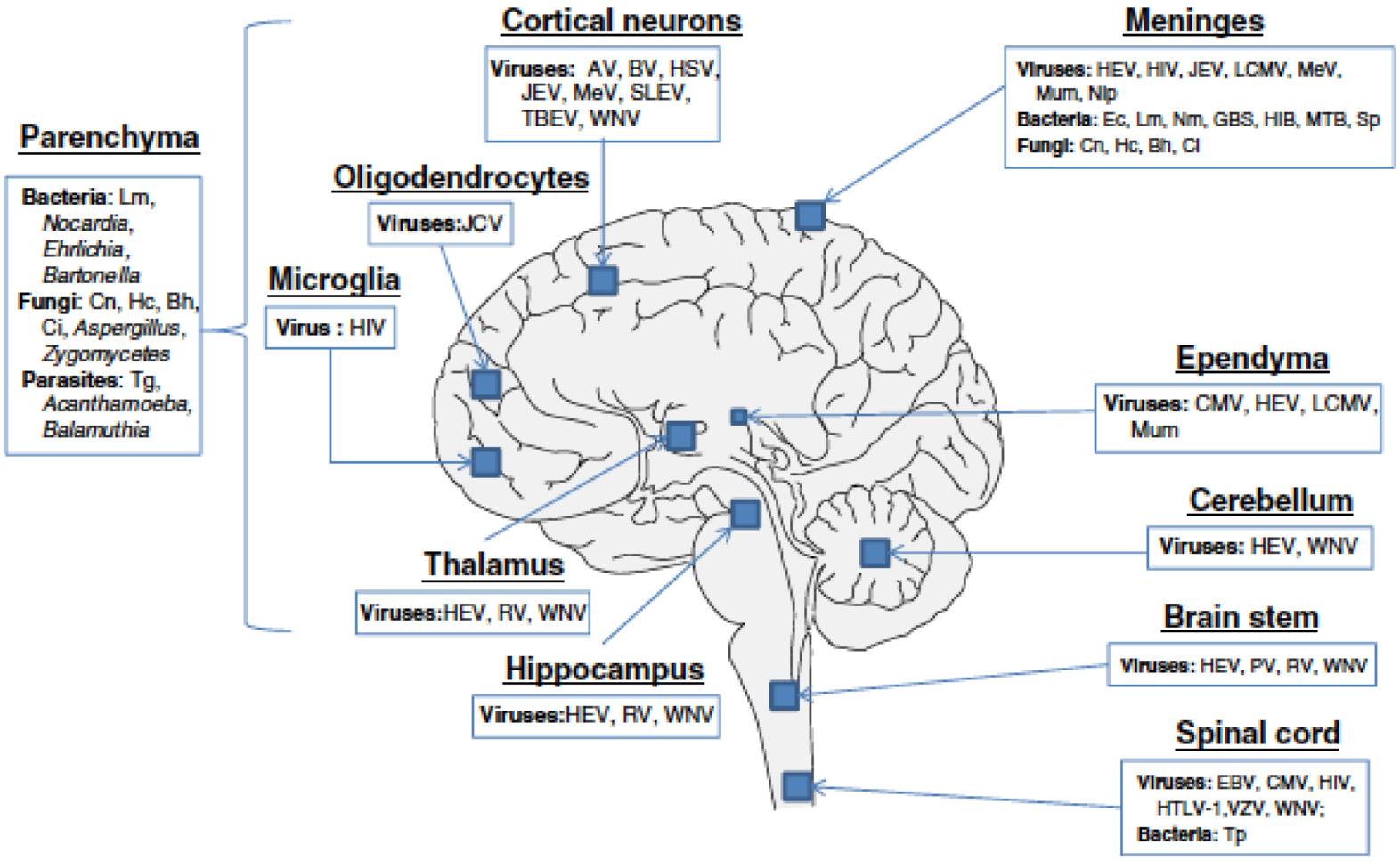
CNS infections



He T et al., Curr Infect Dis Rep (2016) 18: 35

The laboratory diagnostic landscape in acute CNS infections

- CSF standard exam

- CSF microscopy
- CSF (and blood) culture
- CSF antigen detection
- **CSF molecular assays: nucleic acid amplification (e.g., PCR) and sequencing**

- Antibody detection in CSF and plasma (IgG, IgM)
- Intrathecal ab synthesis (CSF and plasma)

- CSF biomarkers (inflammation, BBB and neuronal damage)

CSF molecular assays for diagnosis of acute CNS infections in 2022

Achievements

- Fully entered the microbiology diagnostic labs (especially viral diagnostics): accurate, timely, cost-effective
- Several commercial assays available

Challenges

- Nonviral microbial diagnostics relatively slow to adopt molecular technology
- Standardization (e.g., quantitative PCR results)

→ **Still clinical needs not fully met**

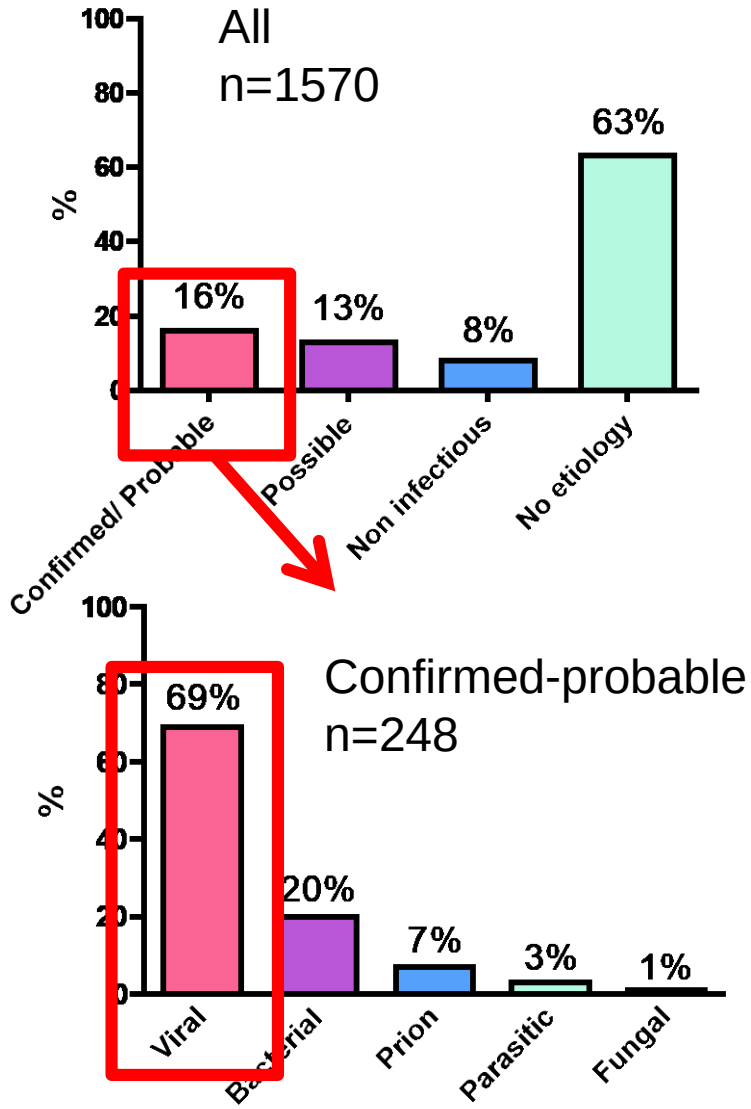
Main diagnostic challenges from a clinical point of view

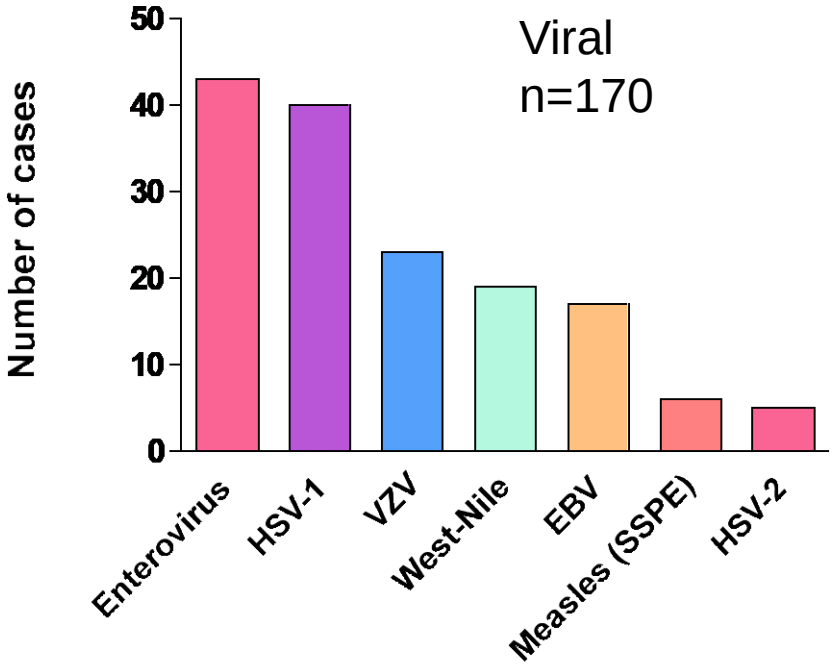
Most cases of acute CNS infections are probably undiagnosed (more viral than bacterial)

- Uncommon or emerging infections with CNS involvement (e.g., WNV, Covid19, MPX)
- Low assay sensitivity (e.g., ongoing antibiotics, culture ‘hostile’ pathogens)

Most cases of encephalitis go undiagnosed

California Encephalitis Project, 1998 – 2005





Glaser et al., CID 2006

‘Broader’ molecular diagnostic approaches

- **Multiplex PCR**
- Broad-range PCR and sequencing (amplification through universal primers of one or several regions of 16s, 18s, ITS*)
- **Metagenomics Next generation sequencing**

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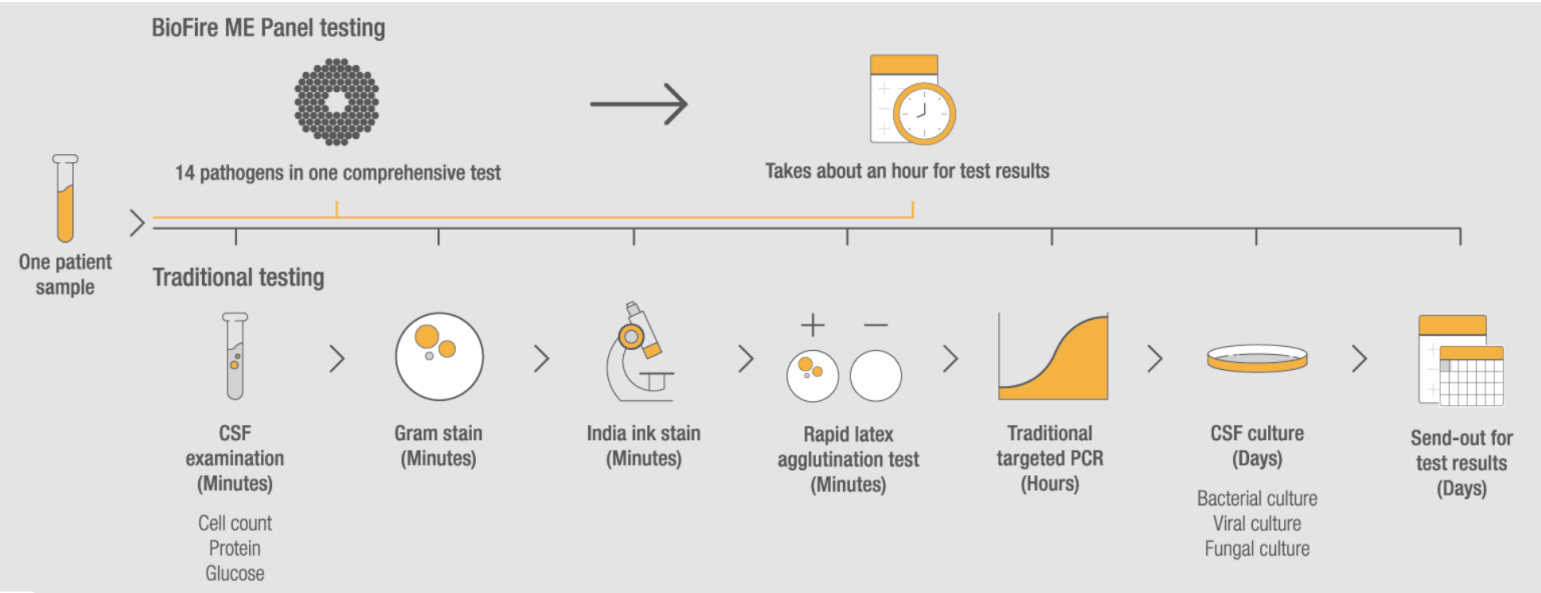
The FilmArray meningitis/encephalitis panel (FA/ME)

- Multiplex PCR that in one reaction amplifies DNA fragments from 14 different CNS pathogens
- Increase diagnostic yield, impact animicrobial treatment, shorten time to diagnosis and hospital stay
- Turn-around time (about 1 hour)



Viruses

- Cytomegalovirus (CMV)
- Enterovirus
- Herpes simplex virus 1 (HSV-1)
- Herpes simplex virus 2 (HSV-2)
- Human herpes virus 6 (HHV-6)
- Human parechovirus
- Varicella zoster virus (VZV)

Bacteria

- *Escherichia coli K1*
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

Yeast

- *Cryptococcus neoformans/gattii*

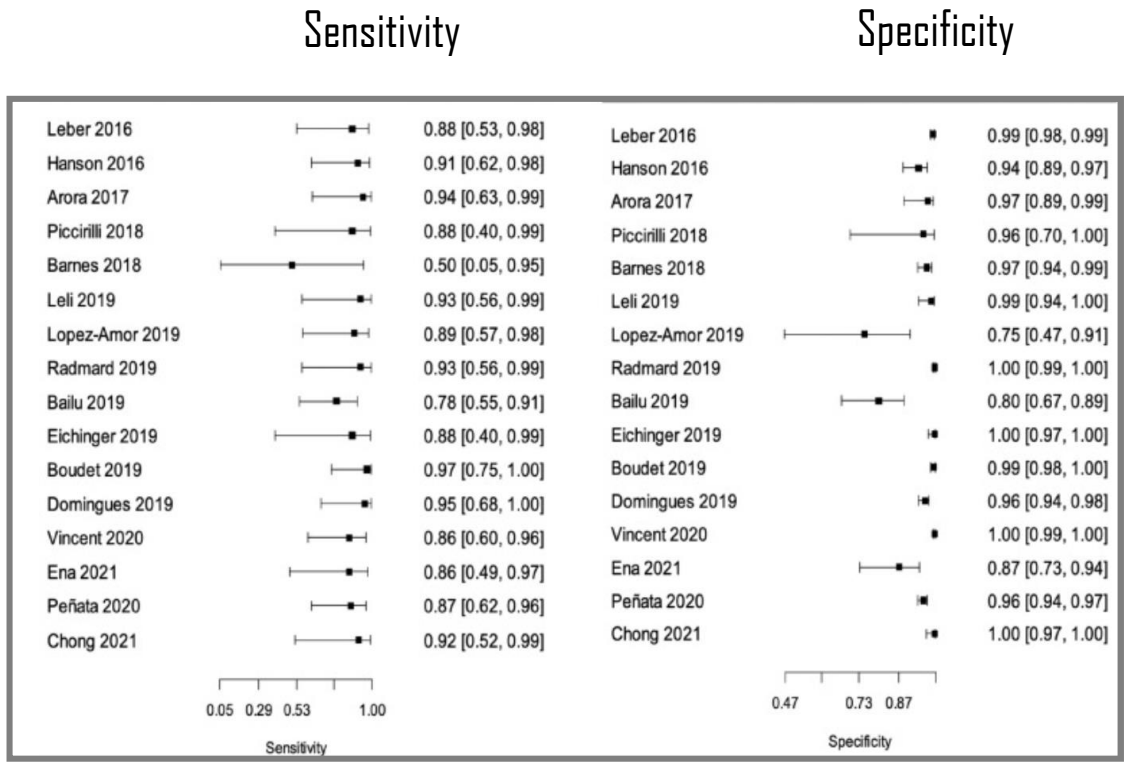
Overall: 94.2% sensitivity and 99.8% specificity

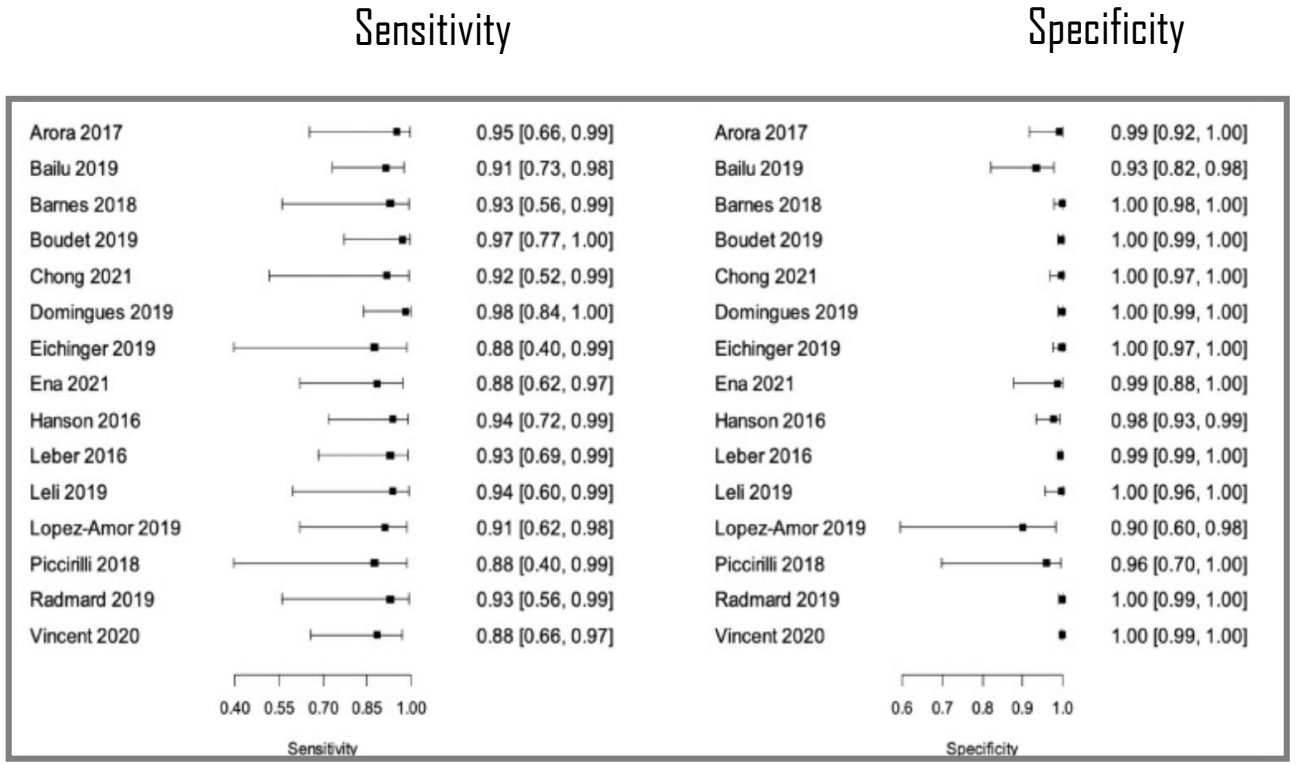
https://www.biofi redx.com/products/the-fi lmarray-panels/fi lmarrayme/

The FilmArray meningitis/encephalitis panel: sensitivitiy and specificity

Meta-analysis of 19 studies (11,351 participants). *Trujillo-Gómez J et al., EClinicalMedicine. 2022)*

Diagnostic value for bacteria





Ref srd 1: CSF or blood culture
Sensitivity 89.5% (95%CI 81.1–94.4)
Specificity 97.4% (95%CI 94–98.9).

Ref srd 2: final dx adjudication based on clinical/laboratory criteria
Sensitivity 92.1% (95%CI 86.8–95.3)
Specificity 99.2 % (95%CI 98.3–99.6)

The FilmArray meningitis/encephalitis panel: sensitivitiy and specificity

Bacteria:
Reference srd 1 (16 studies/6183 patients): sensitivity 89.5% specificity 97.4%
Reference srd 2 (15 studies/5,524 patients): sensitivity 92.1%, specificity 99.2 %

Viruses:
HSV-2, enteroviruses, VZV: sensitivities 75.5 - 93.8%, specificities > 99%

→ Acceptable-to-high sensitivities and high specificities for identifying bacteria, especially *S.pneumoniae*, and viruses, especially HSV-2 and enteroviruses.

→ Sensitivities for *L.monocytogenes, H. influenzae, E.coli*, and HSV-1 suboptimal.

Trujillo-Gómez J et al., EClinicalMedicine. 202

CSF Omics…?

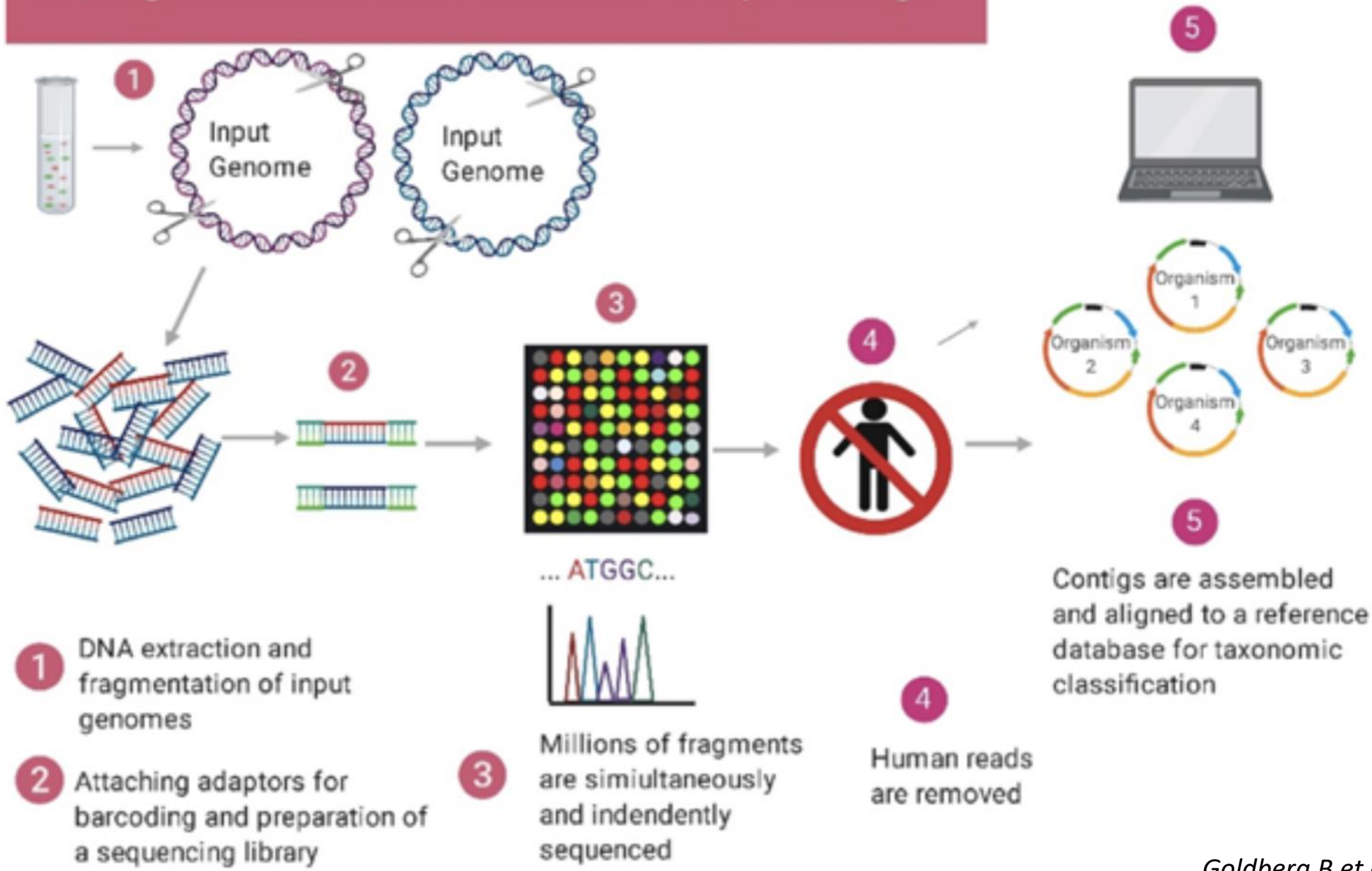
- Genomics
- Lipidomics
- Proteomics
- Foodomics
- Transcriptomics
- Metabolomics
- …
- **Metagenomics**

Metagenomics Next Generation Sequencing (mNGS)

Metagenomics: Analyzing a mixture of microbial genomes, a metagenome, without separating the genomes or culturing the organisms

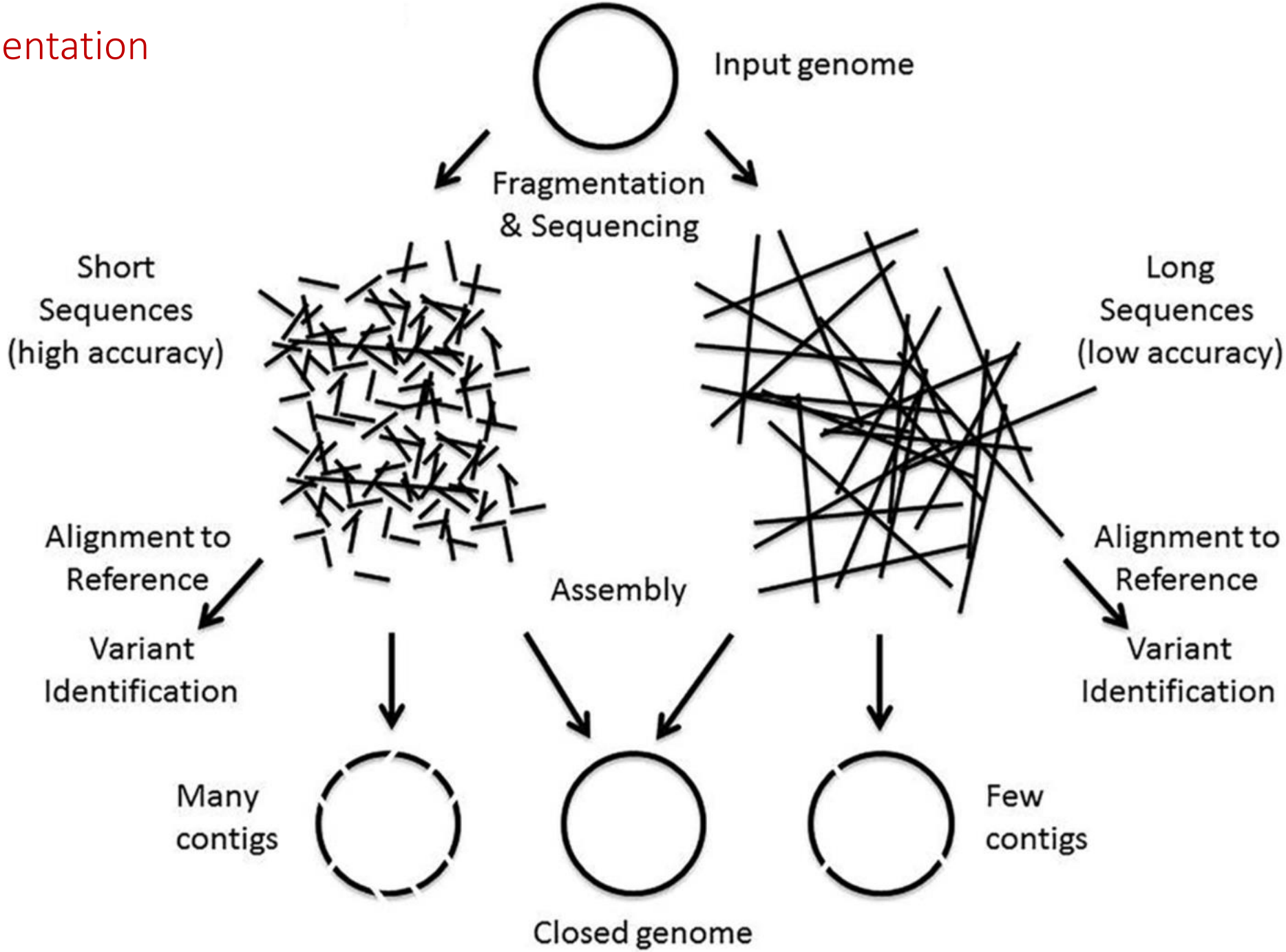
Next-generation sequencing (NGS): A collection of DNA sequencing methods that each use different biochemical approaches and instruments to produce data 1) in vastly larger amounts, 2) at greatly lower cost, 3) in shorter time, and 4) with less manual intervention than previous methods

Metagenomic Next-Generation Sequencing



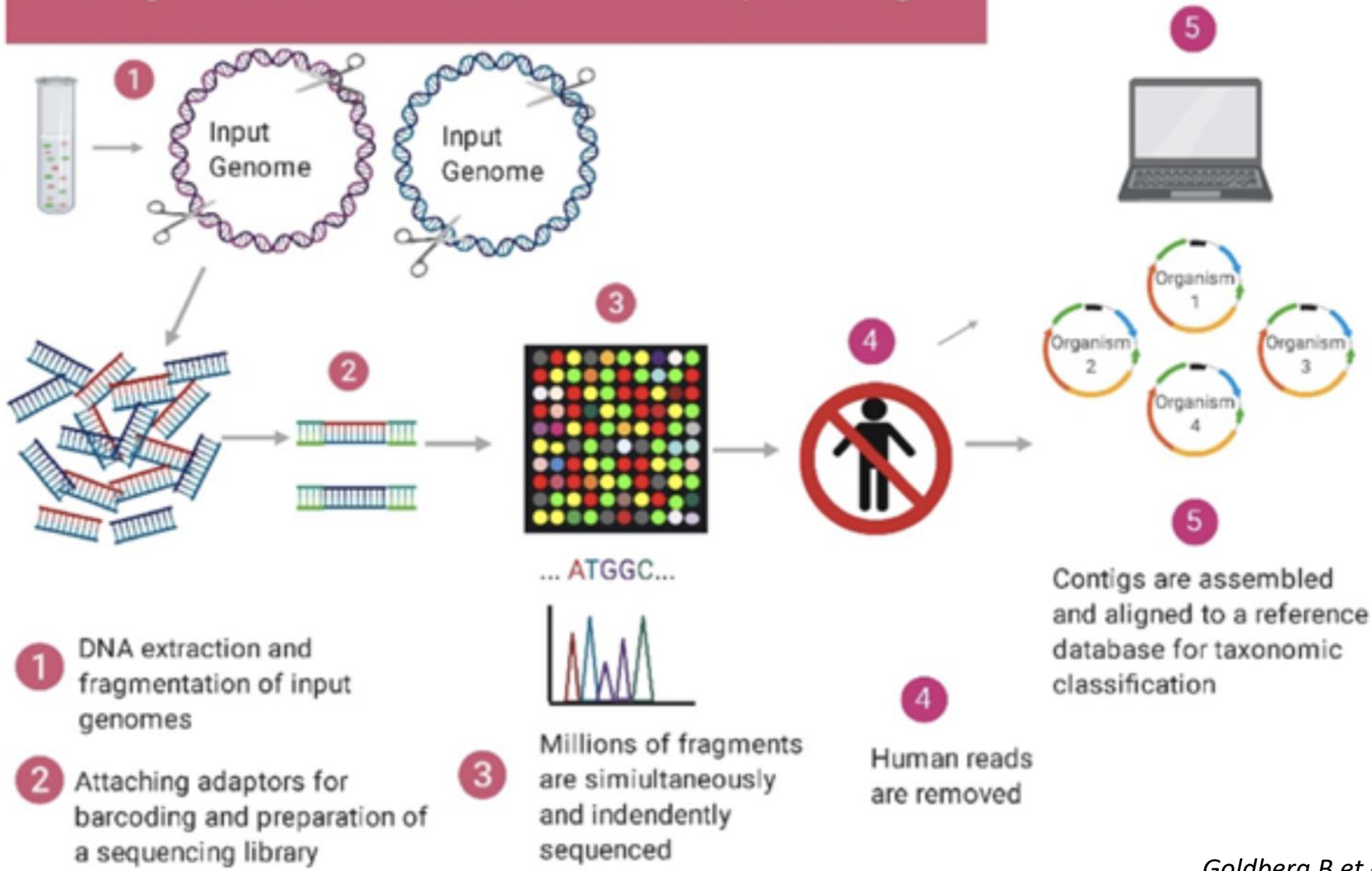
Goldberg B et al., mBio 2015

1 Genome fragmentation



Goldberg B et al., mBio 2015

Metagenomic Next-Generation Sequencing



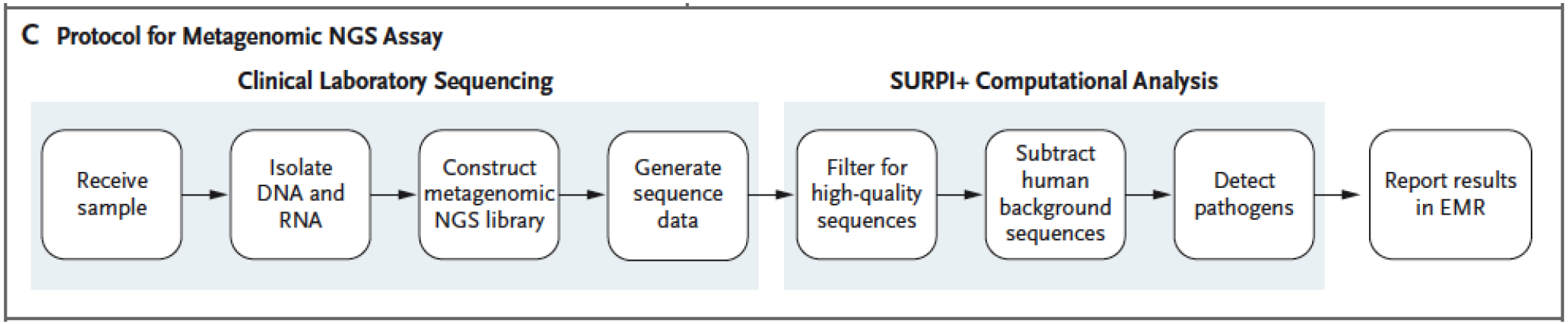
Goldberg B et al., mBio 2015

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

- 1-year, multicenter, prospective study
- 204 patients
  - Mean age 39.6 years; 46 (22.5%) <18 years; 55.9% M
  - Admitted to ICU: 48.5%
  - 30-day mortality: 11.3%

- Meningitis: 70 pts (34.3%)
- Encephalitis: 130 pts (63.7%)
- Myelitis: 4 pts (2.0%)

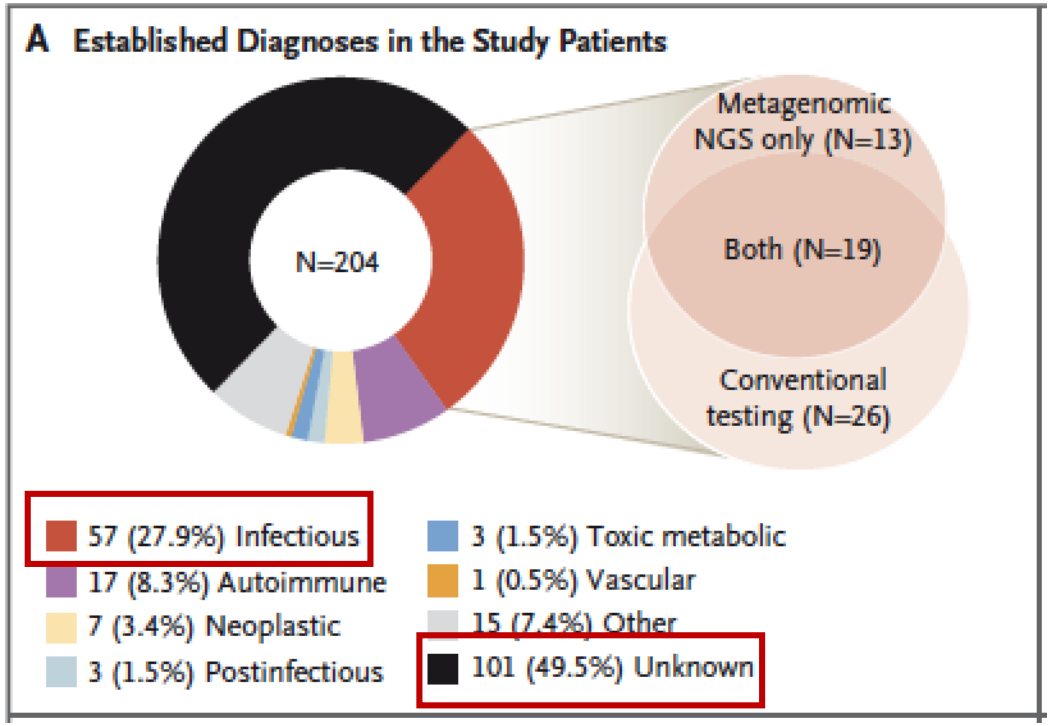
- Acute: 176 pts (86.3%)
- Acute exacerbation of a chronic condition: 28 pts (13.7%)



Mean turn-around time: 90 hours

Wilson MR et al., NEJM 2019

Infections Diagnosed by Metagenomic NGS



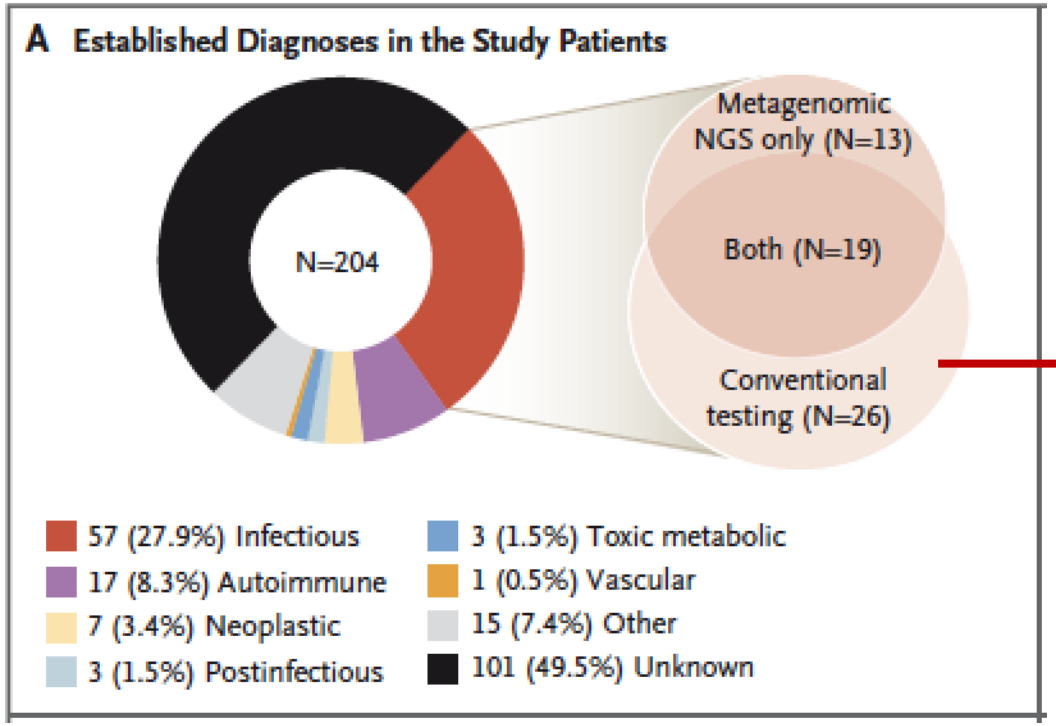
Etiologic diagnosis in 103 of 204 patients (50.5%)

	Concurrent diagnosis by mNGS and conventional microbiologic testing (n=19)
1	*Angiostrongylus cantonensis*
2	*Angiostrongylus cantonensis*
3	Coxsackievirus B5
4	*Cryptococcus neoformans*
5	*Cryptococcus neoformans*
6	*Cryptococcus neoformans*
7	EBV (encephalitis)
8	EBV (PTLD-associated)
9	Echovirus 11
10	HHV-6
11	HHV-6
12	HIV-1 (encephalopathy)
13	HIV-1 (HIV escape)
14	HSV-1
15	HSV-2
16	JC polyomavirus
17	VZV
18	VZV
19	VZV

	Diagnosis by mNGS only (n= 13)
1	*Candida tropicalis*
2	EBV (lymphoma-associated)
3	Echovirus 6
4	Echovirus 30
5	*Enterobacter aerogenes*
6	*Enterococcus faecalis*
7	HEV
8	MW polyomavirus
9	*Neisseria meningitidis*
10	*Nocardia farcinica*
11	St Louis Encephalitis Virus (SLEV)
12	*Streptococcus agalactiae*
13	*Streptococcus mitis*

Wilson MR et al., NEJM 2019

Infections **NOT** Diagnosed by Metagenomic NGS



Etiologic diagnosis in 103 of 204 patients (50.5%)

mNGS 'false negative cases (n=26)

- 11 cases diagnosed by **serologic testing alone**, for which conventional CSF tests were also negative (including WNV, VZV, neurosyphilis)
- 7 cases cases diagnosed from **samples other than CSF** (e.g., brain biopsy)
- 8 cases with **low pathogen titers in CSF**, as by conventional microbiologic tests that were borderline + or had discordant results (including infections from *M. bovis*, *M. tuberculosis*, *C. neoformans*, *P. acnes*, fusobacterium, *S. aureus*, CMV, HSV-2)
- *Samples for mNGS frequently obtained later than those examined at first by conventionsl CSF testing*

Wilson MR et al., NEJM 2019

Results of Metagenomic NGS Testing and Clinical Effect

E Clinical Effect (13 cases diagnosed by metagenomic NGS only)

- 7 (54%) Enabled appropriate and targeted treatment
  - *N. farcinica* — long-term treatment with oral moxifloxacin and minocycline
  - *Candida tropicalis* — treatment with high-dose fluconazole and liposomal amphotericin B (started empirically for elevated 1,3-β-D-glucan level)
  - HEV — successful treatment with IV ribavirin after patient was readmitted with liver failure and consideration of liver transplantation
  - *E. aerogenes* — narrowing of antibiotic therapy to IV cefepime and oral trimethoprim–sulfamethoxazole
  - *Enterococcus faecalis* — narrowing of antibiotic therapy to IV vancomycin; discontinuation of meropenem
  - *S. mitis* — narrowing of antibiotic therapy to IV cefepime; continuation of antibiotics for 4 wk to treat CNS infection
  - *S. agalactiae* — treatment with an additional 4 wk of therapy with IV ceftriaxone and vancomycin
- 1 (8%) Helped to rule out coinfections; enabled patient to proceed with chemotherapy (EBV-associated lymphoma)
- 1 (8%) Supported clinical decisions to narrow coverage (neisseria)
- 2 (15%) Had no effect, because patient already discharged from hospital (enterovirus)
- 1 (8%) Had no effect, because clinical significance unclear (MW polyomavirus)
- 1 (8%) Provided reassurance to patient or surrogate (SLEV)

Wilson MR et al., NEJM 2019

Supplementary mNGS Analyses

C Supplementary Metagenomic NGS Analyses (15 cases discussed during CMSB meetings)

- 5, Viral genotyping (SLEV, HEV, enteroviruses [3 cases])
- 1, Analysis of antibiotic-resistance genes (*Enterobacter aerogenes*)
- 2, Prediction of resistance to antiviral drugs (HIV-1)
- 3, Detection and tracking of new or rare infectious agents (MW polyomavirus, *Angiostrongylus cantonensis* [2 cases])
- 2, Detection of pathogen reads below reporting threshold (*Mycobacterium tuberculosis* complex, astrovirus MLB1)
- 2, Accurate species identification (*Nocardia farcinica*, *Streptococcus mitis*)

Wilson MR et al., NEJM 2019

Advanced laboratory diagnosis of acute CNS infections: wrap-up

Past and present: hypothesis-driven diagnostics

Future (mNGS): additional potential of unbiased hypothesis-free diagnostics

Real-life study: the highest diagnostic yield from a combination of CSF mNGS and conventional testing!

mNGS strength: ability to detect many microorganisms directly

mNGS weakness:

- Differently from culture (that indicates the presence of living organisms), the presence of microbial DNA is less definitive (DNA 'contamination' or 'innocent bystander' effect)
- Feasibility and costs still an issue

mNGS of CSF is a potential step forward in the diagnosis of acute CNS infections

- Identify unexpected or hard-to-diagnose or emerging CNS infections
- Clinical effect: guide earlier and more targeted treatments for CNS infections
- Supplementary analyses: characterise disease phenotypes for clinical and epidemiological purposes
- Accelerate workup and treatment for noninfectious causes

Back-up

	Diagnosis by metagenomic NGS only (13 infections)	Orthogonal Confirmatory Testing
1	Candida tropicalis	Serum and CSF 1,3-β-D-glucan (+); CSF culture (−) CSF fungal 28S rRNA and ITS PCR assay, C. tropicalis (+) (UW)
2	EBV (lymphoma-associated)	CSF qPCR assay for EBV (+), 700 IU/ml (Viracor)
3	Echovirus 6	CSF RT-PCR assay for EV (+), confirmed by Sanger sequencing
4	Echovirus 30	CSF RT-PCR assay for EV (+), confirmed by Sanger sequencing
5	Enterobacter aerogenes	CSF bacterial culture (−); CSF bacterial 16S rRNA PCR assay (−) (UW) CSF PCR assay for E. aerogenes (renamed Klebsiella aerogenes) (+), confirmed by Sanger sequencing
6	Enterococcus faecalis	CSF bacterial culture (−); FilmArray Meningitis/Encephalitis panel (−) CSF bacterial 16S rRNA PCR assay (−) (UW); **brain biopsy**, E. faecalis by culture (+)
7	HEV	CSF IgM assay for HEV (+); CSF IgG assay for HEV (−); CSF RT-PCR assay for HEV (+), 5.96 million copies/ml
8	MW polyomavirus	CSF PCR assay for MW polyomavirus (+), confirmed by Sanger sequencing
9	Neisseria meningitidis	CSF Gram's stain, gram-negative diplococci (+); CSF bacterial culture (−); CSF N. meningitidis antigen assay (−); FilmArray Meningitis/Encephalitis panel (−) (UCLA) Neisseria, probably not N. meningitidis, by CSF metagenomic NGS and phylogenetic analysis
10	Nocardia farcinica	CSF bacterial culture (−); CSF bacterial 16S rRNA PCR assay (−) (UW) CSF PCR assay for N. farcinica (+), confirmed by Sanger sequencing
11	SLEV	CSF RT-PCR assay for SLEV (+) (CDC)
12	Streptococcus agalactiae	CSF bacterial culture (−) FilmArray Meningitis/Encephalitis panel, S. agalactiae (+) (BioFire) (SJCRH) CSF bacterial 16S rRNA PCR assay, S. mitis group (+) (UW)
13	S. mitis	CSF bacterial culture (−); FilmArray Meningitis/Encephalitis panel (−)

	Concurrent diagnosis by metagenomic NGS and conventional microbiologic testing (19 infections)	Relevant Clinical Microbiologic Test
1	Angiostrongylus cantonensis	CSF PCR assay for A. cantonensis (+) (CDC)
2	A. cantonensis	CSF PCR assay for A. cantonensis (+) (CDC)
3	Coxsackievirus B5†	CSF RT-PCR assay for EV (+), 800 copies/ml
4	Cryptococcus neoformans	CSF culture for C. neoformans (+); CSF cryptococcal antigen assay (+), 1:640
5	C. neoformans	CSF culture for C. neoformans (+); FilmArray Meningitis/Encephalitis; panel, C. neoformans (+)
6	C. neoformans	CSF culture for C. neoformans (+)
7	EBV (encephalitis)‡	CSF PCR assay for EBV (+), 2000 copies/ml
8	EBV (PTLD-associated)	CSF qPCR assay for EBV (+), <50 IU/ml (Viracor)
9	Echovirus 11†	FilmArray Meningitis/Encephalitis panel, EV (+) (BioFire)
10	HHV-6	FilmArray Meningitis/Encephalitis panel, HHV-6 (+) (BioFire)
11	HHV-6	CSF PCR assay for HHV-6 (+), 536,000 copies/ml (Viracor)
12	HIV-1 (encephalopathy)	CSF PCR assay for HIV-1 (+), 6900 copies/ml (ARUP Laboratories)
13	HIV-1 (HIV escape)	CSF PCR assay for HIV-1 (+), 36,000 copies/ml (ARUP Laboratories)
14	HSV-1	CSF PCR assay for HSV-1 (+) by Simplexa (UCSF)
15	HSV-2	CSF PCR assay for HSV-2 (+), 166,000 copies/ml (Viracor)
16	JC polyomavirus	CSF PCR assay for JC polyomavirus (+), 162,000 copies/ml (Viracor)
17	VZV	FilmArray Meningitis/Encephalitis panel, VZV (+) (BioFire)
18	VZV	FilmArray Meningitis/Encephalitis panel, VZV (+) (BioFire)
19	VZV	CSF PCR assay for VZV (+), (BioFire)

Supplementary Metagenomic NGS Analyses

A. Prediction of antiviral drug resistance (HIV)

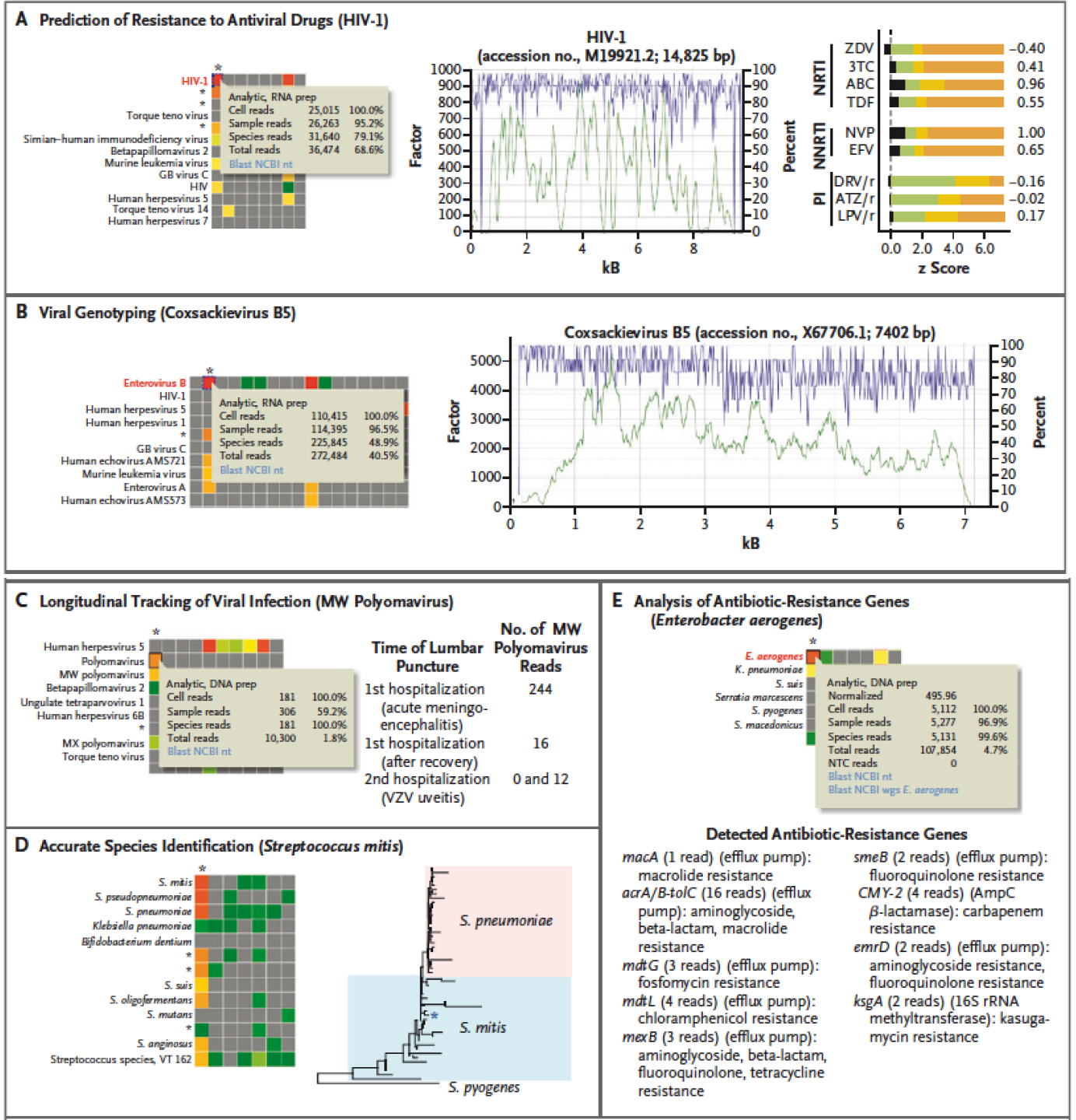
B. Viral genotyping (EV)

C. Longitudinal tracking of viral infection (MW polyomavirus)

D. Accurate specimen identification (*Streptococcus mitis*)

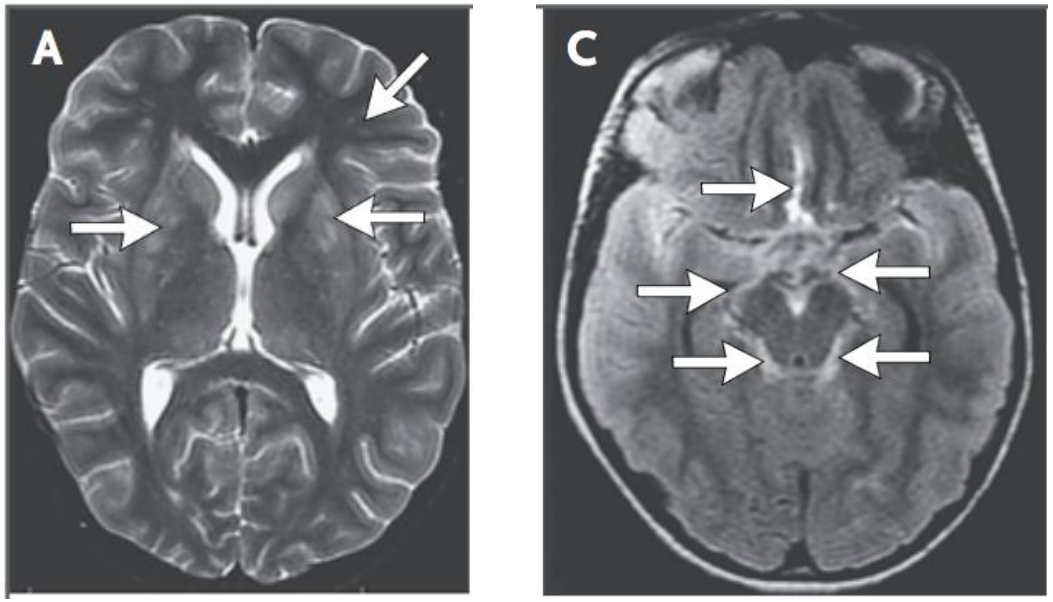
E. Analysis of antibiotic resistant genes (*Enterobacter aerogenes)*

Wilson MR et al., NEJM 2019

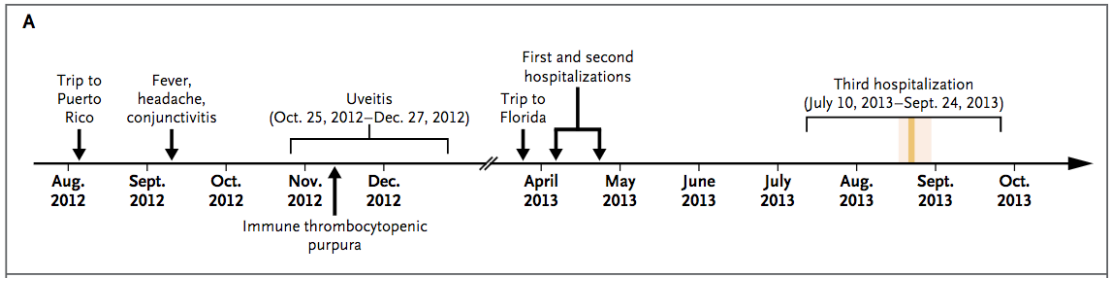


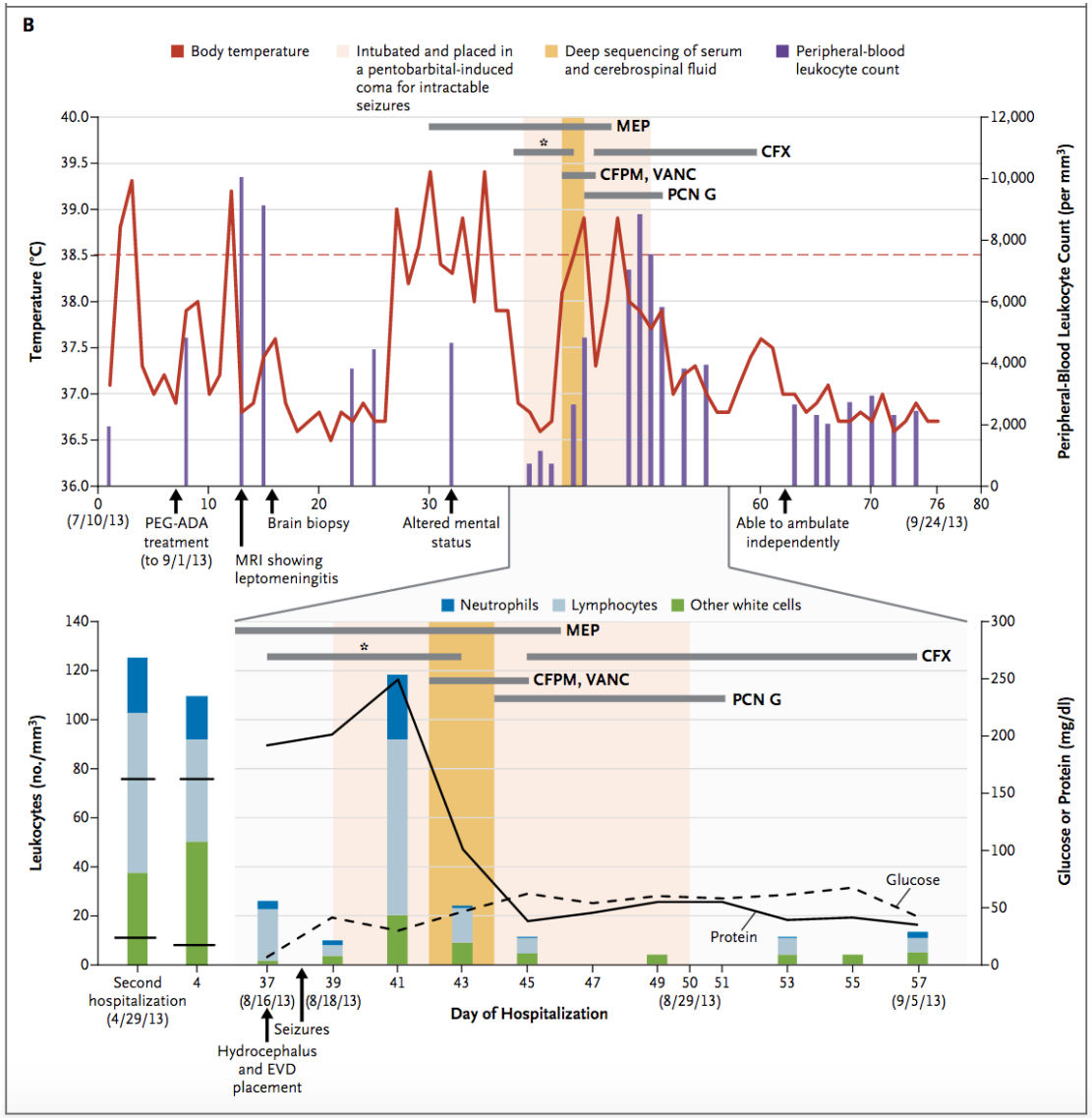
Diagnosis of CNS Infection by CSF Next-Generation Sequencing (NGS) analysis

A 14-year-old boy with SCID and partial immune reconstitution after two haploidentical bone marrow transplantations



Wilson et al., NEJM 2014





Diagnosis of Leptospira Infection by CSF Next-Generation Sequencing (NGS) analysis

Nucleic acid extraction from 750 µL of CSF

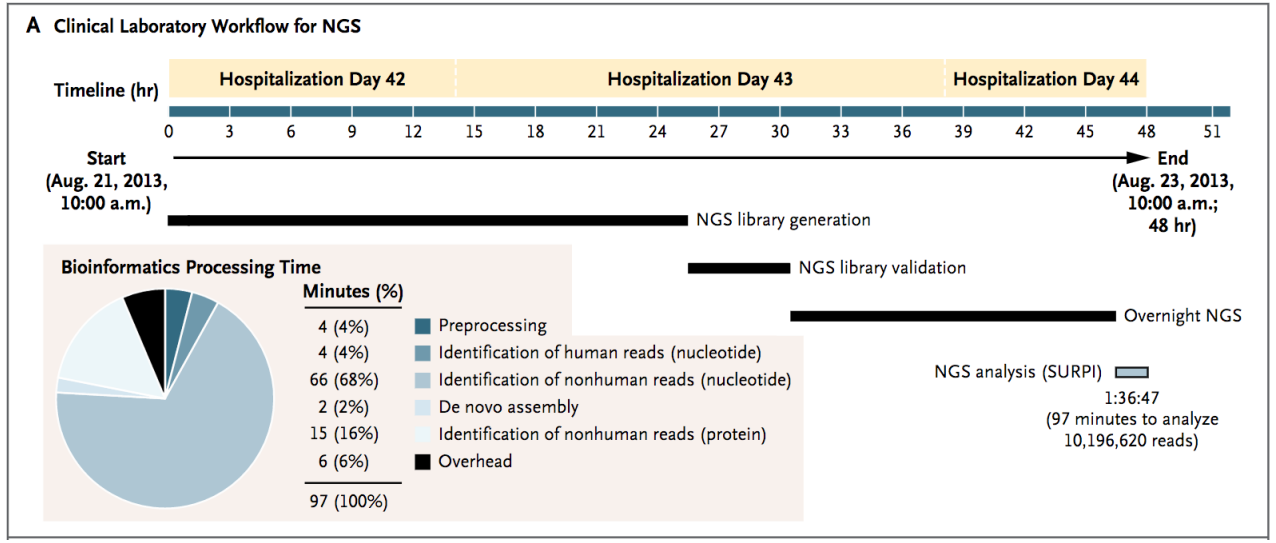
8,187,737 reads derived from patient's serum and CSF

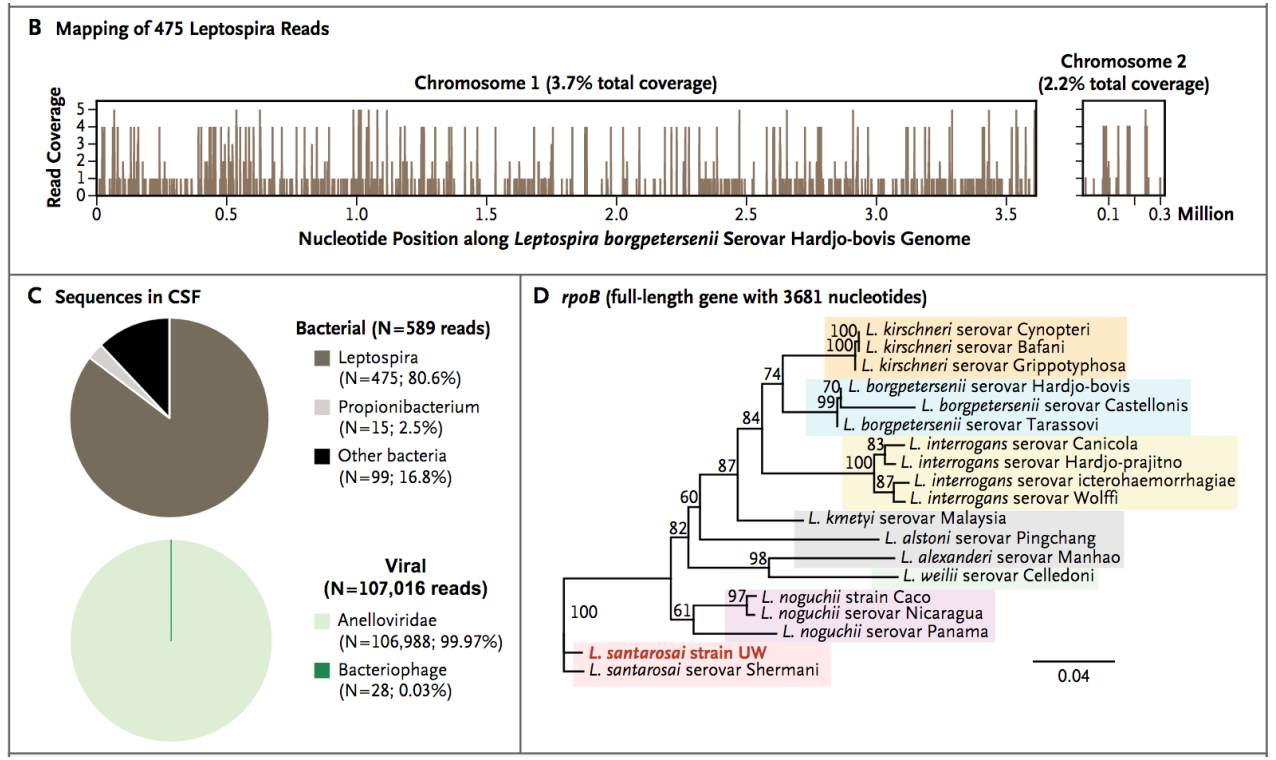
In CSF, the majority of bacterial reads (475 of 589 reads; 80.6%) corresponded to the Leptospiraceae family

(Turnaround time: 48 h)

PCR analysis and sequencing revealed that CSF harbored *L. Santarosai*

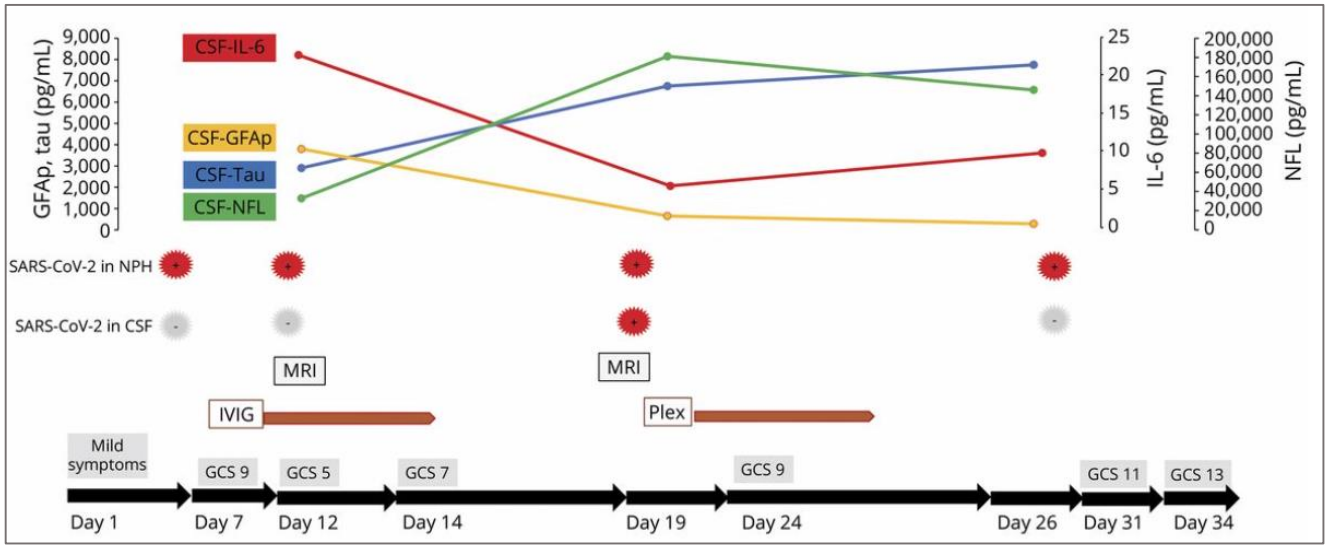
Wilson et al., NEJM 2014

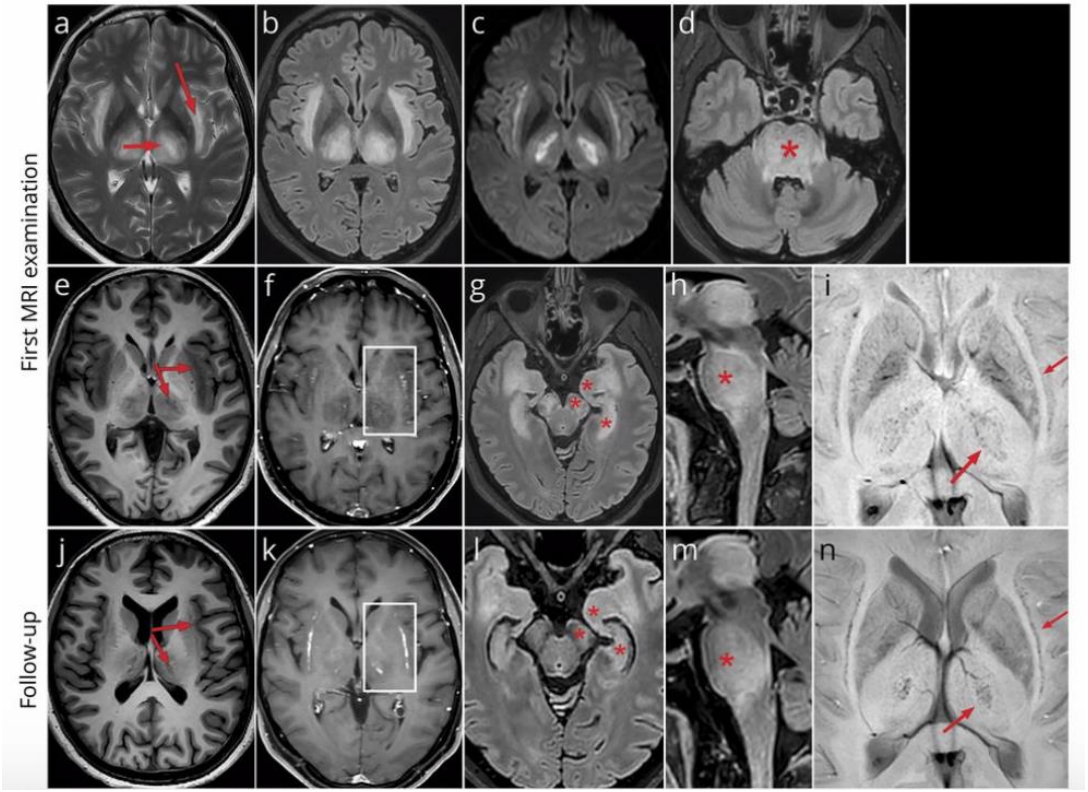




Acute necrotizing encephalopathy with SARS-CoV-2 RNA in CSF

- F, 55, Covid-19 pneumonia and SARS-CoV2 positive NP swab
- Letargy → coma
- MRI: symmetric signal alteration in central thalami, subinsular regions, medial temporal lobes and brain stem.
- SARS-CoV2 in CSF 19 days after symptom onset after testing negative twice.
- Extremely high CSF oncentrations of NFL and tau, gFAP.
- Treatment with IV immunoglobulins and PLEX
- Improvement with extubation 4 weeks after symptom onset.





Virhammar et al., Neurology, September 2020

Undetectable SARS-CoV-2 RNA in CSF in patients with COVID-19 and neurological manifestations

Neurological outcomes	Number of cases	Days between CSF withdrawn and symptoms onset	RT-qPCR in nasopharyngeal swab	RT-qPCR in CSF	Other viruses tested negative in CSF	References
Meningoencephalitis (1), Encephalitis (1), Facial palsy (2), delirium (2), intracranial hypertension (1), new daily persistent headache (1)[a]	8	2 to 10	positive	negative	HSV-1/2, VZV, CMV, EBV, HHV-6, Influenza A and B viruses	This study[b]
Meningoencephalitis	1	9	negative[c]	positive	n.i.	Moriguchi et al. (2020)
Meningoencephalitis	2	5 and 17	positive	negative	Enterovirus, HSV-1/2, VZV, CMV, HHV-6, Parechovirus	Bernard-Valnet et al. (2020)
Autoimmune meningoencephalitis	6	n.i.	positive	negative	Common seasonal viruses (not specified)	Dogan et al. (2020)
Acute disseminated encephalomyelitis	1	n.i.	positive	negative	Neurotropic viruses (not specified)	Zanin et al. (2020)
GBS	5	9 to 13	4 positive and 1 negative[c]	negative	n.i.	Toscano et al. (2020)
GBS	1	10	positive	negative	Enterovirus, HSV-1/2, VZV, CMV, HHV-6, Parechovirus	Coen et al. (2020)
GBS	1	10	positive	negative	n.i.	Ottaviani et al. (2020)
GBS	1	7	positive	negative	n.i.	Alberti et al. (2020)
Facial diplegia (GBS variant)	1	11	positive	negative	n.i.	Juliao Caamaño and Alonso Beato (2020)
Perfusion abnormalities in brain MRI	7	n.i.	positive	negative	n.i.	Helms et al. (2020)
Acute cerebrovascular disease	2	7 and 10	positive	negative	n.i.	Al Saiegh et al. (2020)
Encephalitis	1	19	positive	negative	n.i.	Ye et al. (2020)
Encephalitis	1	5	positive	negative	Enterovirus, HSV-1/2, VZV, EBV, HHV-6, HHV-8, Adenovirus	Pilotto et al. (2020)

de Melo Espíndola et al., Int J Inf Dis, May 2020