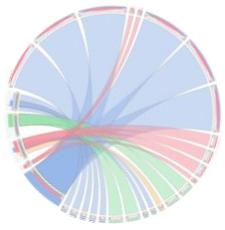




Métagénomique clinique pour le diagnostic microbiologique

Dr Théo GHELLENSTEIN-FERREIRA

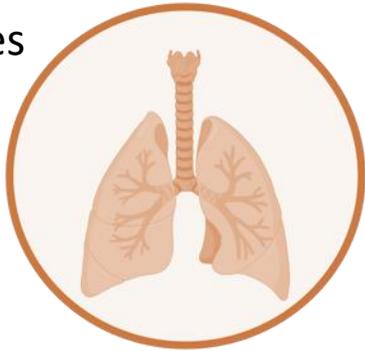
AHU Parasitologie - Mycologie
Hôpital Saint Louis, AP-HP
Paris, France



Nécessité de nouveaux outils de diagnostic

Pathologies infectieuses chez les patients hospitalisés

Pneumonies



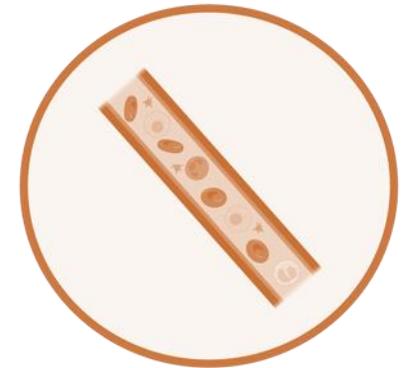
15-20%
sans étiologie

Méningites et
Encéphalites



40-60%
sans étiologie

Sepsis



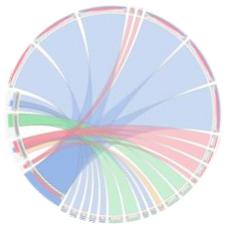
~20%
sans étiologie

Nécessité d'outils complémentaires pour le diagnostic microbiologique



Wilson et al., *N Engl J Med*, 2019, Ewig S et al., *European Respiratory Journal*, 2002

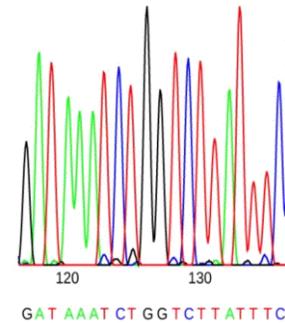
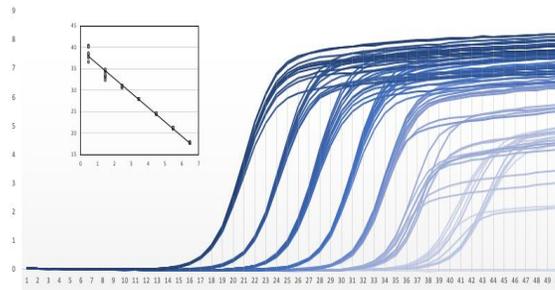
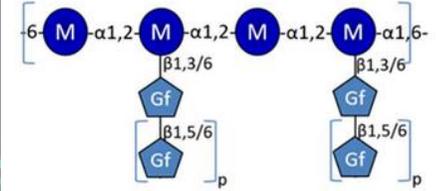
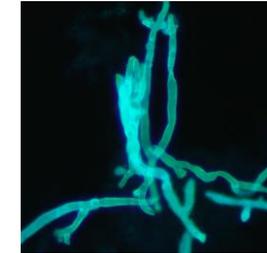
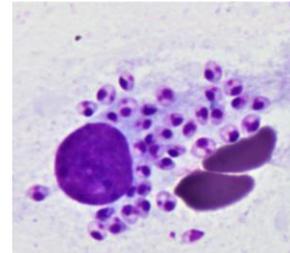
33^e Congrès National STPI

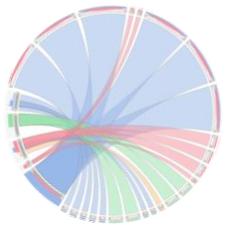


Outils de diagnostic conventionnels



- Examen direct, culture ? Histologie ?
- Antigènes, Anticorps ?
- PCR temps-réel spécifiques ? Multiplex ?
- Séquençage Sanger (16S, ITS, 18S) ?



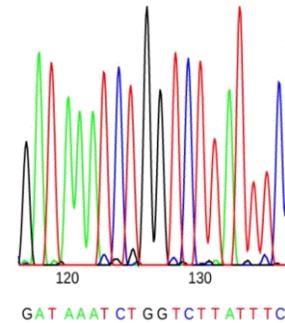
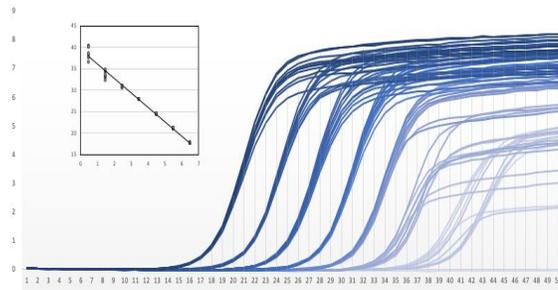
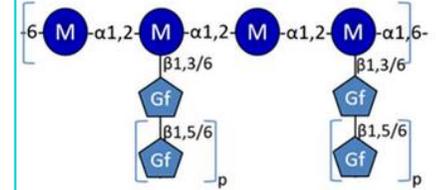


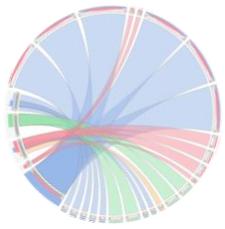
Outils de diagnostic conventionnels

- Examen direct, culture ? Histologie ?
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- PCR temps-réel spécifiques ? Multiplex ?
- Séquençage Sanger (16S, ITS, 18S) ?



Bactéries
 Champignons
 Parasites
 Virus
 Polymicrobiens
 Nouveau pathogène
 Résistance





Outils de diagnostic conventionnels

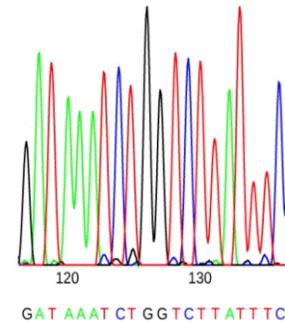
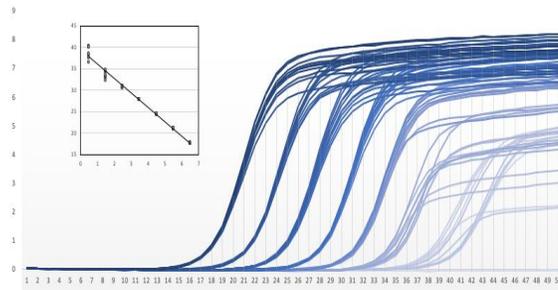
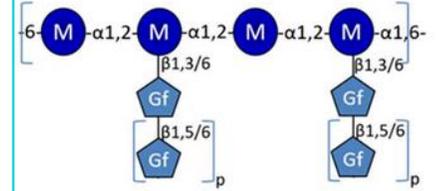


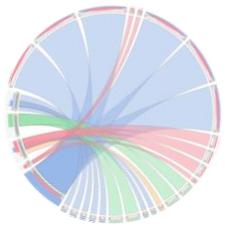
- Examen direct, culture ? Histologie ?
- Antigènes, Anticorps ?
- PCR temps-réel spécifiques ? Multiplex ?
- Séquençage Sanger (16S, ITS, 18S) ?



- Bactéries ✓
- Champignons ✓
- Parasites ✓
- Virus ✓
- Polymicrobiens ✓
- Nouveau pathogène ✗
- Résistance ✓

Mais recherche ciblée !

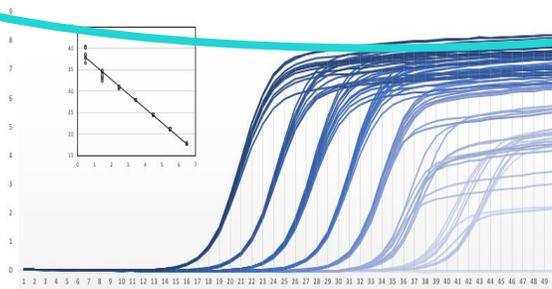
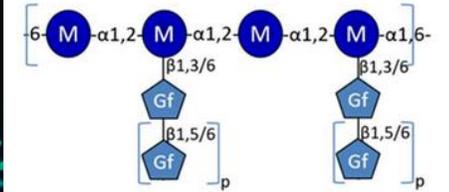
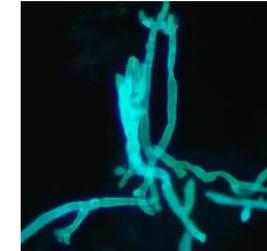
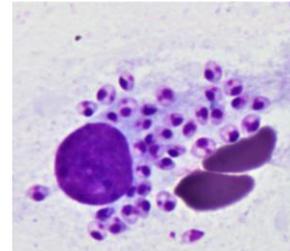




Outils de diagnostic conventionnels



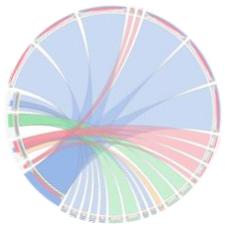
- Examen direct, culture ? Histologie ?
- Antigènes, Anticorps ?
- PCR temps-réel spécifiques ? Multiplex ?
- Séquençage Sanger (16S, ITS, 18S) ?



Bactéries	!
Champignons	!
Parasites	×
Virus	×
Polymicrobiens	×
Nouveau pathogène	×
Résistance	✓

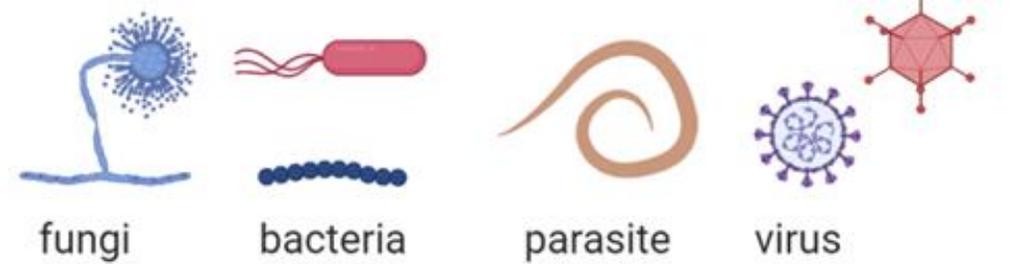
Mais recherche ciblée !





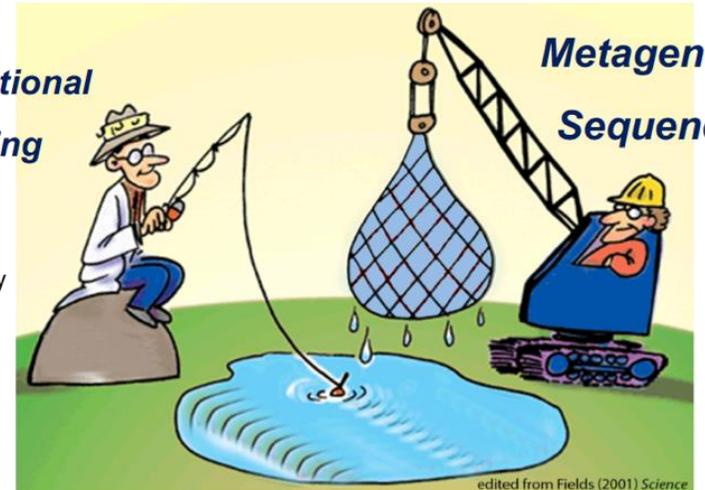
Les promesses de cette nouvelle technique

- Non ciblé/ sans *a priori*
- Détection des micro-organismes de culture difficile
- Détection des micro-organismes non recherchés ou non connus
- Prédiction de la sensibilité aux anti-infectieux
- Génotypage



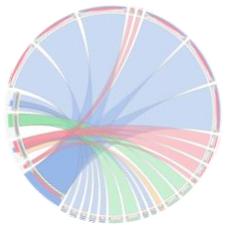
**Conventional
Testing**

Sero
PCR
Culture
Microscopy
...



**Metagenomic
Sequencing**





La métagénomique clinique ou *shotgun*

Métagénomique clinique ?

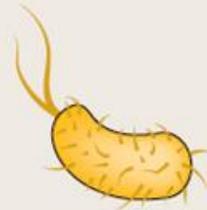
Séquençage non ciblé de l'ensemble du matériel génétique présent dans un échantillon clinique

Metagenomic sequencing

Total nucleic acid extraction



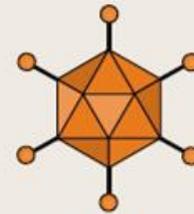
RNA
viruses



Bacteria



Fungi



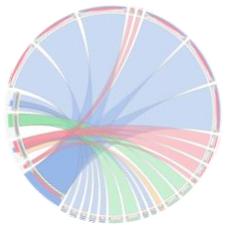
DNA
viruses



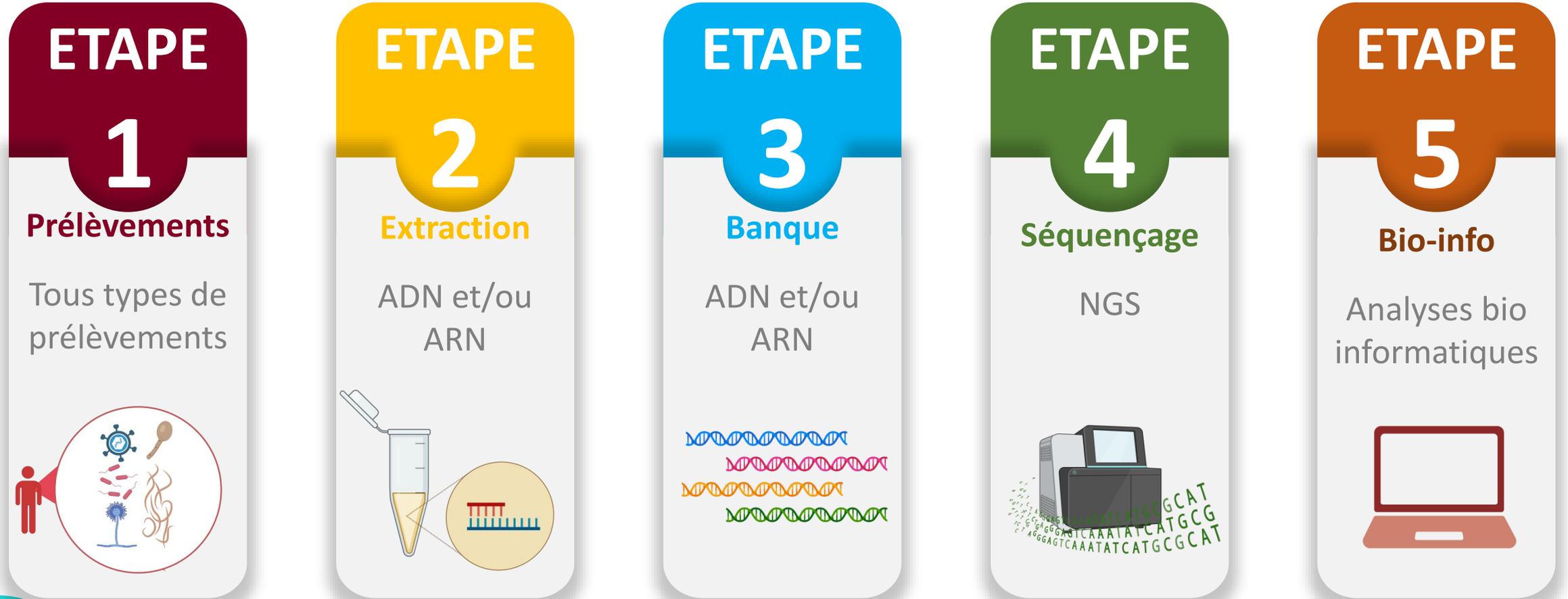
Parasites

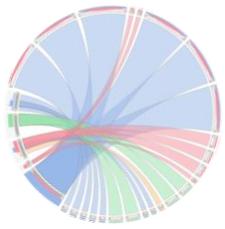


Human



Mode d'emploi





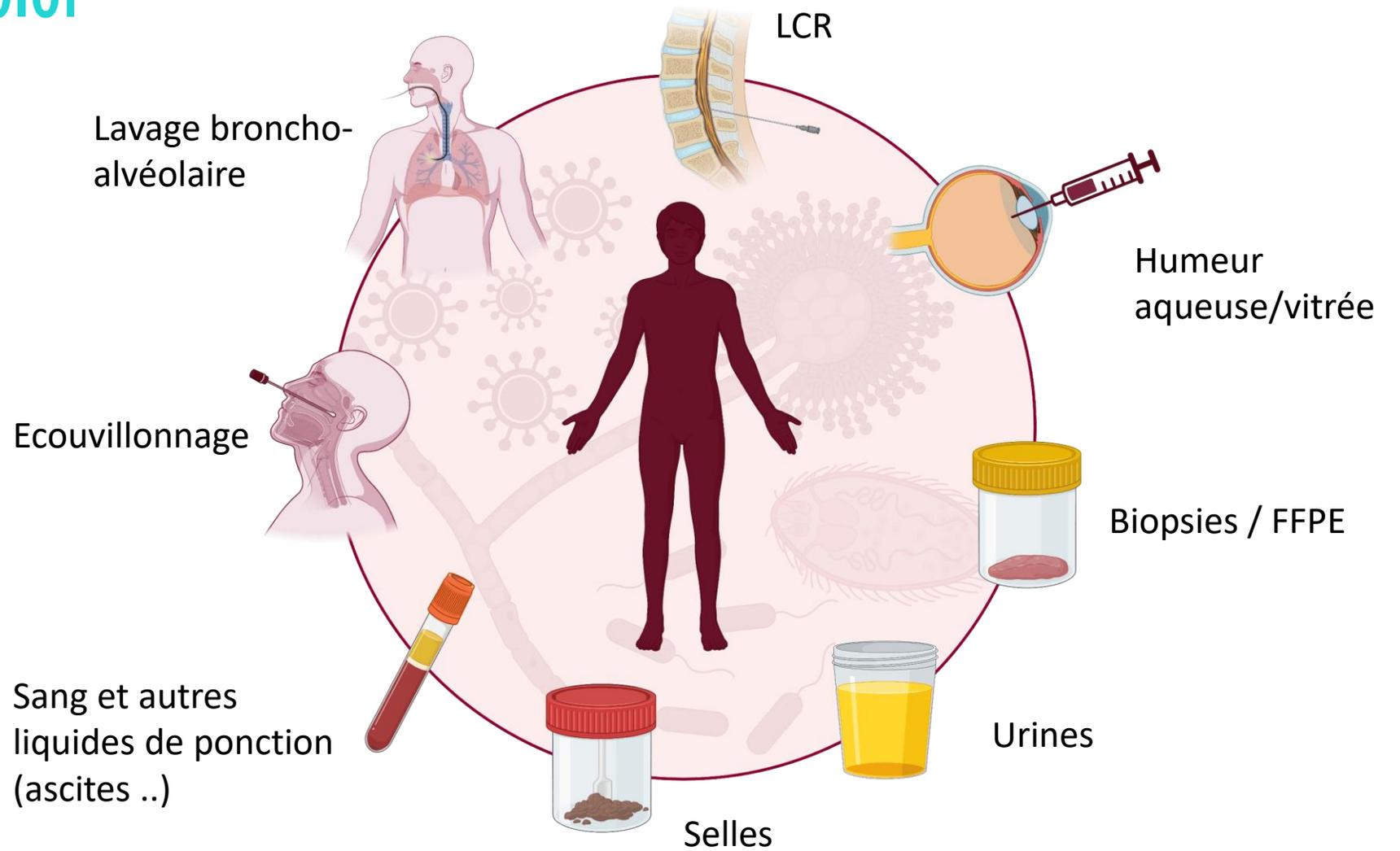
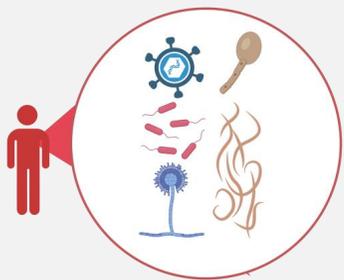
Mode d'emploi

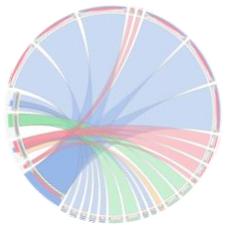
ETAPE

1

Prélèvements

Tous types de
prélèvements





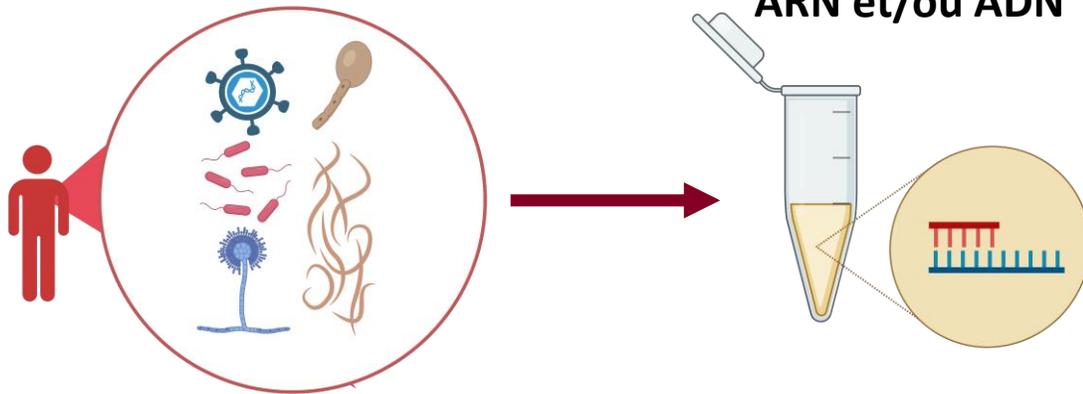
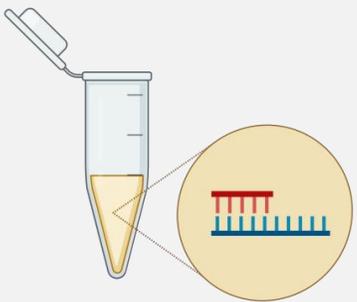
Mode d'emploi

ETAPE

2

Extraction

ADN et/ou
ARN

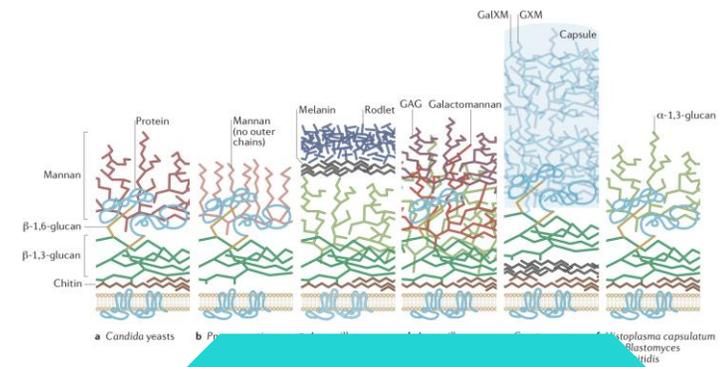


Etape clé dans une analyse de mNGS

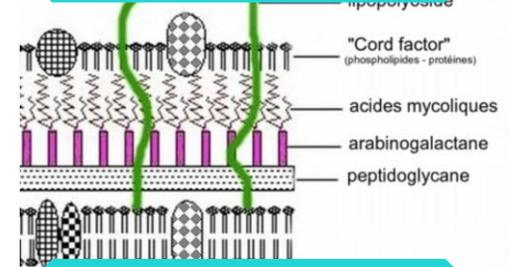
Méthodes d'extraction qui diffèrent selon :

- Le type de prélèvement
- Le pré-traitement éventuel
- Le type d'acide nucléique souhaité

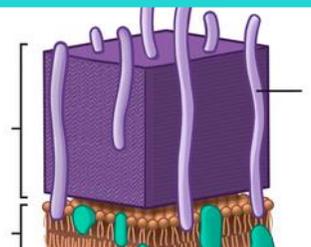
BESOIN D'UNE EXTRACTION UNIVERSELLE pour tous les pathogènes



Eucaryotes

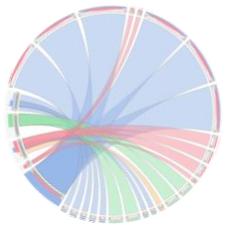


Mycobactéries



Bactéries Gram +

33^e Congrès National STPI



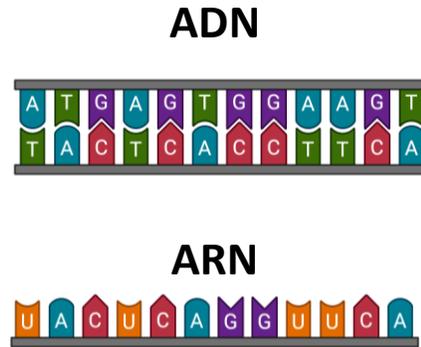
Mode d'emploi

ETAPE

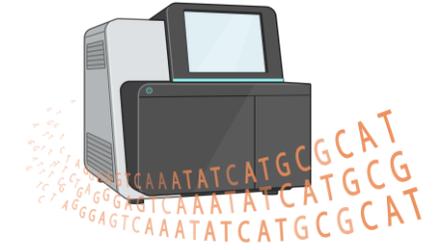
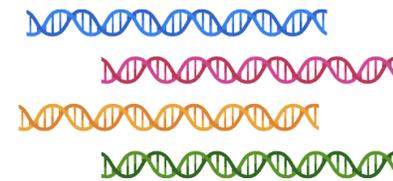
3

Banque

ADN et/ou
ARN

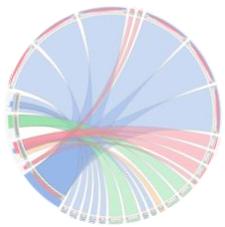


Banque



Traitement des acides nucléiques pour être séquencés

- Fragmentation
- Amplification universelle
- Indexage
- Déplétion en AN humain



Mode d'emploi

ETAPE

4

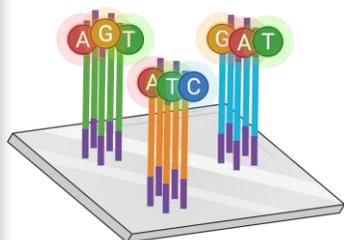
Séquençage

NGS



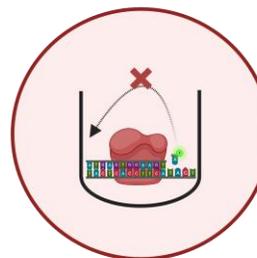
AGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT
GAGGAGTCAAAATATCATGCGCAT

Séquençeurs de 2^{ème} génération

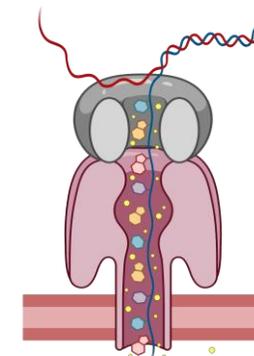


Amplification en pont (Bridge PCR)

Séquençeurs de 3^{ème} génération

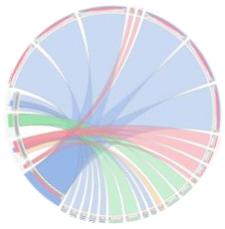


Single molecule
Real time



Nanopore

33^e Congrès National STPI

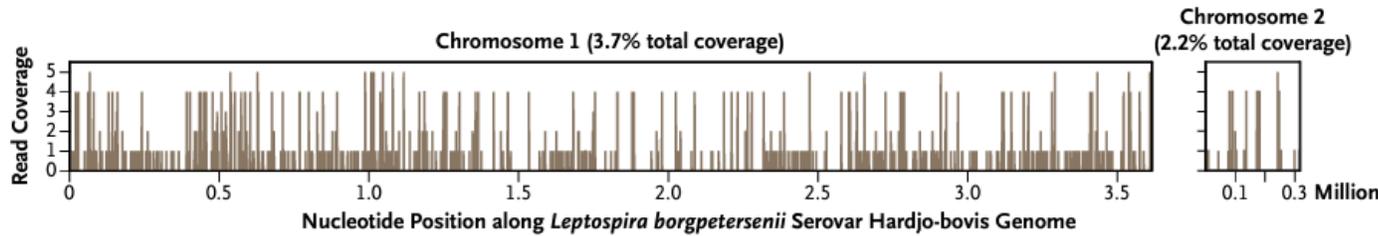


Preuves de concept

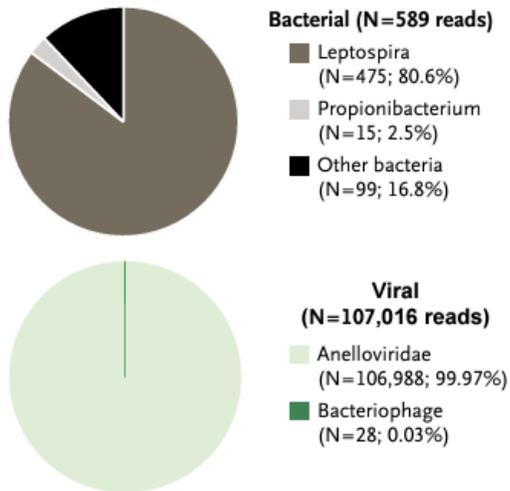


Méningoencéphalite
à *Leptospira santarosai*
chez un enfant de 14 ans immunodéprimé
- déficit immunitaire combiné sévère

B Mapping of 475 Leptospira Reads



C Sequences in CSF



D rpoB (full-length gene with 3681 nucleotides)

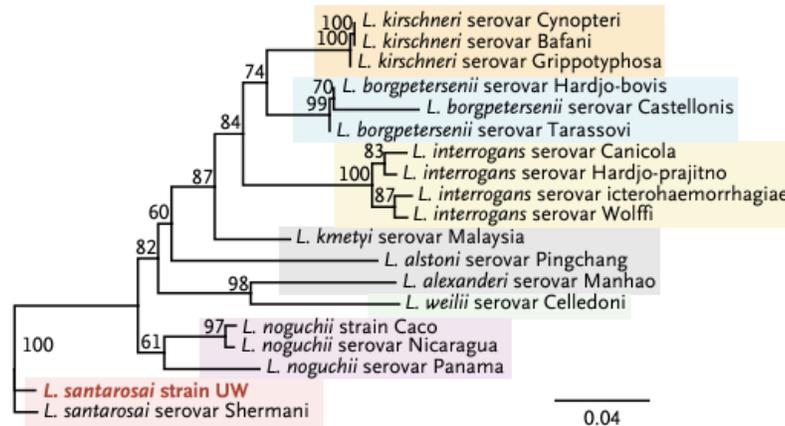
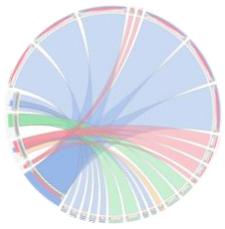


Figure 3. Diagnosis of Leptospira Infection by Means of Unbiased Next-Generation Sequencing (NGS).

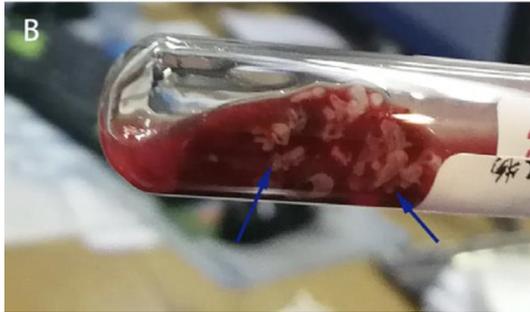


Wilson et al., *N Engl J Med*, 2014



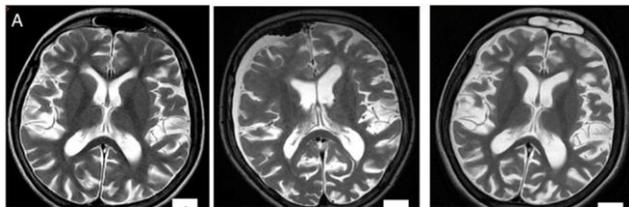
Preuves de concept

DÉTECTION DE PATHOGÈNES RARES



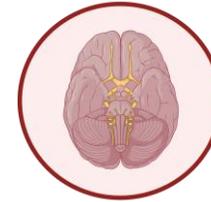
Sparganose à *Spirometra mansoni*

DÉTECTION DE PATHOGÈNES INHABITUELS DANS LE SITE DE PRÉLÈVEMENT



Astrovirus dans une biopsie cérébrale

DÉTECTION DE PATHOGÈNES INHABITUELS DANS LA ZONE GÉOGRAPHIQUE



Epidémie Virus de l'encéphalite Japonaise en Australie 2022

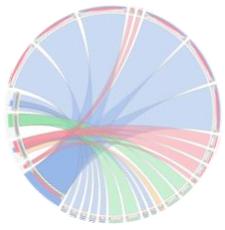
CARACTERISATION DE NOUVEAUX PATHOGÈNES



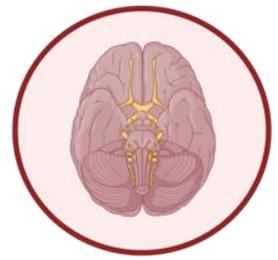
SARS-CoV2 caractérisé par métagénomique



Hu et al., *Int. J. Infect. Dis.*, 2023; Frémond ML et al., *J Pediatric Infect Dis Soc.* 2015, Wu F et al., *Nature.* 2020, Howard-Jones AR et al *Viruses.* 2022

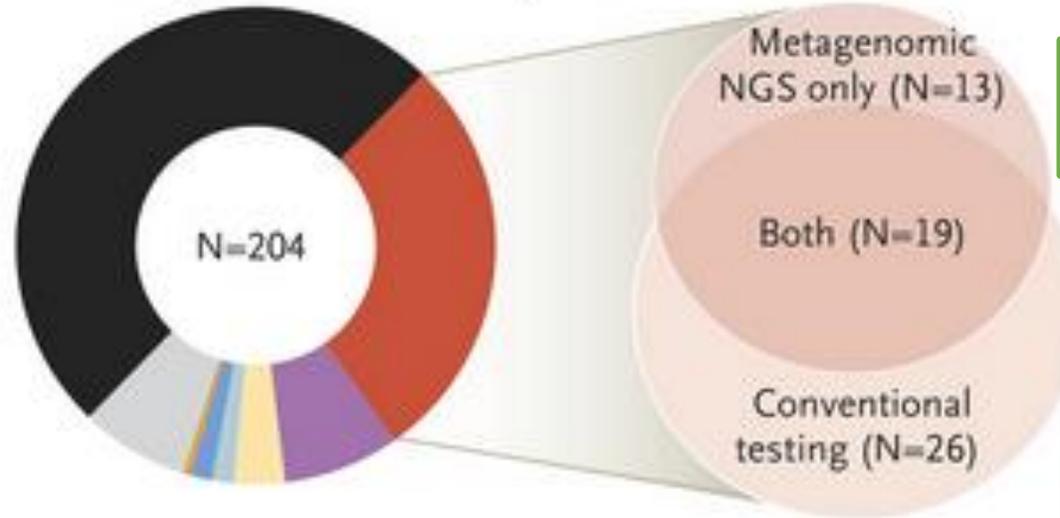


Utilisation en clinique



1^{re} étude prospective multicentrique

204 patients de 8 hôpitaux avec une suspicion d'infection du SNC



8/13 modification de la prise en charge

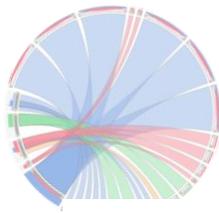
Diagnostic sérologique
Diagnostic non LCR
Faible charge

- 57 (27.9%) Infectious
- 17 (8.3%) Autoimmune
- 7 (3.4%) Neoplastic
- 3 (1.5%) Postinfectious
- 3 (1.5%) Toxic metabolic
- 1 (0.5%) Vascular
- 15 (7.4%) Other
- 101 (49.5%) Unknown



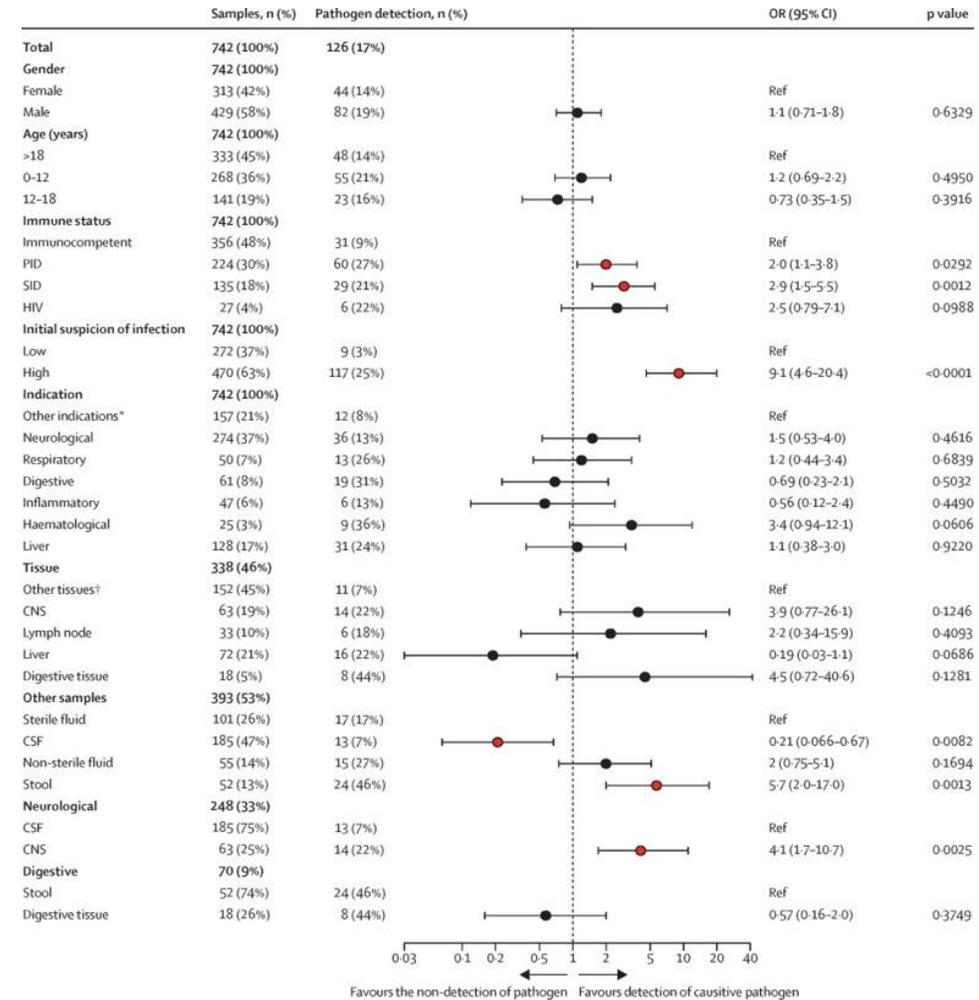
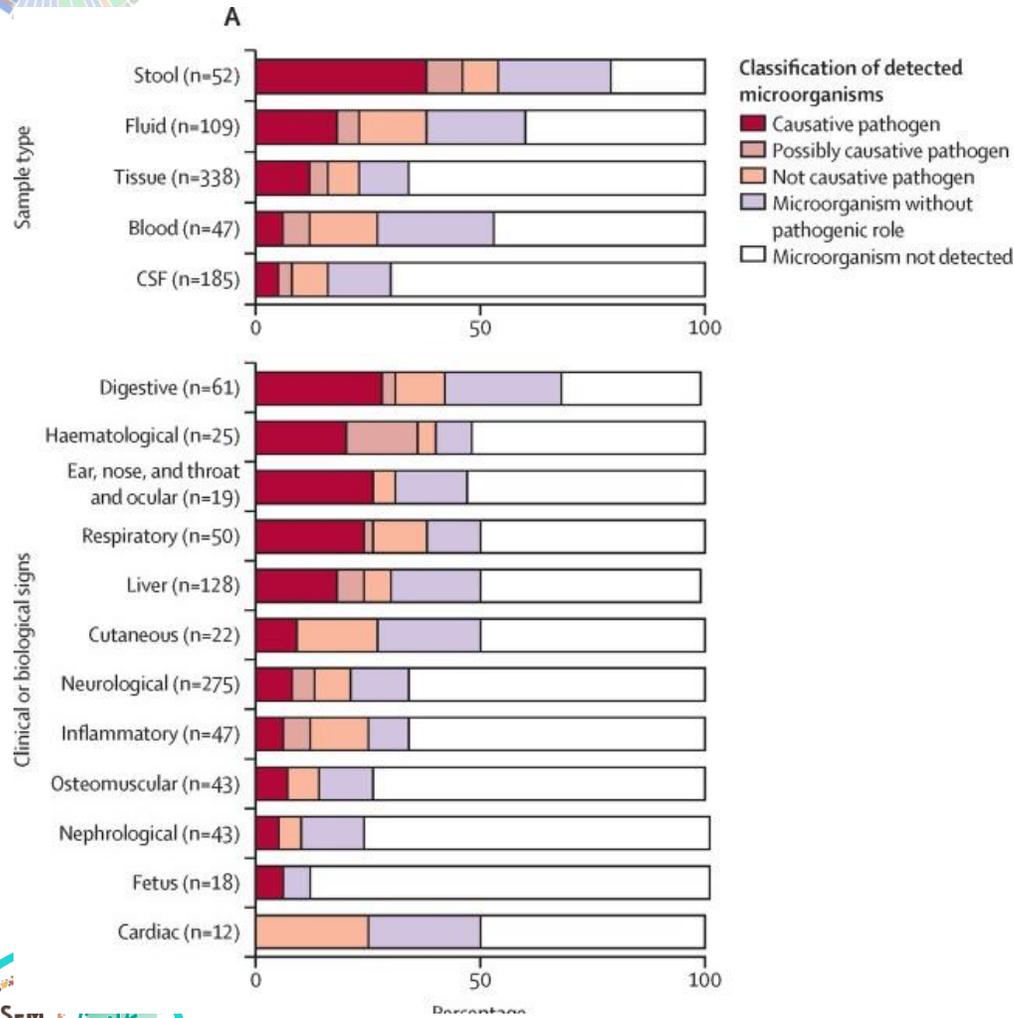
Wilson et al, NEJM 2019

33^e Congrès National STPI



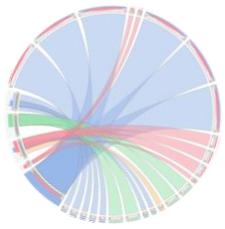
Utilisation en clinique

742 échantillons , 4 ans, Hôpital Necker

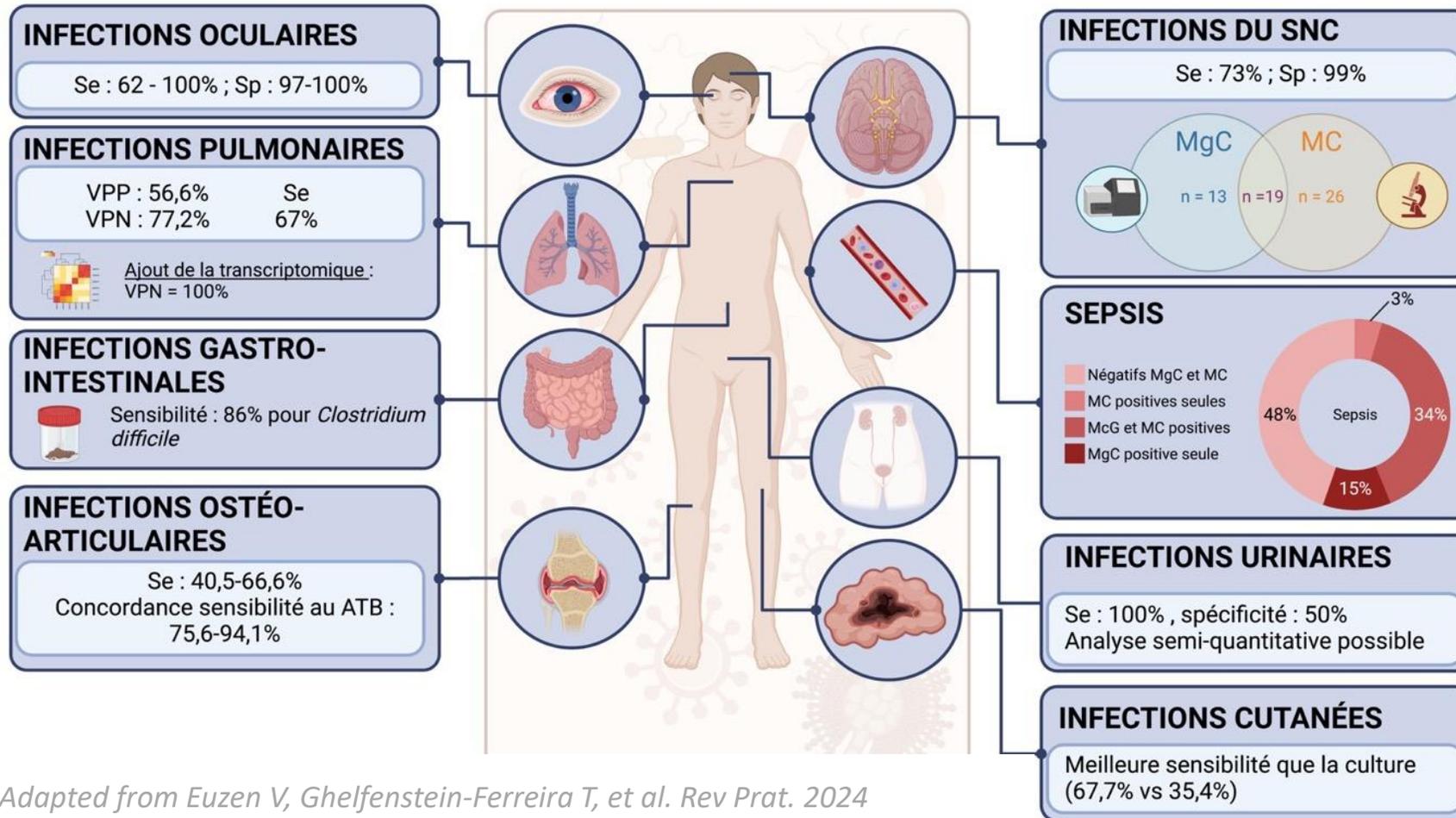


Fourgeaud et al, The Lancet Microbe 2023

33^e Congrès National STPI



Utilisation en clinique

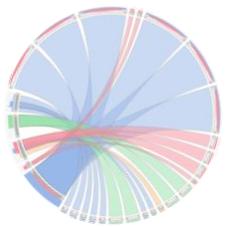


Adapted from Euzen V, Ghelfenstein-Ferreira T, et al. Rev Prat. 2024

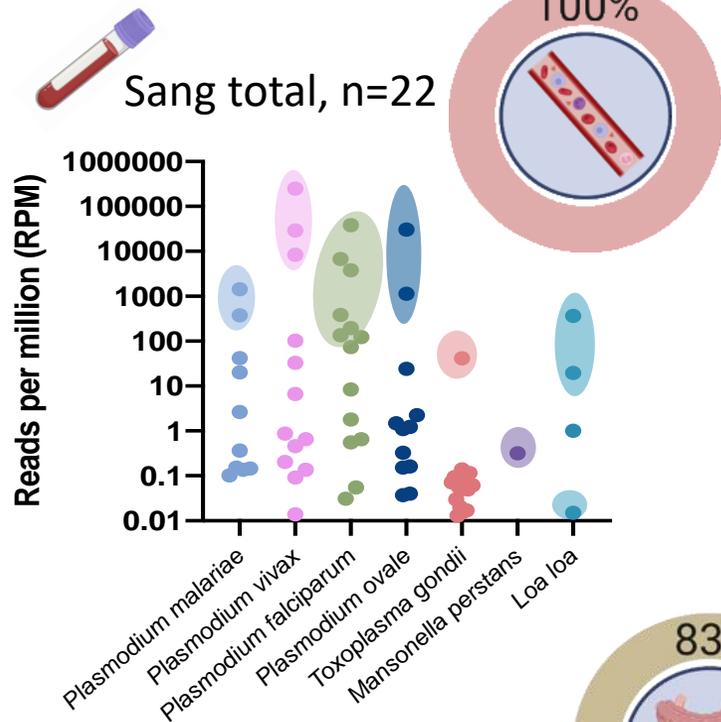


Lalitha P, et al. Ophthalmology. 2021; Lalitha P, et al. Ophthalmology. 2019; Janes VA, et al. Lancet Microbe. 2022; Rodriguez C et al. Ann Dermatol Vénérologie. 2016; Blauwkamp TA Nat Microbiol. 2019; Niles DT. Et al. Diagn Microbiol Infect Dis. 2023

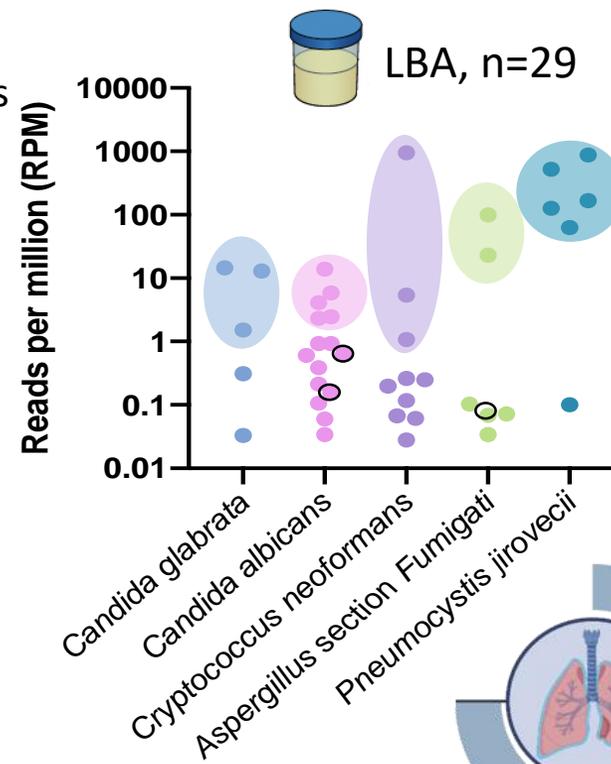
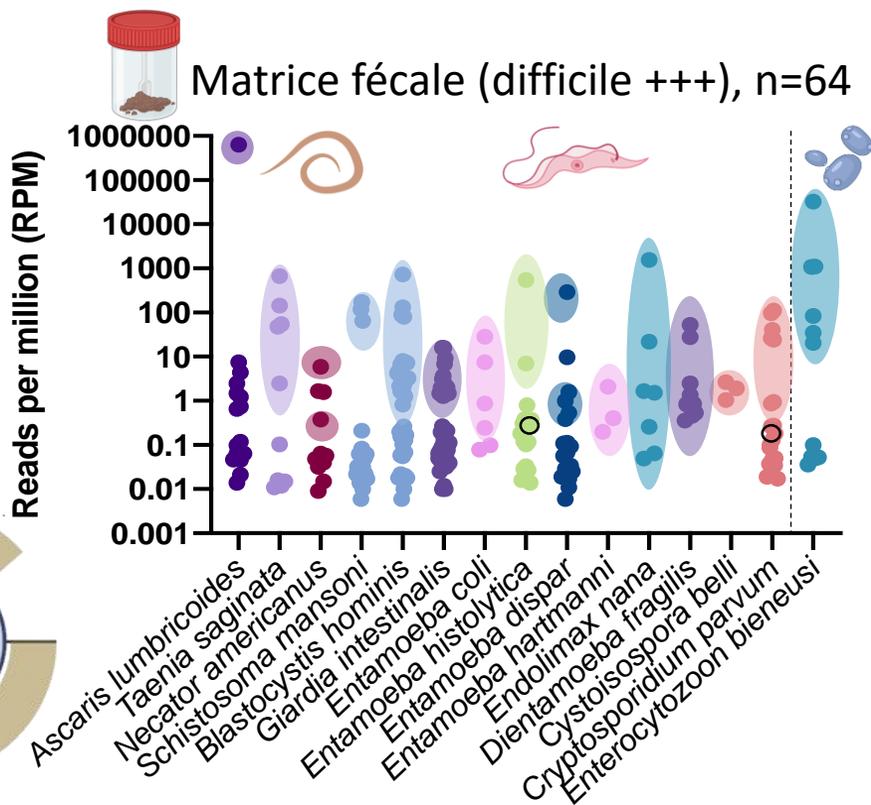
33^e Congrès National STPI



Utilisation en clinique, infections fongiques / parasitaires



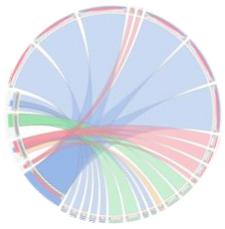
- Echantillons ⊕ manqués en MG
- Echantillons ⊕ en MG & techniques conventionnelles



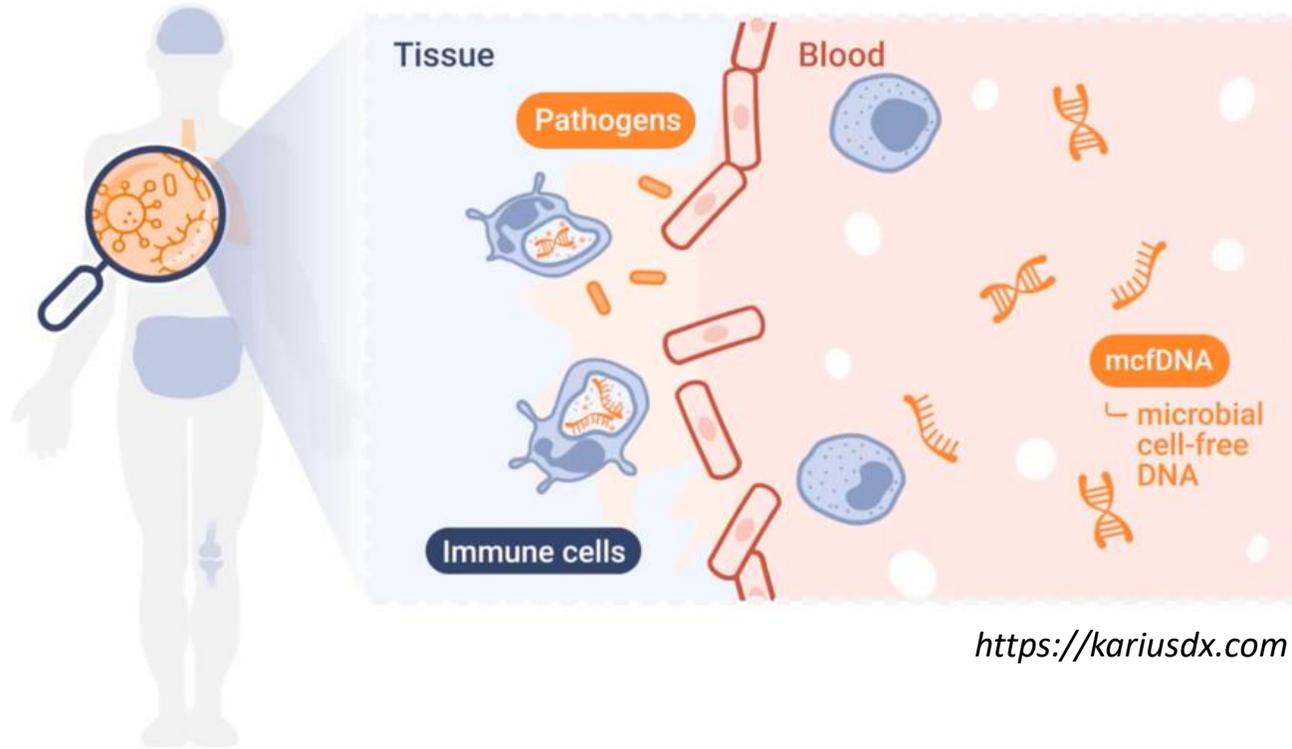
82% de concordance globale entre MG et techniques conventionnelles pour la recherche de parasites & champignons. Variable selon la matrice.

Ghelfenstein-Ferreira et al. ECCMID 2023

National STPI



ADN libre circulant



<https://kariusdx.com>



5 mL blood sample

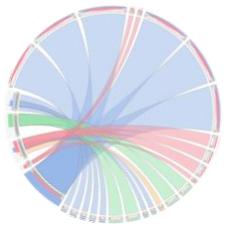
Cell-free DNA in plasma:

- fetal DNA (10%)
- tumor DNA (~0.1%)
- microbial DNA (~0.001%)

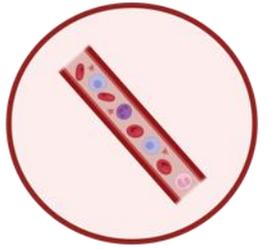
KARIUS[®]

noscendo





ADN libre circulant



Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease

[Timothy A. Blauwkamp](#) , [Simone Thair](#), [Michael J. Rosen](#), [Lily Blair](#), [Martin S. Lindner](#), [Igor D. Vilfan](#), [Trupti Kawli](#), [Fred C. Christians](#), [Shivkumar Venkatasubrahmanyam](#), [Gregory D. Wall](#), [Anita Cheung](#), [Zoë N. Rogers](#), [Galit Meshulam-Simon](#), [Liza Huijse](#), [Sanjeev Balakrishnan](#), [James V. Quinn](#), [Desiree Hollemon](#), [David K. Hong](#), [Marla Lay Vaughn](#), [Mickey Kertesz](#), [Sivan Bercovici](#), [Judith C. Wilber](#) & [Samuel Yang](#)

Nature Microbiology 4, 663–674 (2019) | [Cite this article](#)

Research | [Open Access](#) | Published: 13 April 2022

Clinical impact of a metagenomic microbial plasma cell-free DNA next-generation sequencing assay on treatment decisions: a single-center retrospective study

[Akira A. Shishido](#) , [Myint Noe](#), [Kapil Saharia](#) & [Paul Luethy](#)

BMC Infectious Diseases 22, Article number: 372 (2022) | [Cite this article](#)

Patient characteristics (n = 348)	NGS positive	NGS negative	Agreement (%)	95% CI (%)
Positive by composite reference standard	169	13	92.9	88.1–96.1
Negative by composite reference standard	62	104	62.7	54.8–70.0

> 22% de volontaires sains positifs

Diagnostic microbiologique 65% des cas
Impact positif → 43% des cas

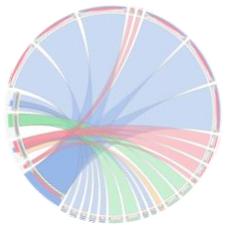
- Désescalade thérapeutique ++
- SOT
- Sepsis
- antiinfectieux <7j



80 patients (45% ID)

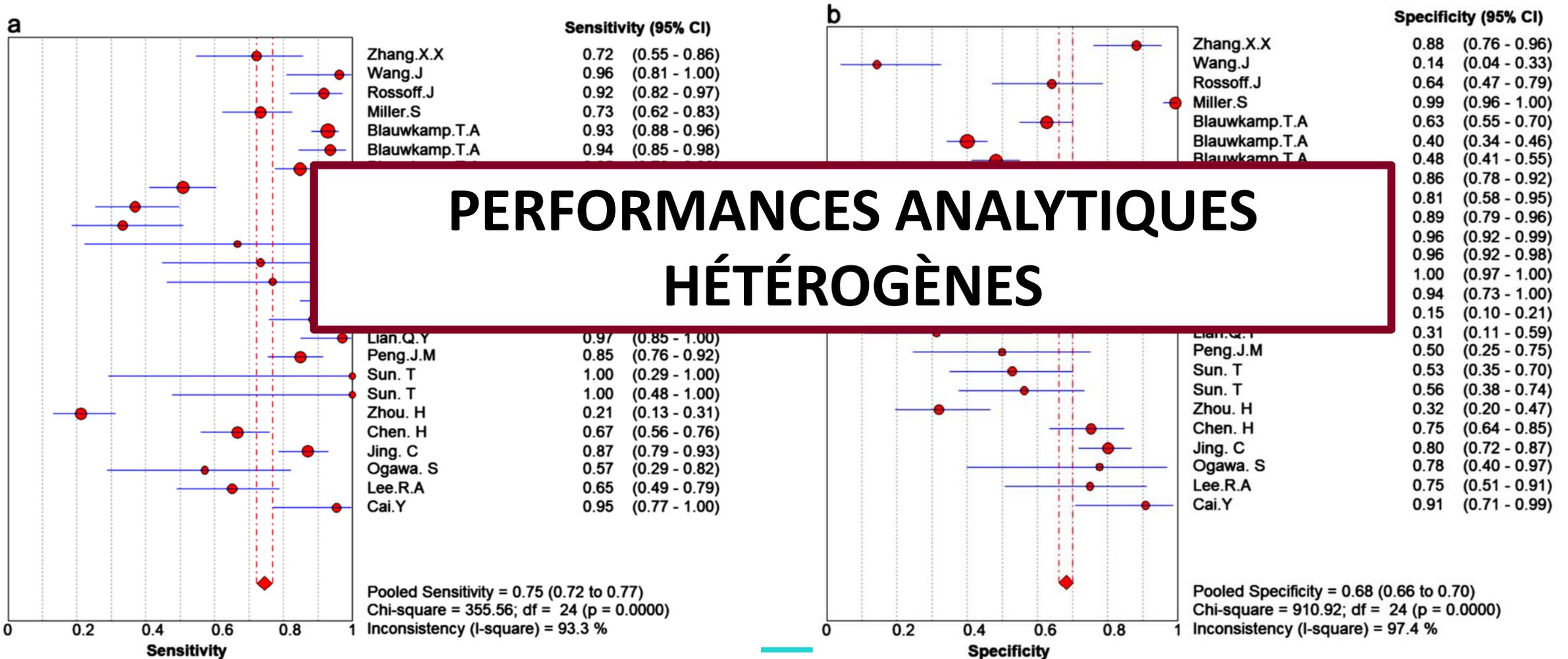


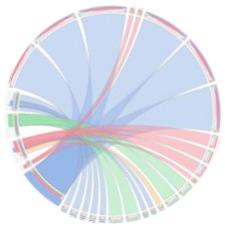
33^e Congrès National STPI



Performances analytiques ?

Meta-analyse 20 études avec 2716 patients





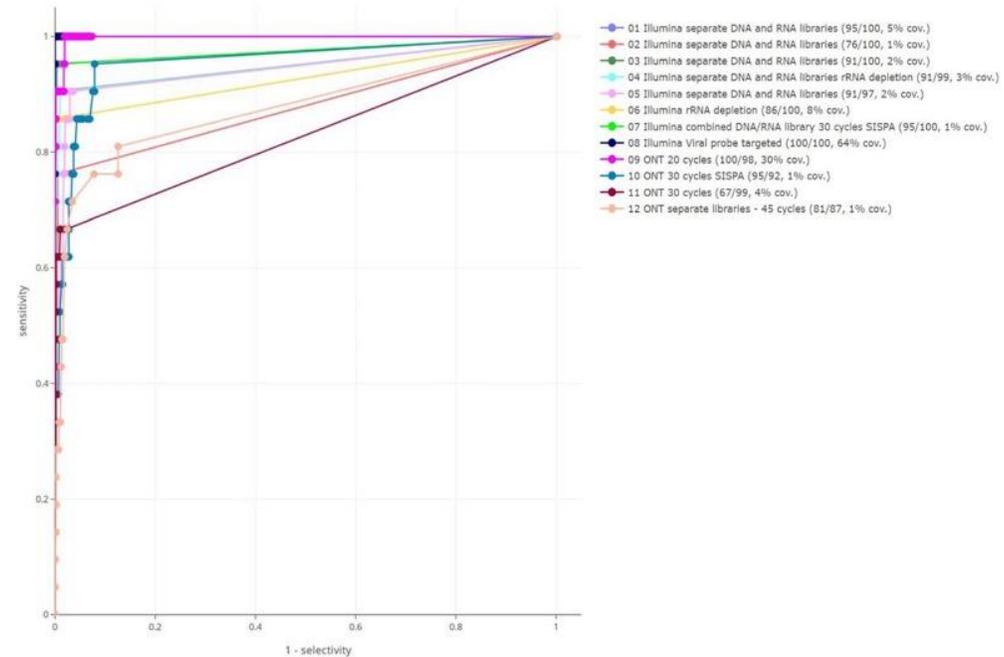
Vers une harmonisation des performances?

- EIL Virus
- Recommandations ENNGS
- Challenge ENNGS Virus
 - Wet et Dry Lab

- Marquage CE IVD kits diagnostic



European Society
for Clinical Virology
ENNGS steering group





KARIUS TEST REPORT

Karius ID: KA-XXXXXX

Page 1 of 4
KARIUS TEST
clearly #1 speed™

SPECIMEN TYPE: PLASMA

SPECIMEN INFORMATION	Collected	Received	Reported	Specimen ID
PATIENT INFORMATION	MRN#	Last Name	First Name	Date of Birth
INSTITUTION INFORMATION	Ordering Physician		Address	

TEST RESULTS

MICROORGANISM DETECTED	DNA MOLECULES PER MICROLITER (MPM)*	REFERENCE INTERVAL (MPM)**
<i>Pneumocystis jirovecii</i>	32,023	< 10

* Molecules Per Microliter = number of DNA fragments present in one microliter of plasma

** Reference Interval = the 97.5th percentile MPM concentration detected in PPT plasma from a cohort of 684 asymptomatic donors

Results can also be accessed via our online secure portal.

Karius medical staff are available to answer questions about these results: Phone: (866) 452-7487 | Email: medical@kariusdx.com
Karius is a covered entity under HIPAA.

TEST DESCRIPTION

The Karius Test can detect:

Bacteria: 982 **DNA viruses:** 106 **Archaea:** 1 **Fungi:** 403 **Eukaryotes:** 68

Full list of organisms is found at: <https://www.kariusdx.com/pathogenlist/3.8.0>

The Karius Test for infectious disease detects **microbial cell free DNA (cfDNA)** in plasma from bacteria, DNA viruses, fungi and protozoa using next-generation sequencing (NGS) [1]. The test reports the presence and abundance of **microbial cfDNA** when statistically significant levels are detected above background.

Microbial cfDNA may be found in plasma when viable microorganisms are not detected in blood by other methods [2]. It can be detected from localized infections or during effective antimicrobial treatment [1, 3, 4]. The reported microorganism(s) may or may not be the cause of patient infection. Results should be interpreted within the context of clinical data, including medical history, physical findings, epidemiological factors, and other laboratory data.

[1] Blauwkamp T, et al. *Nat Microbiol*. 2019;4(4):683-674.

[2] De Vlaminck I, et al. *Cell*. 2013;155(5):1178-1187.

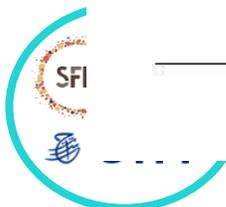
[3] Farnaes L, et al. *Diagn Microbiol Infect Dis* 2019;94(2):188-191.

[4] Rossell J, et al. *Open Forum Infect Dis* 2019;6(8).

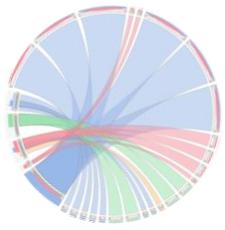
Karius Laboratory | 975 Island Drive, Suite 101, Redwood City, CA 94065
Toll Free: (866) 452-7487 | Fax: (866) 246-6567 | Email: help@kariusdx.com
CLIA # 05D2121236 | CAP # 9497749 | Lab Director: Judith Wilber, PhD, D(ABMM)
v3.8



→ Questionnaire Delphi « Eléments nécessaires pour l'interprétation Microbiologique d'un CF de Métagénomique clinique »
GT Métagénomique clinique, ANRS-MIE 2024 (A Jary, M Salmona)



33^e Congrès National STPI

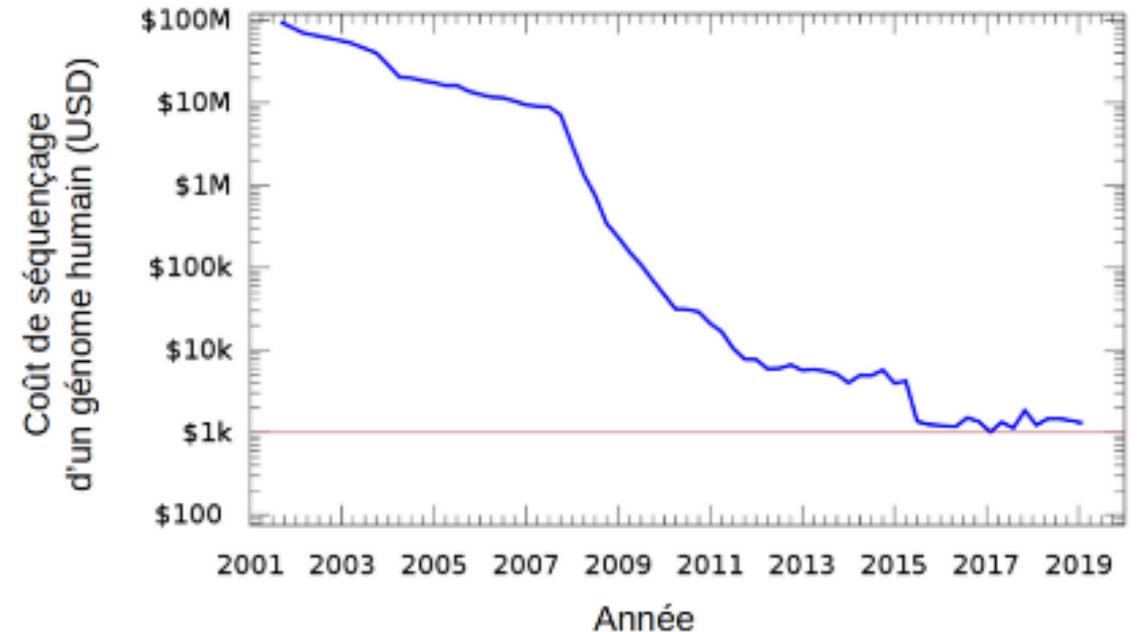


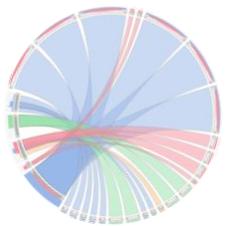
En pratique au laboratoire

- Prix ~ **50 – 300 Euros (170 – 1000 TND)**
/ échantillon
- RIHN 942 → **2500 Euros (8500 TND)**

- Existence contrôles inter-laboratoires
(QCMD/ENNGS)

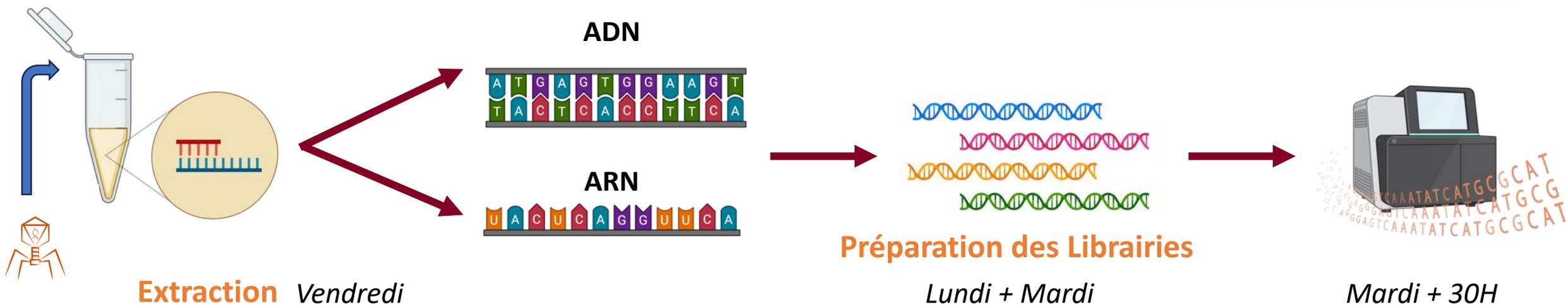
Loi de Moore :
doublement des capacités informatiques
à prix constant





En pratique au laboratoire

Temps 6h à 72h — MAIS en pratique ? | — 3 semaines



Extraction *Vendredi*

+ Témoin environnemental

Préparation des Bibliothèques

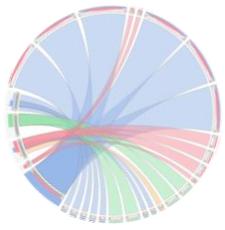
Lundi + Mardi

Mardi + 30H

Pipeline d'analyse « Maison »
Technique accrédité Cofrac depuis avril 2024



33^e Congrès National STPI



Rapport biologiste



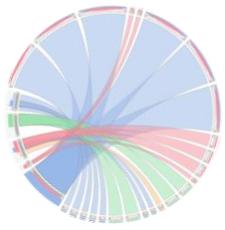
Fiabilité des résultats?



Intégration de :

- Contrôles internes (ARN et ADN)
- Contrôle négatif
- Score de fiabilité (code couleur)
- Graph de couverture





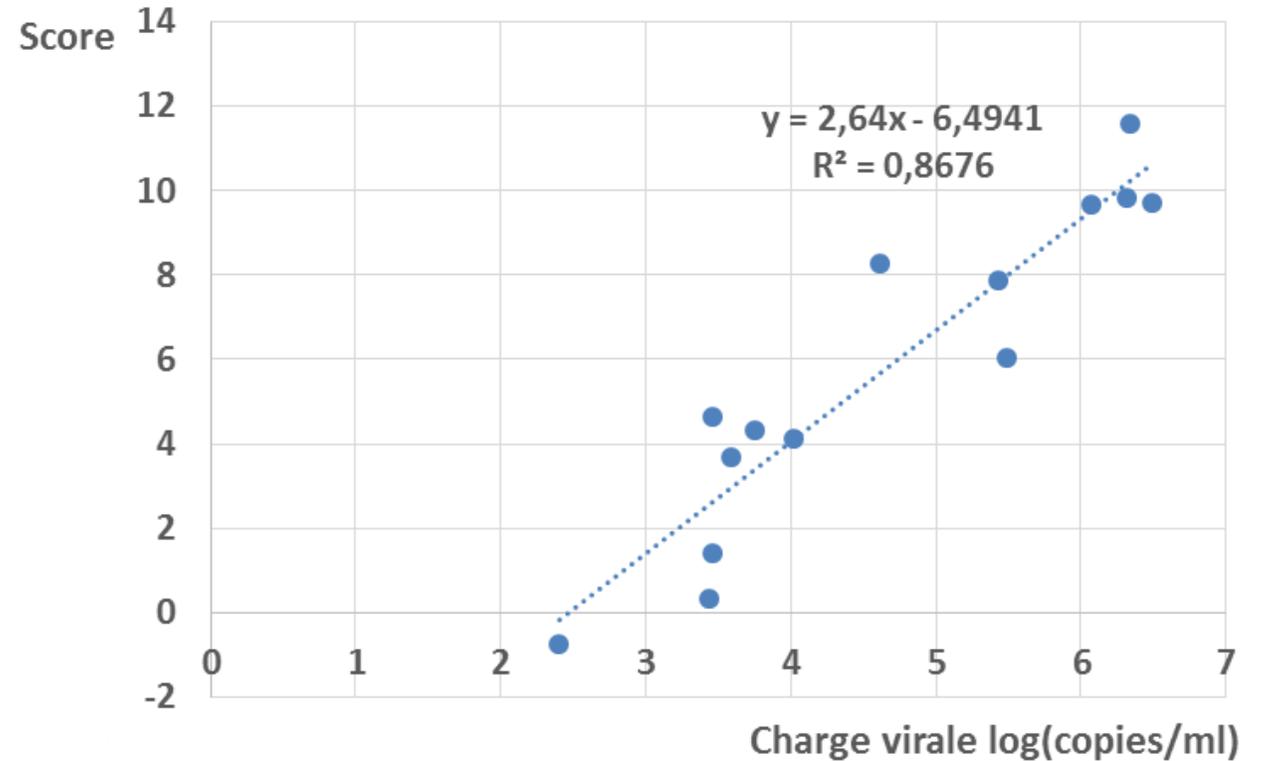
Score de confiance

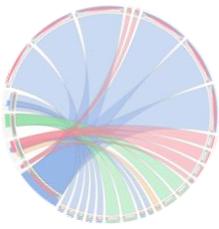
Calculé à partir

- de la couverture/taille du pathogène
- du nombre de RPM
- de la contamination cellulaire

Score corrélé à la charge virale

- 14 LCR + HSV1 ou HSV2
- Quantification (qPCR)



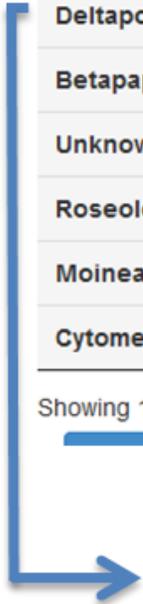


Search:

	Sample Reads	NTC Reads
Alphatorquevirus	10985	0
Molluscipoxvirus	6091	0
Deltapolyomavirus	2254	0
Betapapillomavirus	855	0
Unknown Genus	149	2
Roseolovirus	26	0
Moineauvirus	19	0
Cytomegalovirus	6	0

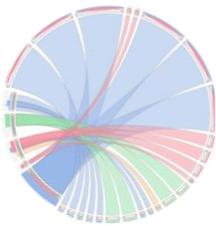
Showing 1 to 8 of 8 entries

Previous **1** Next



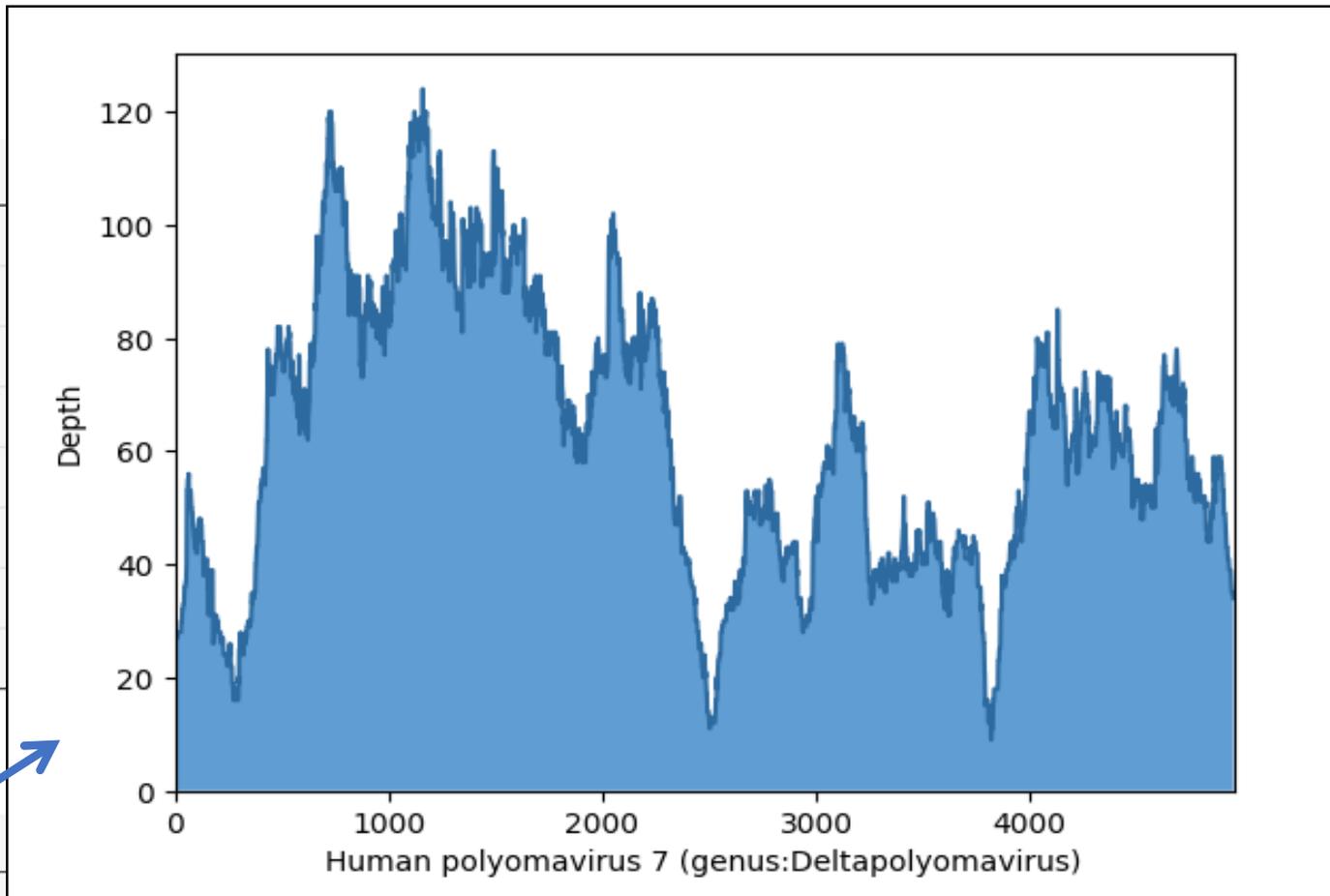
	Sample			NTC		
	Verified Reads	Kraken	Coverage %	Verified Reads	Kraken	Coverage %
Human polyomavirus 7	2254	2254	99.97981	0	0	0





- Alphatorquevirus
- Molluscipoxvirus
- Deltapolyomavirus
- Betapapillomavirus
- Unknown Genus
- Roseolovirus
- Moineauvirus
- Cytomegalovirus

Showing 1 to 8 of 8 entries

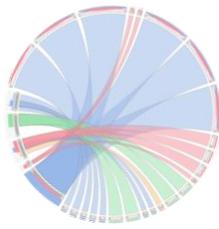


	Verified Reads	Kraken	Coverage %	Verified Reads	Kraken	Coverage %
Human polyomavirus 7	2254	2254	99.97981	0	0	0

Séquences (fasta)

Permet des analyses supplémentaires





Rapport clinique

	Résultat	Unité	Val. de Réf.
Dossier n° :	762400910391		
Prélevé le :	13/02/2024 00:00		
Saisi le :	26/03/2024 12:32		

Laboratoire de Virologie - Accueil : 01 42 49 94 79 - Hotline : 01 42 38 50 28

VIROLOGIE MOLECULAIRE

Pathogènes par Métagénomique clinique

Prélèvement: Autre PIED GRAINS MYCETOME EXTRAIT ADN/ARN

Conclusion virus ADN

Séquençage Haut Débit ADN (ILLUMINA)
Pipeline META_VIRO.1.1

Indélectable

Conclusion bactéries ADN

Séquençage Haut Débit ADN (ILLUMINA)
Pipeline META_VIRO.1.1

Présence d'ADN de Alcaligenes sp, Citrobacter koseri, Streptococcus pyogenes, Staphylococcus aureus et de Finegoldia magna.

Conclusion champignons ADN

Séquençage Haut Débit ADN (ILLUMINA)
Pipeline META_VIRO.1.1

Présence d'ARN de Chaetomium globosum

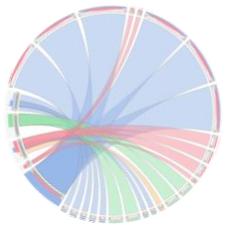
Conclusion virus ARN

Séquençage Haut Débit ARN (ILLUMINA)
Pipeline META_VIRO.1.1

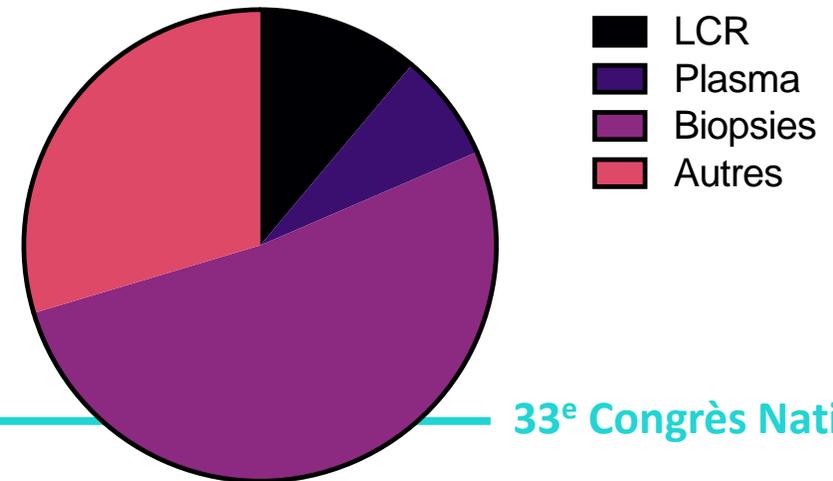
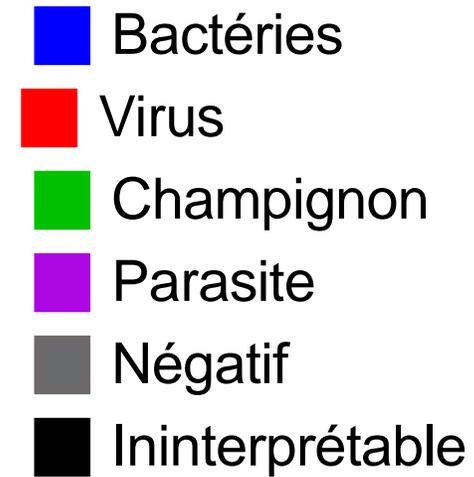
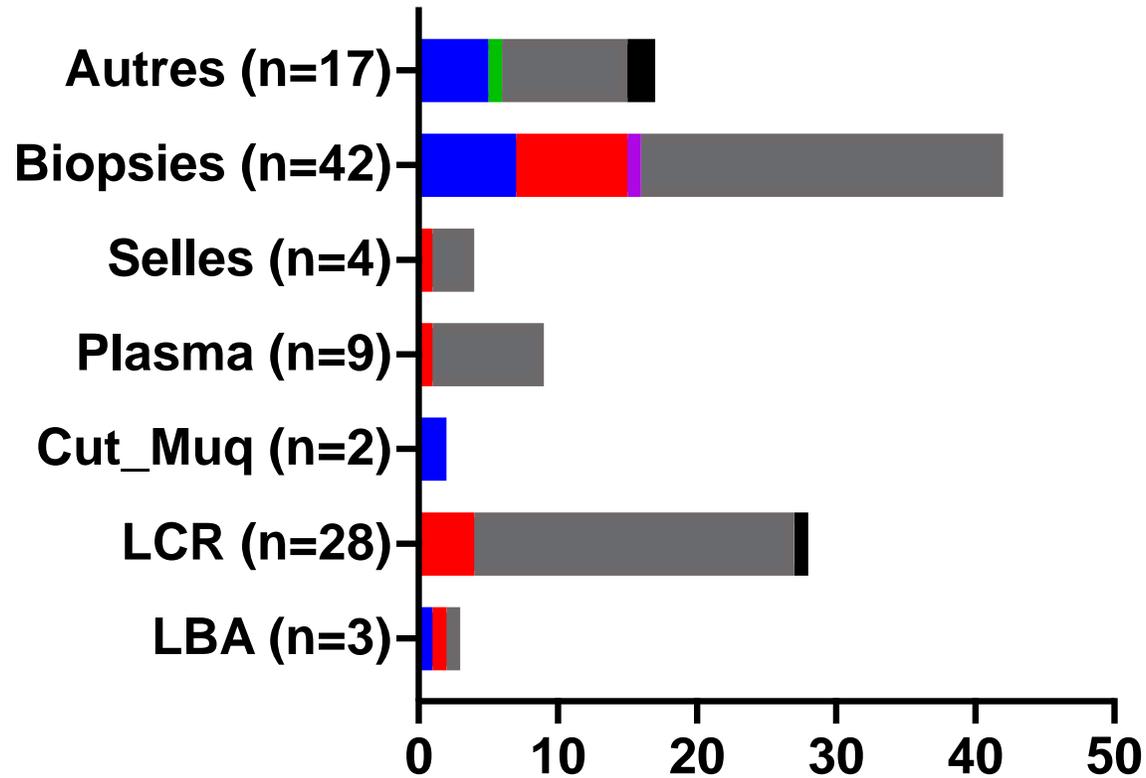
Indélectable

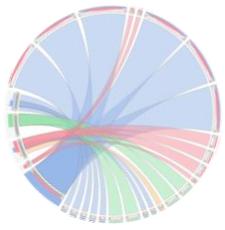
Les données administratives, sociales et médicales qui sont collectées sont utiles pour assurer votre prise en charge et la gestion de votre dossier. Pour toute information, vous pouvez consulter la politique d'information de l'Assistance Publique - Hôpitaux de Paris disponible sur le site <https://www.aphp.fr/patient-public/vos-droits/protection-des-donnees-personnelles-information-patient>.



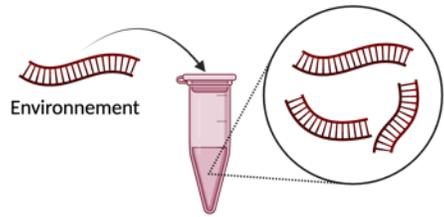


Les derniers mois sur SLS

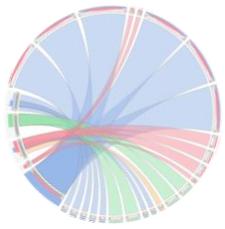




Les limites de la métagénomique



Contamination



Contamination

- Le «*Labome*» et le «*Kitome* »
- Cross contamination

- Contrôles négatifs
- Marche en avant
- Surveillance des lots de réactifs
- Suivi de la contamination environnementale

Acinetobacter	316
Staphylococcus	27
Corynebacterium	26
Paracoccus	8
Nocardioides	6
[Propionibacterium]	5
Blastococcus	5
Brevundimonas	5

Showing 1 to 8 of 8 entries

CSV Copy

	Verified Reads	Sample
Acinetobacter lwoffii WJ10621	81	1
Acinetobacter johnsonii ANC 3681	52	6
Acinetobacter johnsonii TG19605	45	17
Acinetobacter johnsonii SH046	35	7

Bacteria

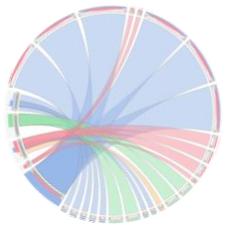
Search:

	Sample Reads
Cutibacterium	653
Corynebacterium	320
[Propionibacterium]	260
Staphylococcus	257
Paracoccus	72
Dietzia	45
Micrococcus	32
Nocardioides	31
Blastococcus	29
Modestobacter	23

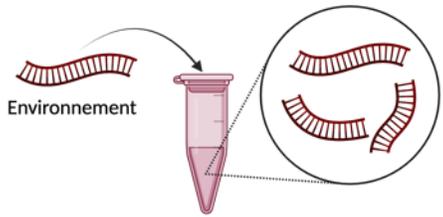
Showing 1 to 10 of 23 entries

Previous 1 2 3

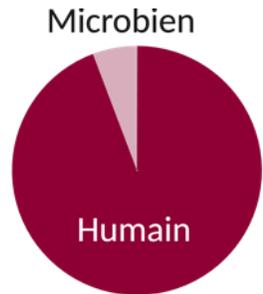




Les limites de la métagénomique

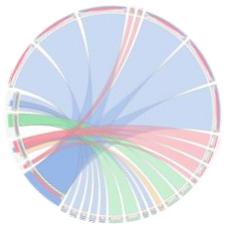


Contamination

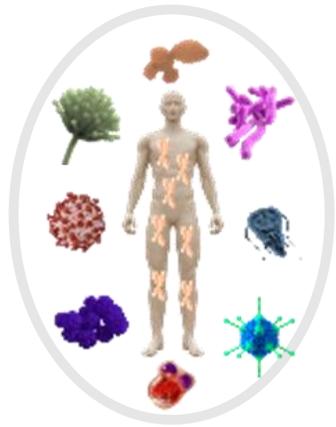


Sensibilité





Des charges microbiennes parfois faible...



LBA, Aspirations



Liquide divers
(pus, abcès)



Biopsie



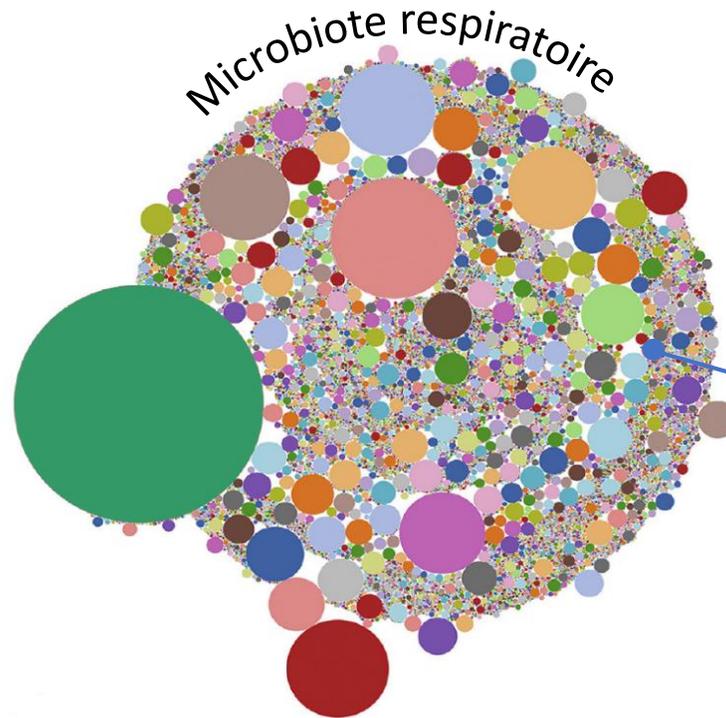
Serum/plasma



Sang



LCR



Fungi

Pneumocystis jirovecii
Pneumocystose du sujet non-VIH
Charge *P. jirovecii*, Ct 30-34

Alanio et al. 2017.

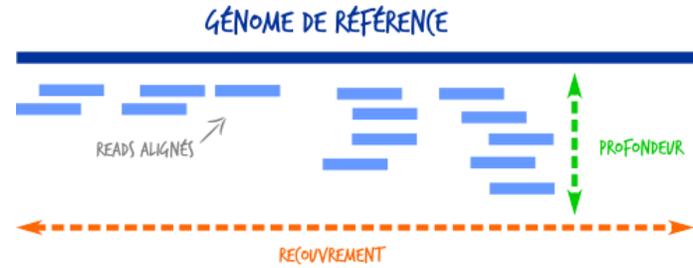
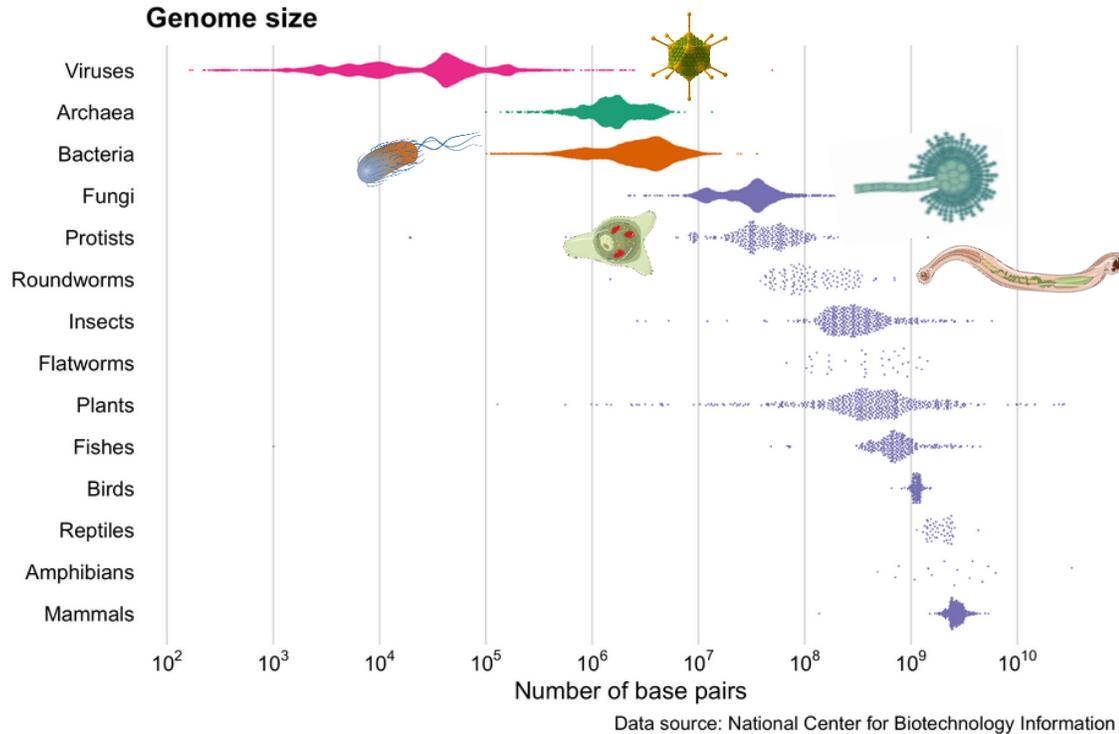
En métagénomique « shotgun » : tous les ADN/ARN sont amplifiés
Par ordre de fréquence : ADN/ARN humains ++++ (>99%) >> ADN/ARN bactériens ++ >
ADN/ARN viraux >> ADN/ARN fongiques





... combinées à des tailles de génomes variables

Taille des génomes (paire de base)

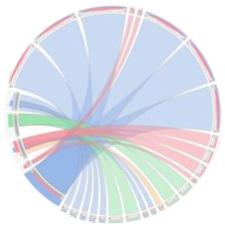


Petit génome + faible charge -> faible nombre de séquences obtenues

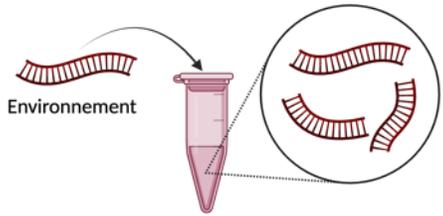
Gros génome + faible charge -> séquençage et identification moins performants



Si possible, ↗ la profondeur de séquençage
De 10 à 20 millions de reads (arbitraire) ?



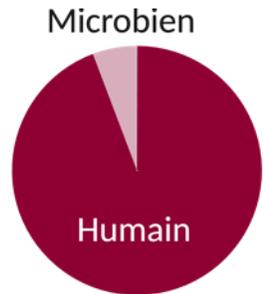
Les limites de la métagénomique



Contamination



Coûts élevés

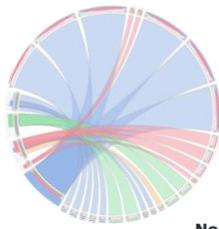


Sensibilité



Infrastructure et
personnel spécialisés



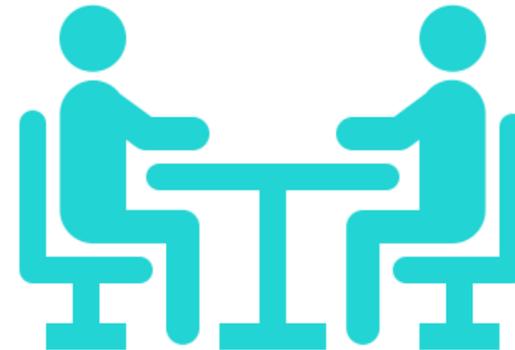


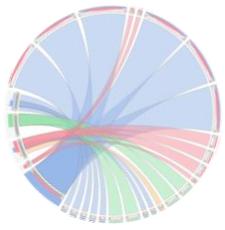
Le rendu de la métagénomique clinique

Nom	N° taxonomique	Nombre de séquences
<i>Veillonella parvula</i>	29466	10
<i>Faecalitalea cylindroides</i>	39483	10
<i>Lactobacillus mucosae</i>	97478	10
<i>Pseudomonas sp. LG1D9</i>	2083054	10
<i>Bacteroides ovatus</i>	28116	9
[<i>Eubacterium</i>] <i>siraeum</i>	39492	9
<i>Bifidobacterium dentium</i>	1689	9
butyrate-producing bacterium SS3/4	245014	9
<i>Roseburia hominis</i>	301301	9
<i>Dialister pneumosintes</i>	39950	8
<i>Lactobacillus casei</i>	1582	8
<i>Lactobacillus gasseri</i>	1596	8
<i>Blautia hansenii</i>	1322	8
<i>Alistipes shahii</i>	328814	7
<i>Streptococcus parasanguinis</i>	1318	7
<i>Odoribacter splanchnicus</i>	28118	6
<i>Bacteroides caecimuris</i>	1796613	6
[<i>Clostridium</i>] <i>bolteae</i>	208479	6
<i>Acinetobacter schindleri</i>	108981	6
<i>Acinetobacter johnsonii</i>	40214	6
<i>Parabacteroides distasonis</i>	823	6
<i>Pseudomonas aeruginosa group</i>	136841	6
<i>Desulfovibrio fairfieldensis</i>	44742	6
<i>Enterobacter hormaechei</i>	158836	6
<i>Monoglobus pectinilyticus</i>	1981510	6
<i>Lachnospirillum sp. YL32</i>	1834196	6
<i>Lachnospirillum phocaense</i>	1871021	5
<i>Murdochella vaginalis</i>	1852373	5
<i>Flavonifractor plautii</i>	292800	5

Rendu pluridisciplinaire

- Experts métagénomique clinique
- Experts microbiologistes toutes les spécialités
- Contexte clinique +++ (prélèvement adapté)

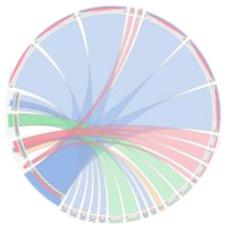




Là où ça ne marche pas...



- **Prélèvement inapproprié**
 - Ex : Biopsie cérébrale / LCR
- **Diagnostic sérologique uniquement**
 - MgC = Technique directe
- **Faible quantité de pathogène**
 - Nécessité d'augmenter la profondeur de séquençage — le coût — le temps d'analyse ... etc
 - Infections fongiques, à mycobactéries

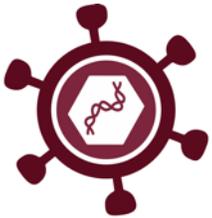


Analyses secondaires

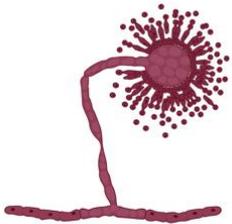
Recherche de supports de résistance antimicrobienne



Concordance de 92% sur *Klebsiella pneumoniae*
Concordance >95% sur *Acinetobacter baumannii*
⚠ Gènes présents chez pathogènes et commensaux (*mecA*)



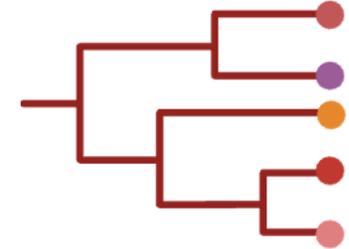
VIH, VHC, CMV, HSV, Virus de la grippe



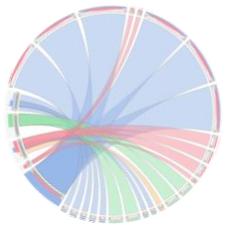
Résistance aux azolés pour *Aspergillus fumigatus*
Résistance aux échinocandines pour *Candida* spp.

Epidémiologie

Etudes phylogénétiques

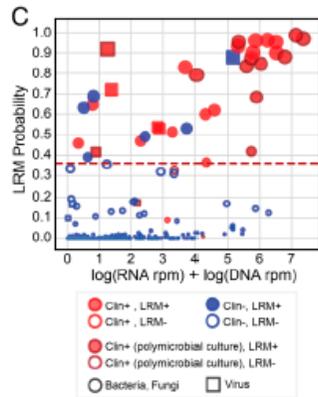


Tamma PD et al., *Antimicrob Agents Chemother.* 2018, Ruppé E et al., *Nat Microbiol.* 2019., Hu X et al., *J Clin Microbiol.* 2023, d'Humières C et al., *Therapeutic Perspectives. Drugs.* 2021; Quick J et al., *Nature.* 2016, Rhodes J et al., *Nat Microbiol.* 2022, Spettel K et al., *PLoS One.* 2019

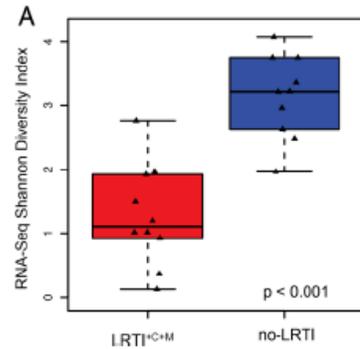


Intégration de la réponse de l'hôte et du microbiote

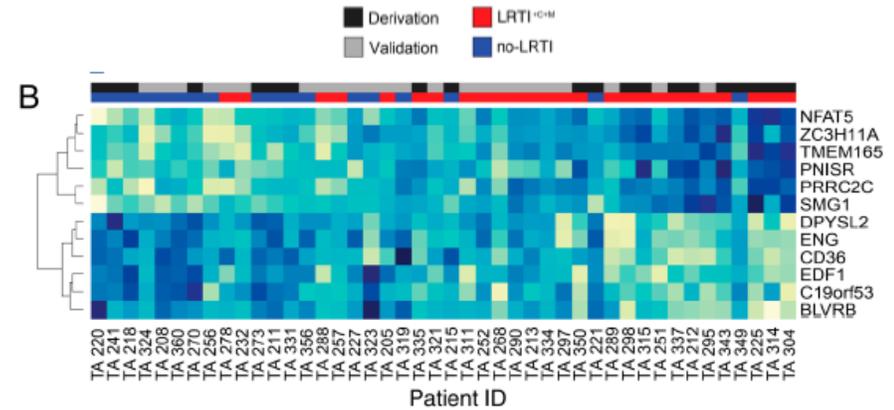
26 patients LRTI +/- 18 patients LRTI -
DNA and RNA sequencing.



Relative abundance of microorganisms



Alpha-diversity of the microbiota



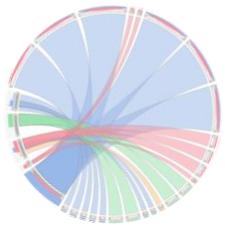
Host's response

Prediction of LRTI with 100% sensitivity (87.5% specificity)



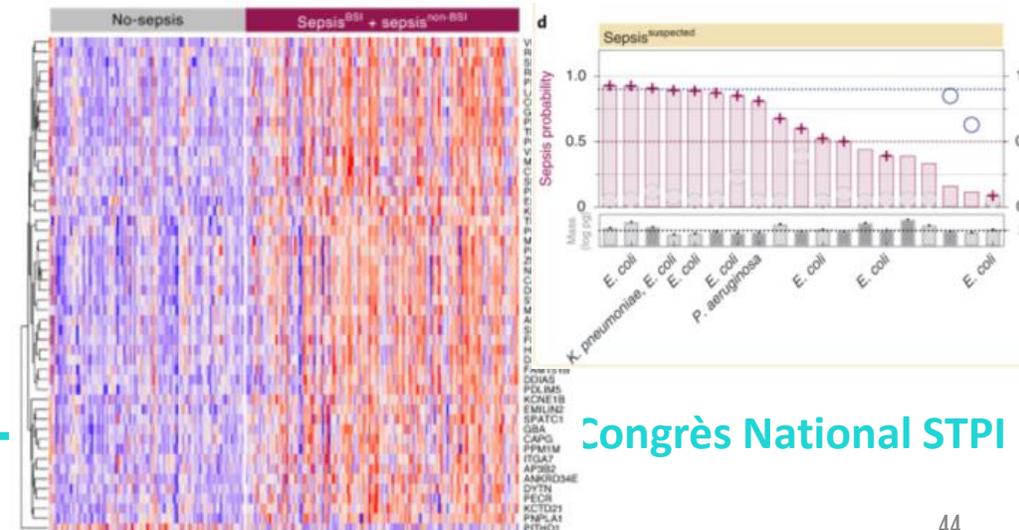
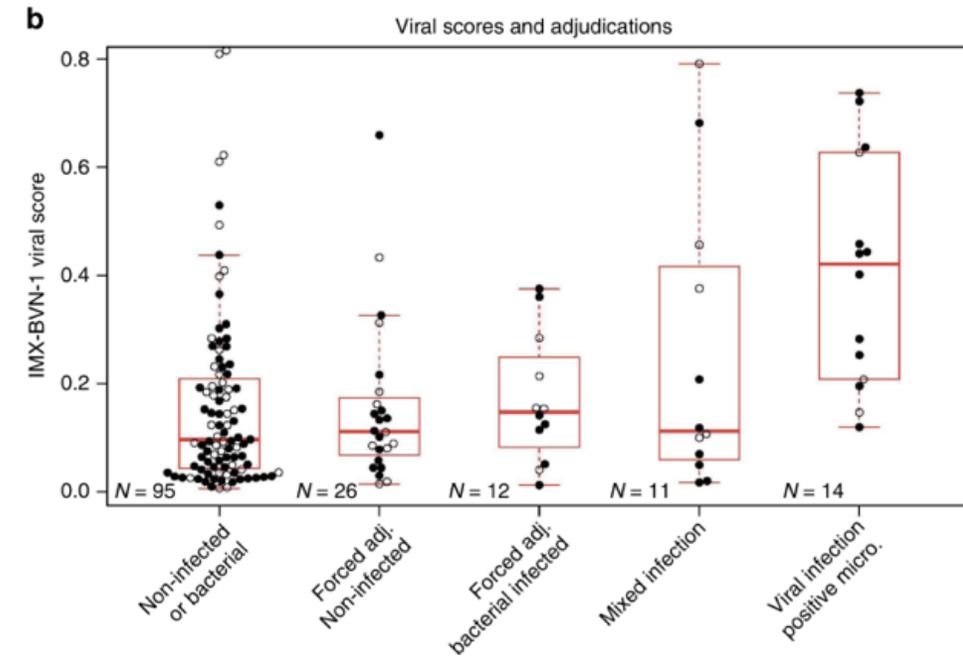
Langelier et al., PNAS 2018

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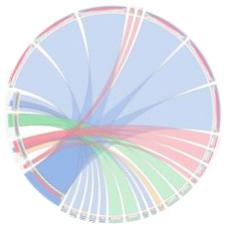


Métagénomique et IA

- Prédire le diagnostic:
 - Différencier Infection virales et bactériennes
 - Réseau de Neurones entraîné sur 18 études transcriptomiques
 - Validé sur une cohorte indépendante avec 29 mRNA
 - AUC-ROC = 0.92
- Coupler analyse de la réponse de l'hôte et métagénomique clinique
 - Sepsis et modèle de ML
 - Identification de
 - 99% des cas de sepsis microbiologiquement confirmés
 - 74% des cas de sepsis suspectés
 - 89% des cas indéterminés



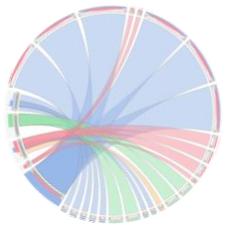
M.B. Mayhew et al, Nature com, 2020 , K.L. Kalantar et al., Nat Microbiol, 2022



Conclusion

- Métagénomique clinique utilisable dans **tous types d'infections**
 - **Complémentaire** aux méthodes conventionnelles
 - Diagnostic et analyses secondaires en 1 seule étape
 - **Performances diagnostiques variables** selon les études et selon le site d'infection
 - Etudes cliniques prospectives pour codifier son utilisation en routine
 - Interprétation difficile
- Importance du **dialogue** entre microbiologistes et cliniciens





L'équipe de métagénomique

Responsable de plateforme



Dr Maud Salmona

Nos étudiants



Équipe technique wet-lab et dry-lab



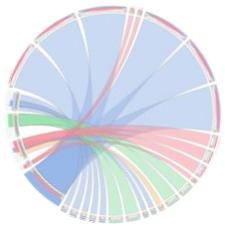
Expertise microbiologique



Hôpitaux Universitaires
SAINT-LOUIS
LARIBOISIÈRE
FERNAND-WIDAL



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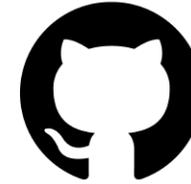
Merci de votre attention



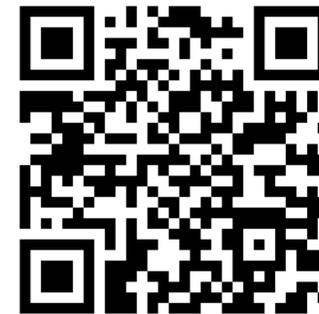
Société Française
de Microbiologie



theo.ferreira@aphp.fr



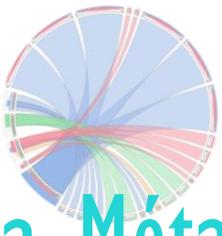
GitHub



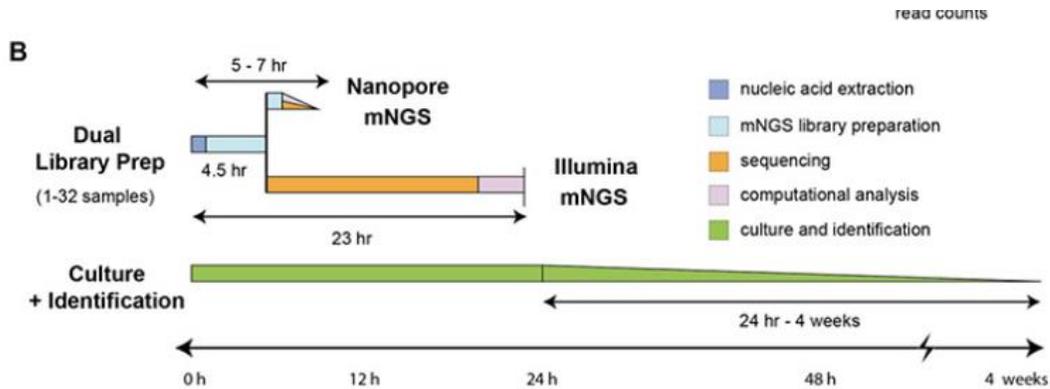
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La Métagénomique « rapide »



Sample to answer median : 6 H
Temps de séquençage médian : 50 min

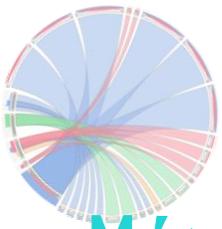
Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids

[Wei Gu](#), [Xianding Deng](#), [Marco Lee](#), [Yasemin D. Sucu](#), [Shaun Arevalo](#), [Doug Stryke](#), [Scot Federman](#), [Allan Gopez](#), [Kevin Reyes](#), [Kelsey Zorn](#), [Hannah Sample](#), [Guixia Yu](#), [Gurpreet Ishpuniani](#), [Benjamin Briggs](#), [Eric D. Chow](#), [Amy Berger](#), [Michael R. Wilson](#), [Candace Wang](#), [Elaine Hsu](#), [Steve Miller](#), [Joseph L. DeRisi](#) & [Charles Y. Chiu](#) ✉

[Nature Medicine](#) 27, 115–124 (2021) | [Cite this article](#)



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La Métagénomique en POC

Real-time metagenomics-based diagnosis of community-acquired meningitis: A prospective series, southern France

Madjid Morsli,^{a,b} Agathe Boudet,^{c,d} Quentin Kerharo,^{a,e} Robin Stephan,^d Florian Salipante,^{d,f} Catherine Dunyach-Remy,^{c,d} Linda Houhamdi,^a Pierre-Edouard Fournier,^{a,g} Jean Philippe Lavigne,^{c,d} and Michel Drancourt^{a,b,e,g}



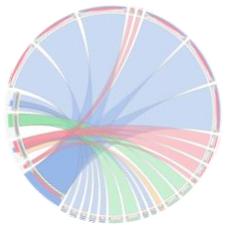
- 52 LCR : RTM (47 +) vs Multiplex (49+)
- RTM : detection + antibiogramme *in silico* (62% concordance) + Genotype
- Analyse RT sur DB de 12 pathogènes + Epi2me NCBI



RTM : 120 min

Filmarray : 90min

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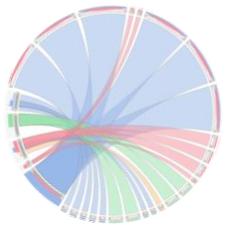
Exemple : Bactérie non attendue

Patient LyB rituximab
Fièvre inexpliquée récurrente
Troubles digestifs et urinaires

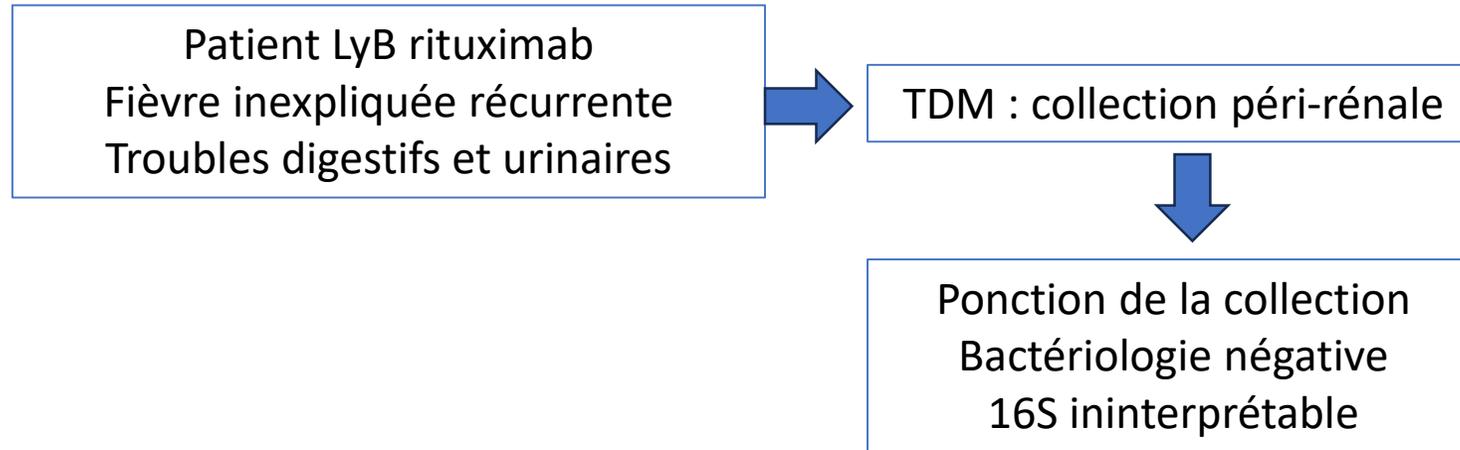


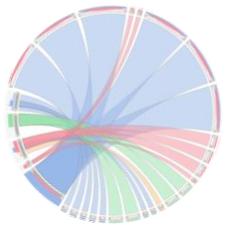
TDM : collection péri-rénale



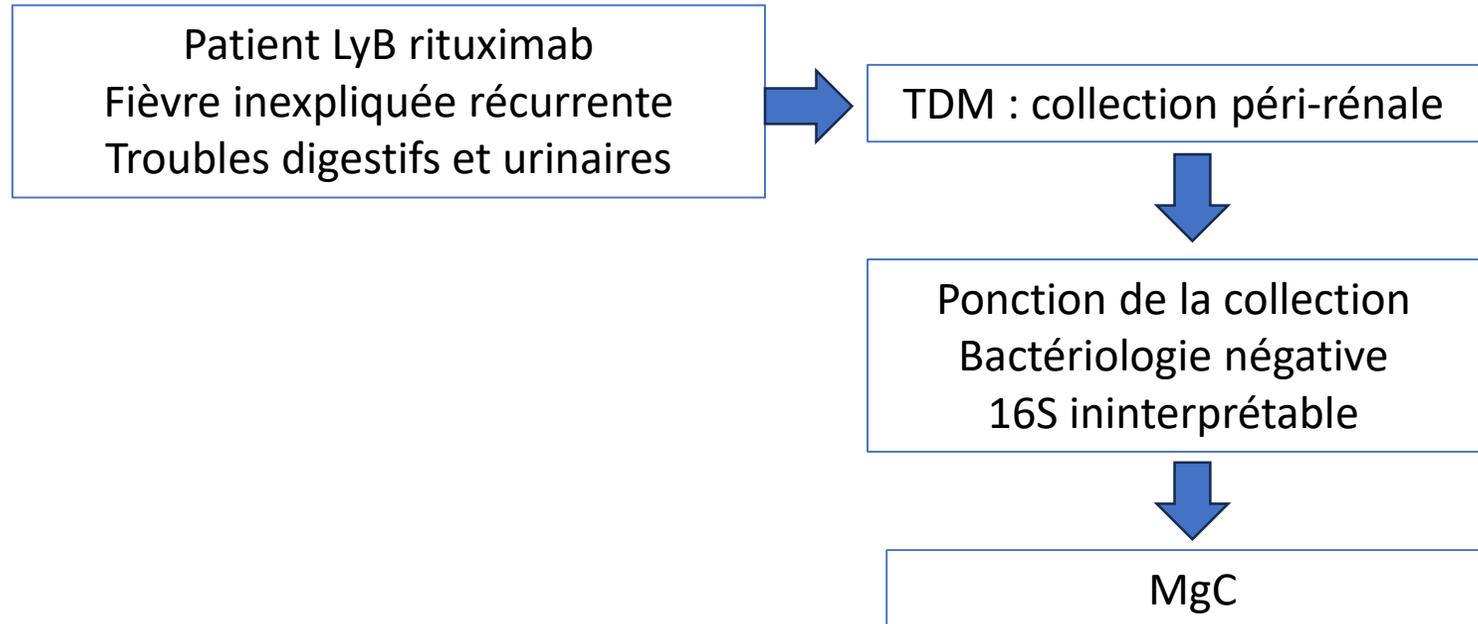


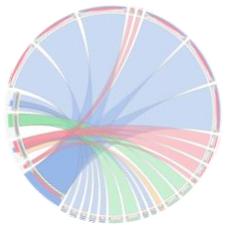
Exemple : Bactérie non attendue





Exemple : Bactérie non attendue





Exemple : Bactérie non attendue

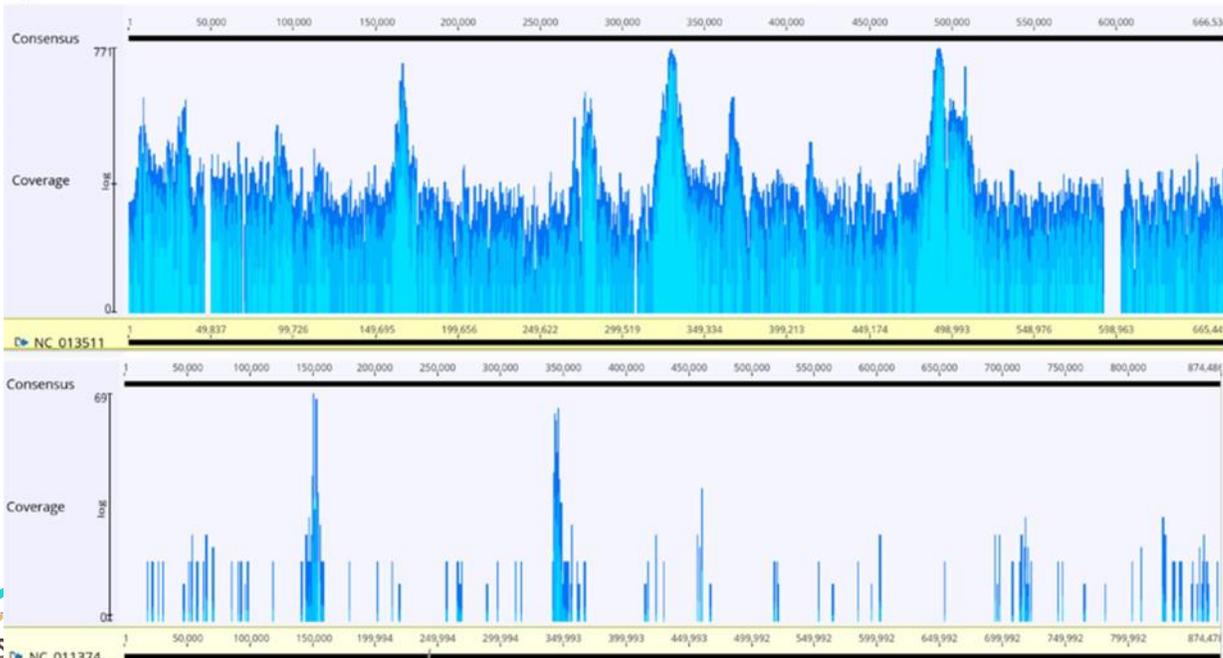
Patient LyB rituximab
Fièvre inexpliquée récurrente
Troubles digestifs et urinaires

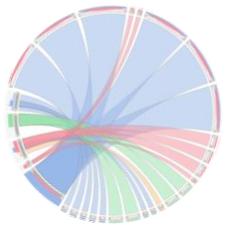
TDM : collection péri-rénale

Ponction de la collection
Bactériologie négative
16S ininterprétable

MgC

Mycoplasma hominis
Ureaplasma urealyticum
Toque Tenovirus





Exemple : Bactérie non attendue

Patient LyB rituximab
Fièvre inexpliquée récurrente
Troubles digestifs et urinaires

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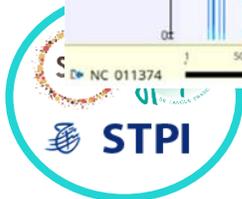
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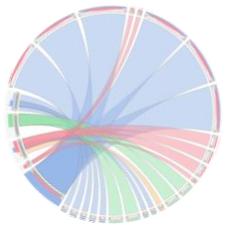
MgC

Mycoplasma hominis
Ureaplasma urealyticum
Toque Tenovirus

Pas de gène de
résistance

FQ + tétracyclines
Évolution favorable

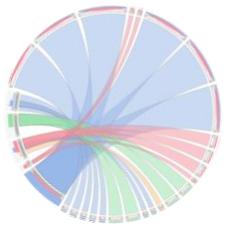




Exemple : Nouveaux virus

Encéphalite chez une patiente avec DI et lymphome





Exemple : Nouveaux virus

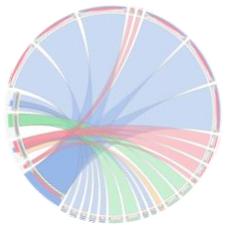
Encéphalite chez une patiente avec DI et lymphome



LCR : diagnostic de routine :neg
MgC : neg



Biopsie cérébrale



Exemple : Nouveaux virus

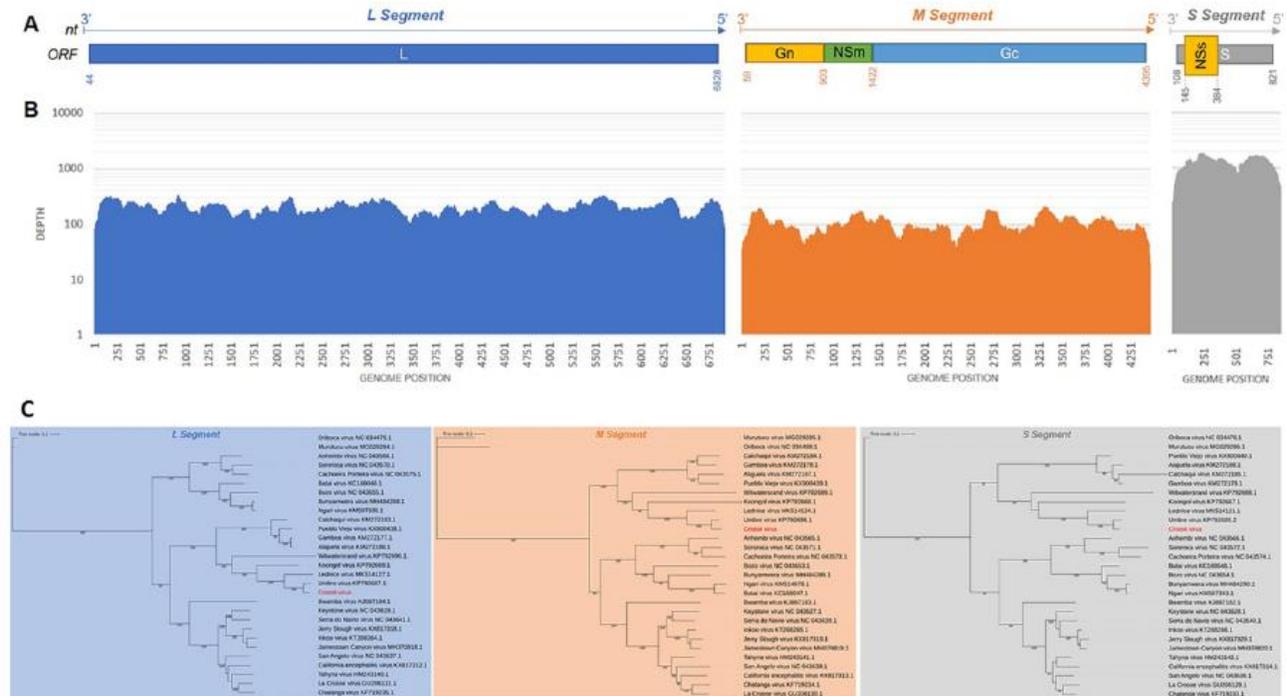
Encéphalite chez une patiente avec DI et lymphome

LCR : diagnostic de routine : neg
MgC : neg

Biopsie cérébrale

Mg C → Cristoli virus

Décès de la patiente



Nouveau virus : CRISTOLI VIRUS

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