Intérêt du NGS en maladies infectieuses

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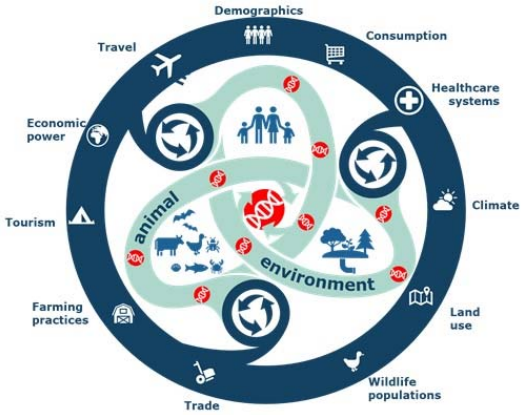
Inserm

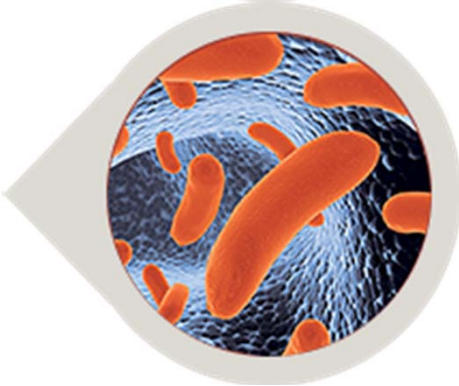
UPEC Connaissance - Action

UNIVERSITÉ PARIS-EST CRÉTEIL VAL DE MARNE

Surveillance

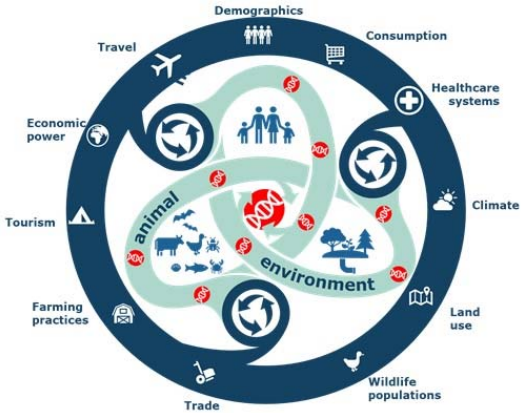


Diagnostic

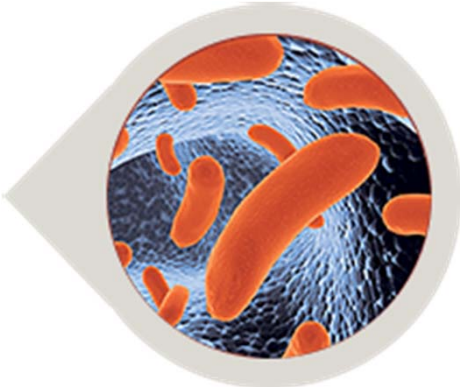


Microbial documentation

Surveillance



Diagnostic



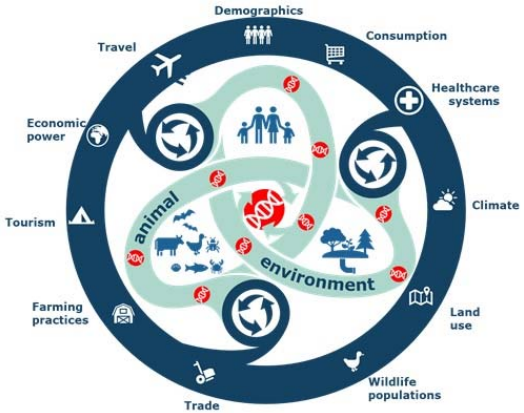
Genome characterization

NGS non ciblé

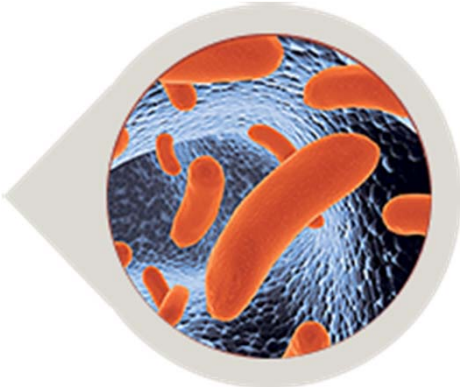
Microbial documentation

NGS non ciblé

Surveillance



Diagnostic



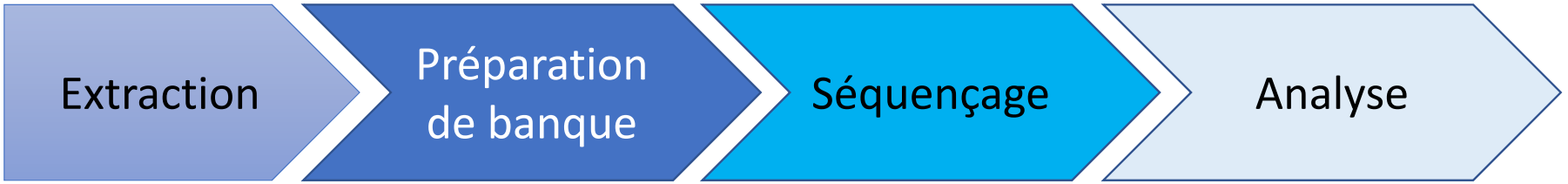
NGS Ciblé

Genome characterization

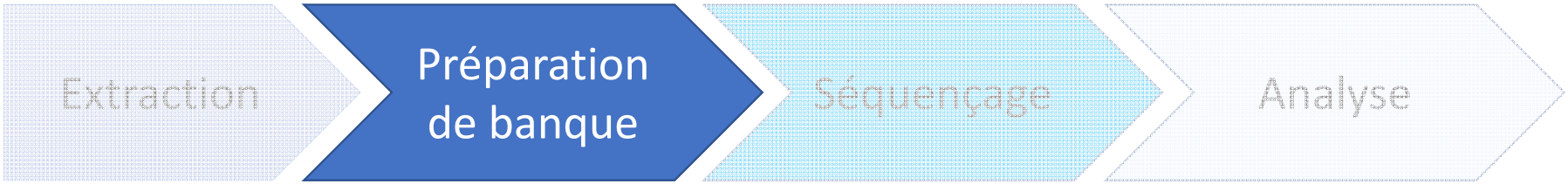
NGS Ciblé/NGS non ciblé

Processus techniques

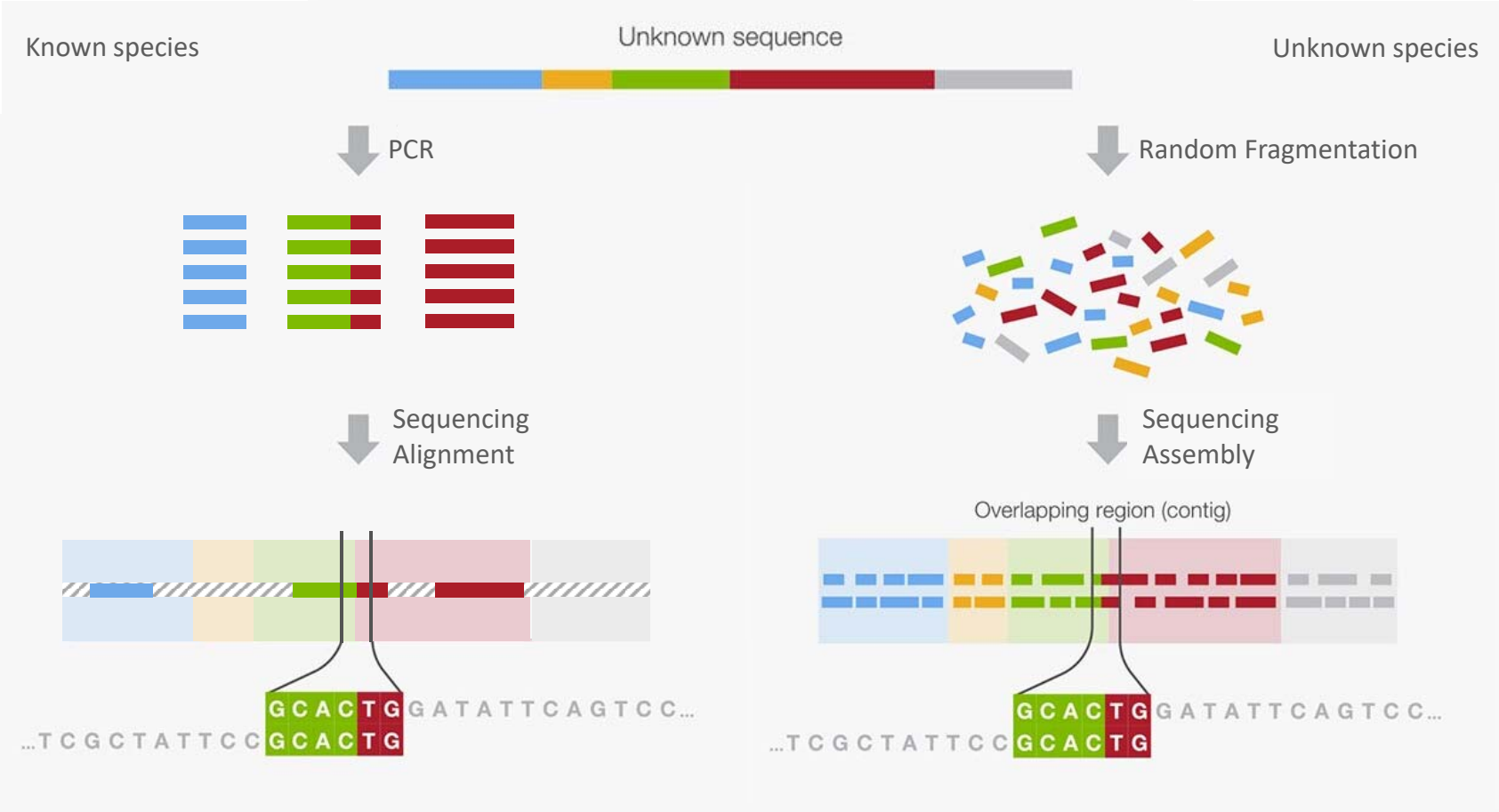
Workflow NGS



Workflow NGS

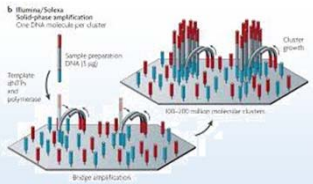
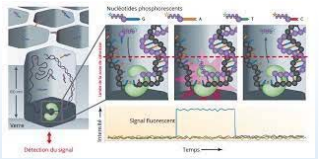
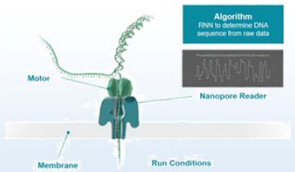
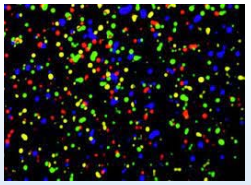
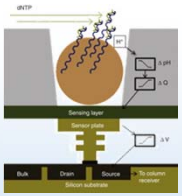
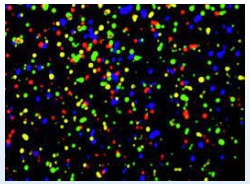
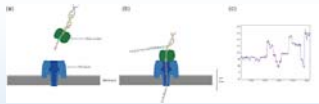


NGS Ciblé vs NGS Non ciblé (Shotgun)

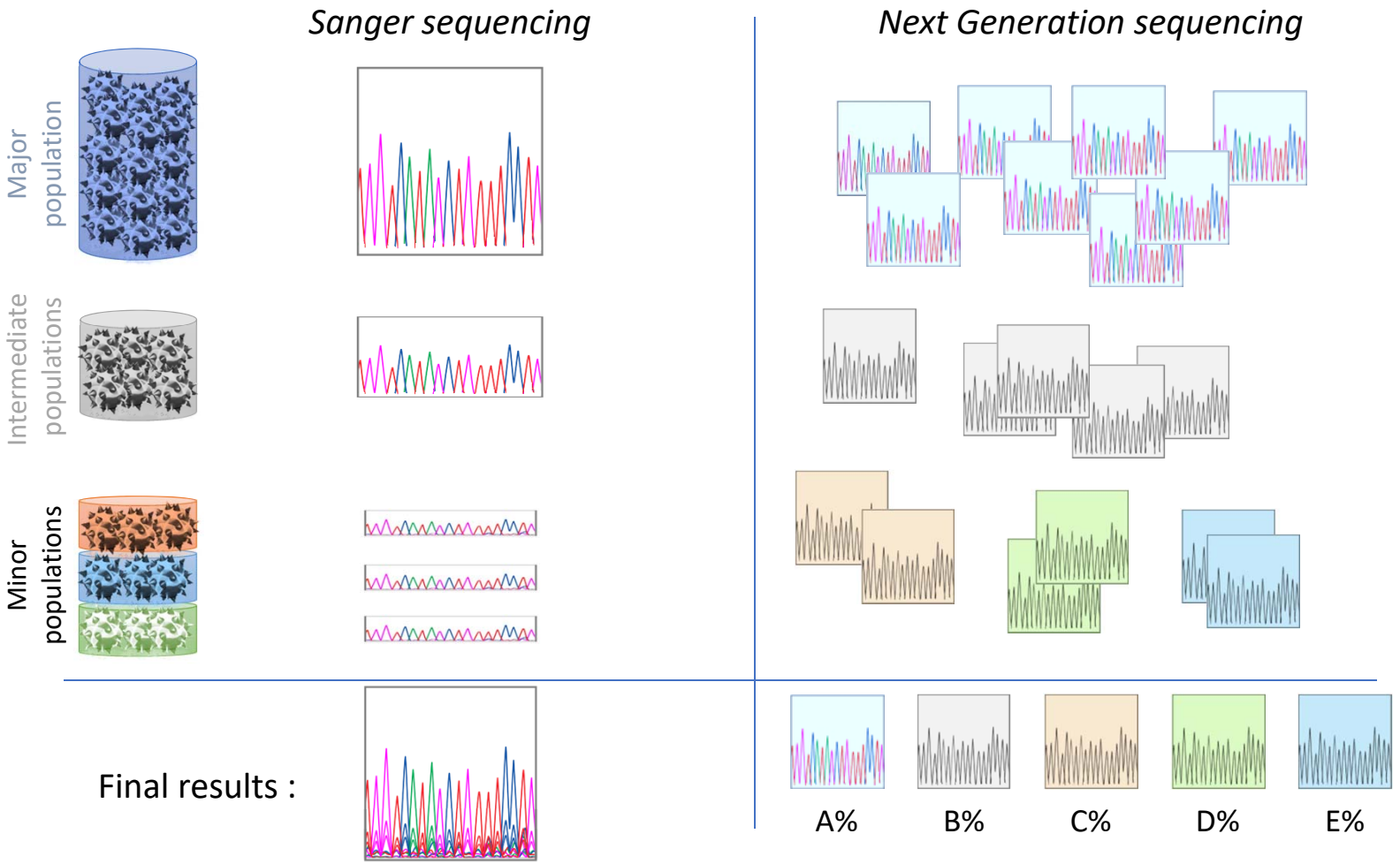


Workflow NGS

C’est quoi le NGS ?

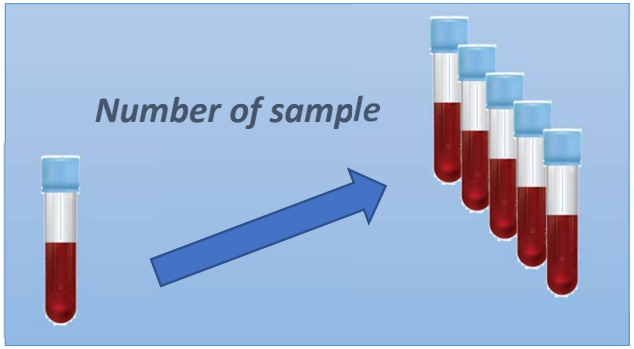
Plateforme	illumina® 	ThermoFisher SCIENTIFIC 	PACBIO® 	Oxford NANOPORE Technologies 
Isolement des séquences, amplification du signal	 ***Bridge PCR***	 ***Emulsion PCR***	 ***ZMW cells***	 ***Nanopore***
Acquisition du signal	 ***Fluorescence***	 ***pHmetrie***	 ***Fluorescence***	 ***Conductimetrie***

Sanger vs NGS



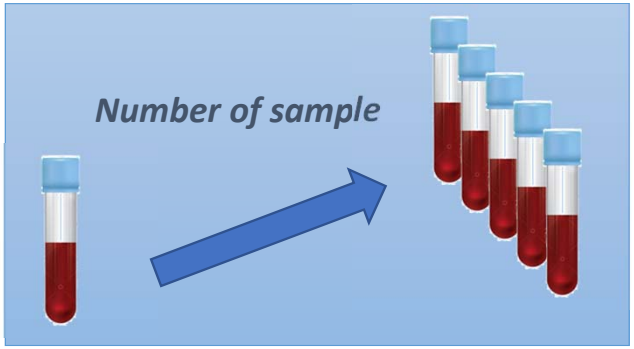
Quels avantages ?

Ciblé : Quantité
(échantillon et profondeur)



Quels avantages ?

Ciblé : Quantité (échantillon et profondeur)



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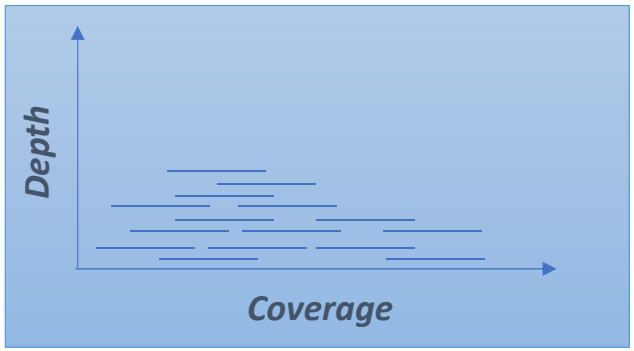
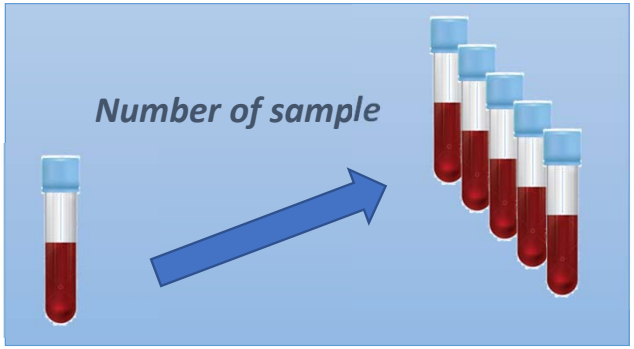


Depth : profondeur de séquençage => nombre de fois qu'une position génomique est séquencée

Couverture : portion du génome d'intérêt « couvert » par au moins une séquence

Quels avantages ?

Ciblé : Quantité (échantillon et profondeur)



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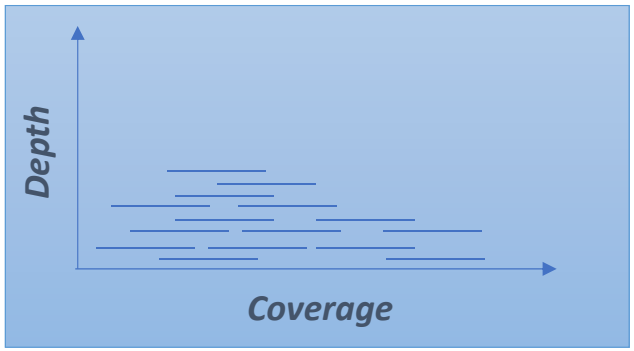
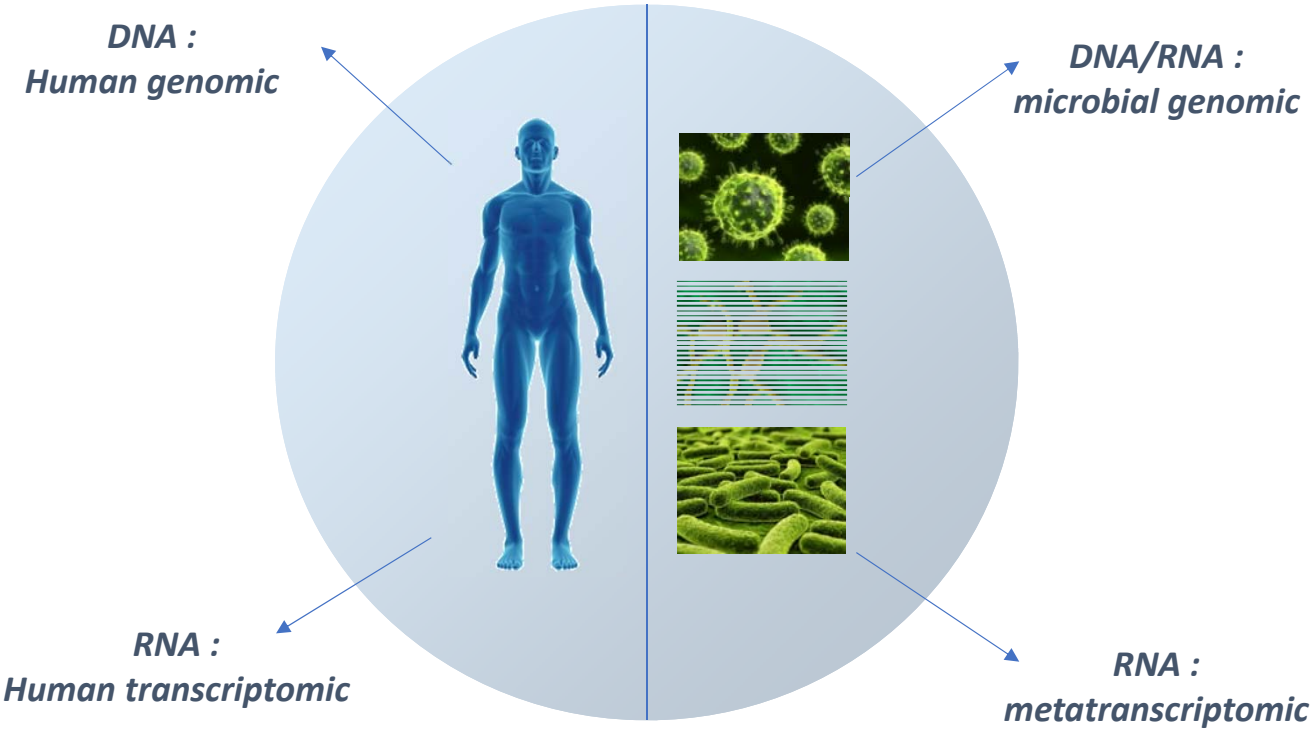


Non ciblé : Qualité (diversification des questions)



Quels avantages ?

Ciblé

1 question avec a priori :

Le VIH de mon patient est-il résistant ?

Non ciblé : Qualité (diversification des questions)

Questions multiples sans a priori :

Est-ce que mon patient est infecté ?
Si oui par quoi ?
Si c'est du VIH est-il résistant ?
…

Applications du NGS ciblé

Applications diagnostiques

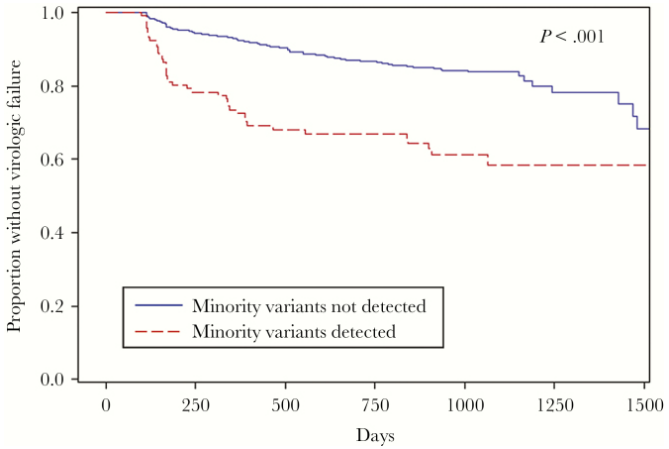
- *Résistance*
- *Microbiote par approche 16S/ITS*
- *Surveillance*

Applications diagnostiques

- *Résistance*
- *Microbiote par approche 16S/ITS*
- *Surveillance*

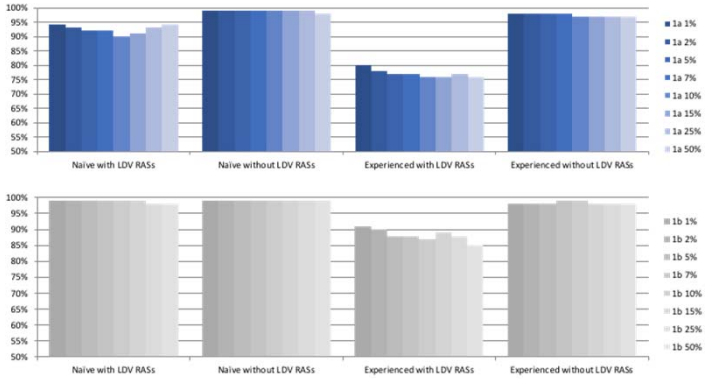
Résistance et variants minoritaires

VIH ARN



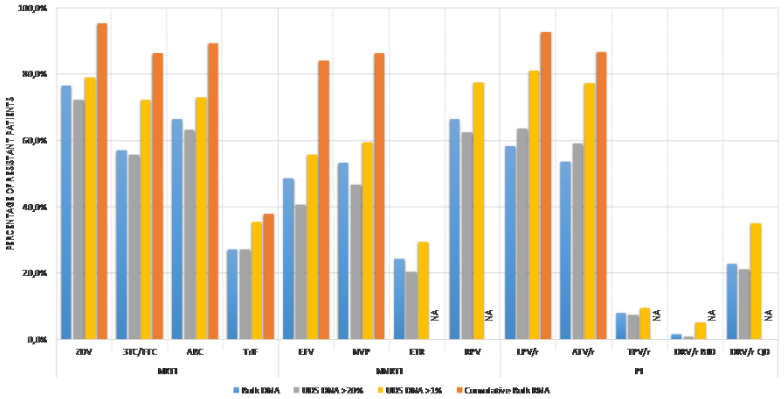
Stella-Ascariz et al; JID 2017

VHC ARN



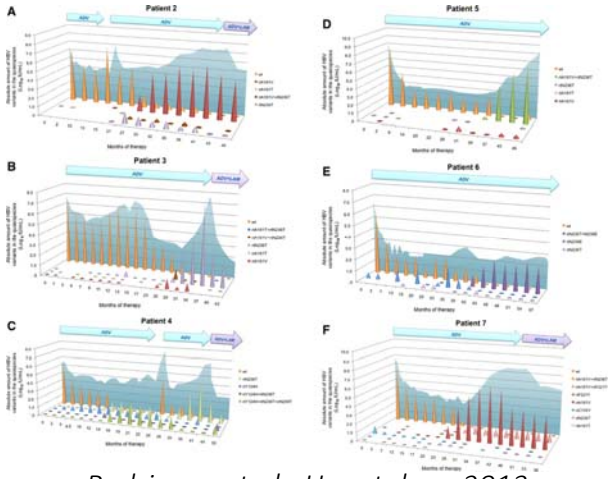
Adapted from Zeuzem et al., J Hepatol 2017

VIH ADN



Rodriguez et al; JAC 2018

VHB ADN



Rodriguez et al; Hepatology 2013

Les reco pour le VIH

Clinical Infectious Diseases

MAJOR ARTICLE

Human Immunodeficiency Virus Drug Resistance: 2018 Recommendations of the International Antiviral Society–USA Panel

Huldrych F. Günthard,[1] Vincent Calvez,[2] Roger Paredes,[3,4] Deenan Pillay,[5] Robert W. Shafer,[6] Annemarie M. Wensing,[7] Donna M. Jacobsen,[8] and Douglas D. Richman[9]

Box 2. Recommendations for Transmission of Minority Variants Harboring Drug-resistant Mutations

- Drug resistance testing to detect minority variants is not currently recommended outside of research settings, but may be considered for nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs; evidence rating AIIa).

Cut-off 1-5%

Günthard et al; CID 2019

Les reco pour le VHC

Guidelines

JOURNAL OF HEPATOLOGY

EASL Recommendations on Treatment of Hepatitis C 2018☆

European Association for the Study of the Liver*

Recommendations

- Testing for HCV resistance prior to treatment is not recommended (**B1**).
- HCV resistance testing prior to retreatment in patients who failed after any of the DAA-containing treatment regimens is useful to guide retreatment by probabilities of response, according to the resistance profile observed in the context of a multidisciplinary team including experienced treaters and virologists (**B2**).

AASLD IDSA **HCV Resistance Primer**
From www.HCVGuidance.org on December 03, 2019

HCV Resistance Primer

3. Assay Summary Points

- Either population sequencing or deep sequencing can be used to detect the presence of RASs in NS3, NS5A, and NS5B.
- For clinical decisions, population sequencing or deep sequencing with at least 15% prevalence of RASs as the cutoff is recommended. The presence of RASs with <15% prevalence should not be considered clinically significant.
- When assessing the potential clinical effect of RASs, it is important to determine the drug-specific RASs.

Resistance Testing in Clinical Practice

Regimen-Specific Recommendations for Use of RAS Testing in Clinical Practice

RECOMMENDED	RATING
Elbasvir/grazoprevir NS5A RAS testing is recommended for genotype 1a-infected, treatment-naive or -experienced patients being considered for elbasvir/grazoprevir. If present, a different regimen should be considered.	I, A
Ledipasvir/sofosbuvir NS5A RAS testing can be considered for genotype 1a-infected, treatment-experienced patients with and without cirrhosis being considered for ledipasvir/sofosbuvir. If clinically important[a] resistance is present, a different recommended therapy should be used.	I, A
Sofosbuvir/velpatasvir NS5A RAS testing is recommended for genotype 3-infected, treatment-naive patients with cirrhosis and treatment-experienced patients (without cirrhosis) being considered for 12 weeks of sofosbuvir/velpatasvir. If Y93H is present, weight-based ribavirin should be added or another recommended regimen should be used.	I, A

Cut-off 15%

Les reco pour le VHB

Clinical Practice Guidelines

CrossMark

EASL | JOURNAL OF HEPATOLOGY

EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection☆

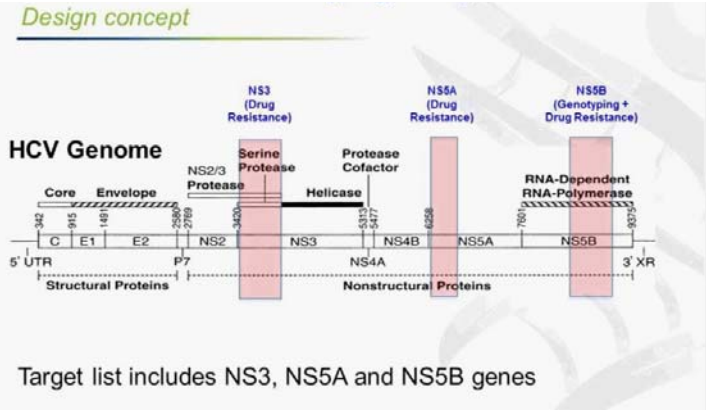
European Association for the Study of the Liver*

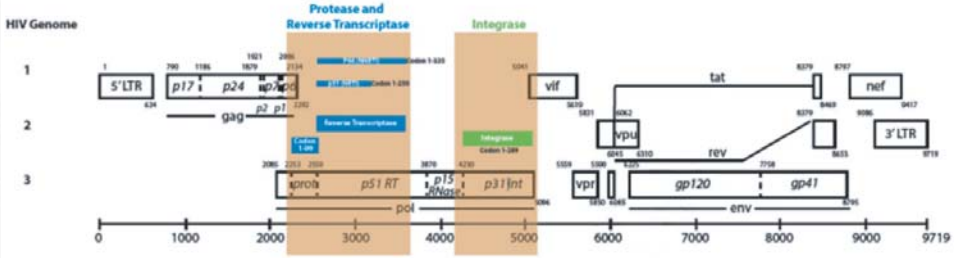
Recommendations

- Prevention of resistance should rely on the use of first line therapy with high barrier to resistance NAs (Evidence level I, grade of recommendation 1).
- Compliance to NA therapy should be checked in all cases of treatment failure (Evidence level II-1, grade of recommendation 1).
- Management of treatment failure should be based on NAs cross-resistance data (Evidence level II-2, grade of recommendation 1).
- Treatment adaptation should be performed as soon as virologic failure under NAs is confirmed (Evidence level II-1, grade of recommendation 1).

Intérêt ?

Automatisation complète du processus !



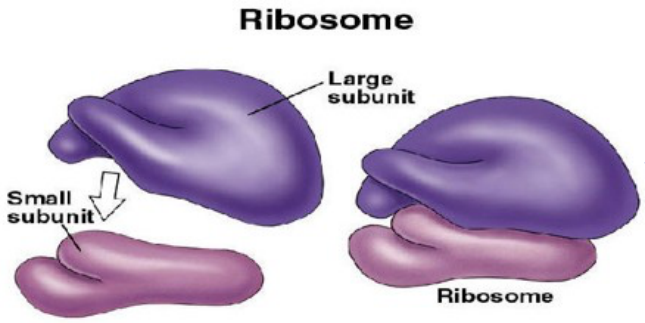


https://www.veladx.com/solutions/next-generation-sequencing.html

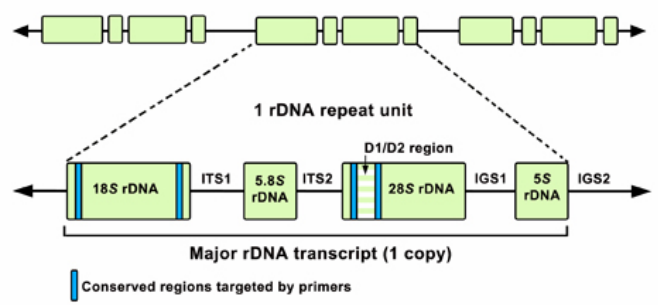
Applications diagnostiques

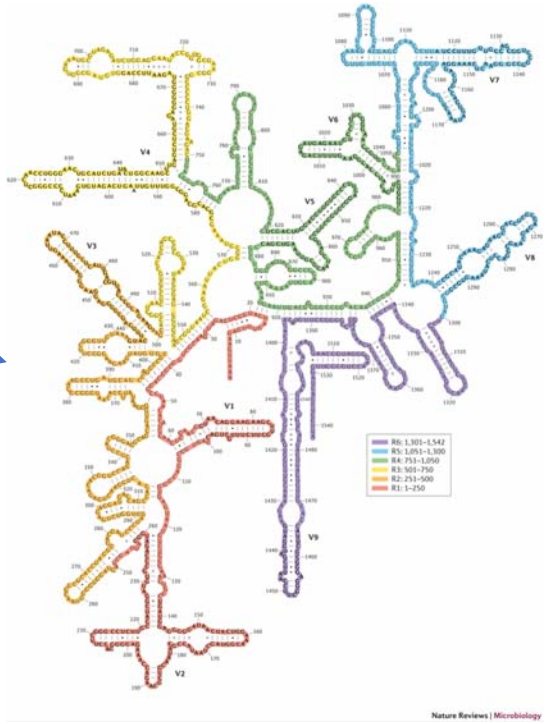
- *Résistance*
- *Microbiote par approche 16S/ITS*
- *Surveillance*

Qu’est ce que le 16S/ITS



Champignons





Bactéries

16S identification

A

Strain	Sequence	Accession number
S. epidermidis	TCCT CTGACCCCTCTAGAGATAGAGT TTT	L37605
S. saccharolyticus	TCCT CTGACCCCTCTAGAGATAGAGT TTT	L37602
S. capitis	TCCT CTGA**T**CCCTCTAGAGATAGAGT TTT	L37599
S. auricularis	TCCT **T**TGACC**G**CTCTAGAGATAGAGT TTT	L37598
S. warneri	TCCT **T**TGACC**G**CTCTAGAGATAGAGT TTT	L37603
S. haemolyticus	TCCT **T**TGAC**AA**CTCTAGAGATAGAG**C** TTT	L37600
S. aureus	TCCT **T**TGAC**AA**CTCTAGAGATAGAG**C** TTT	L37597
S. hominis	TCCT **T**TGACCC**T**TCTAGAGATAGA**AG** TTT	L37601
S. saprophyticus	TCCT **T**TGA**AAA**CTCTAGAGATAGAG**C** TTT	L37596

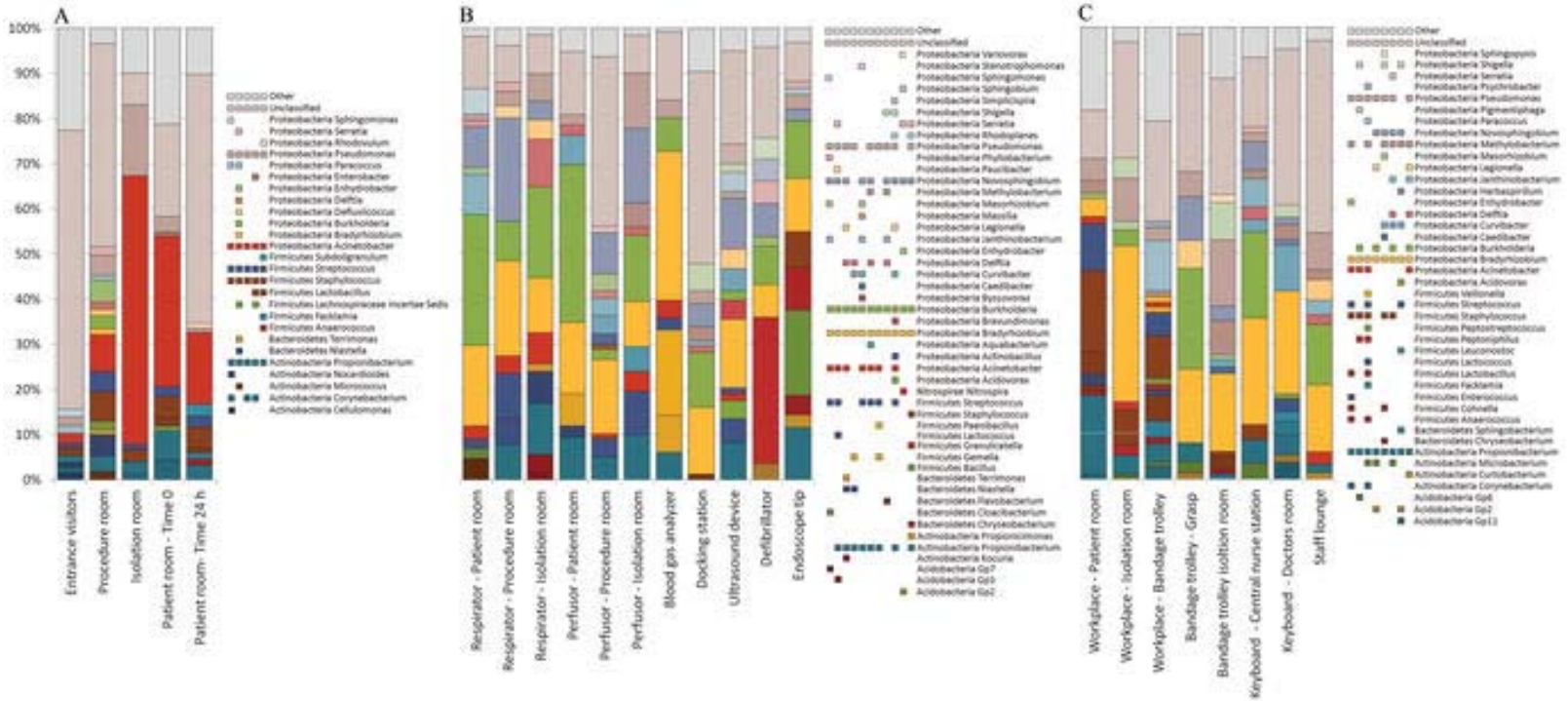
B

Strain	Sequence	Accession number
S. aureus	CGAA CGGACGAGAAGCTTG CTTCTCT GAT	L37597
S. epidermidis	CGAA C**A**GACGAG**G**AGCTTGCT**C**CTCT GAC	L37605
S. capitis	CGAA C**A**GACGAG**G**AGCTTGCTCCTCT GAC	L37599
S. warneri	CGAA C**A**GA**T**AAG**G**AGCTTGCT**C**CTT**T** GAC	L37603
S. haemolyticus	CGAA C**A**GAC**A**AG**G**AGCTTGCT**C**CTT**T** GAC	L37600
S. hominis	CGAA C**A**GACGAG**G**AGCTTGCT**C**CTT**T** GAC	L37601
S. saprophyticus	CGAA C**A**GA**T**AAG**G**AGCTTGCT**C**CTT**T** GAC	L37596

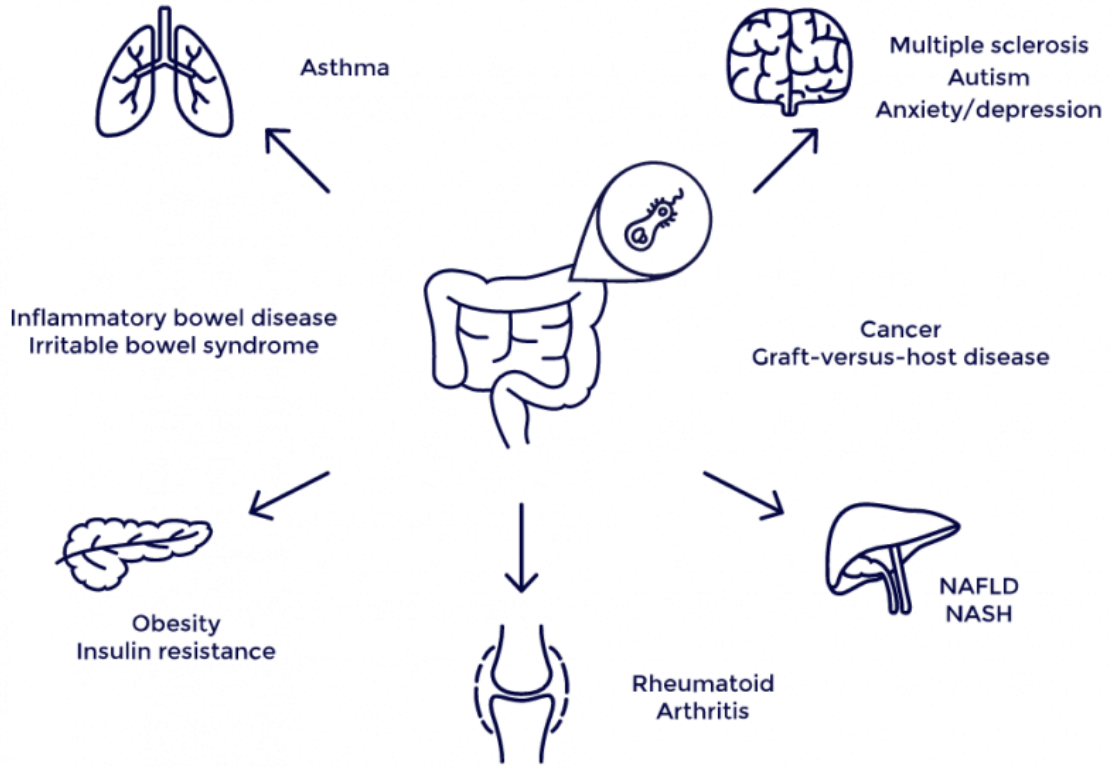
Distinction des bactéries au niveau du genre et parfois au niveau de l'espèce en fonction de la boucle 16S choisie et de l'espèce bactérienne

Cette approche est accessible en séquençage Sanger si une seule espèce est présente dans l'échantillon sinon le NGS sera nécessaire !

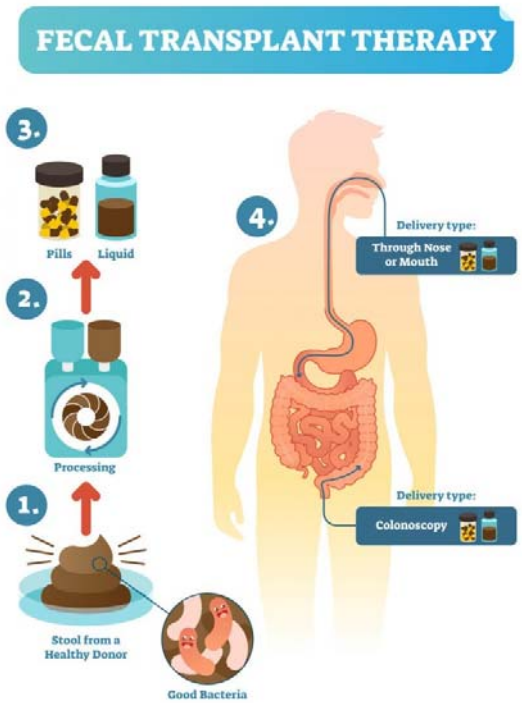
16S Diversité

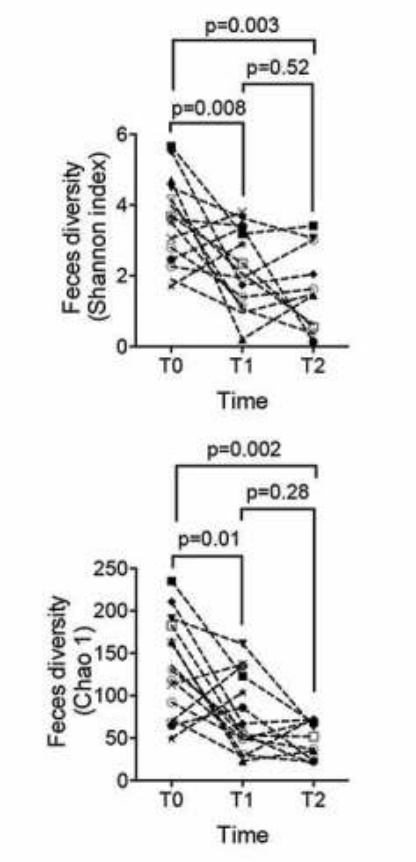


Application 16S : microbiote

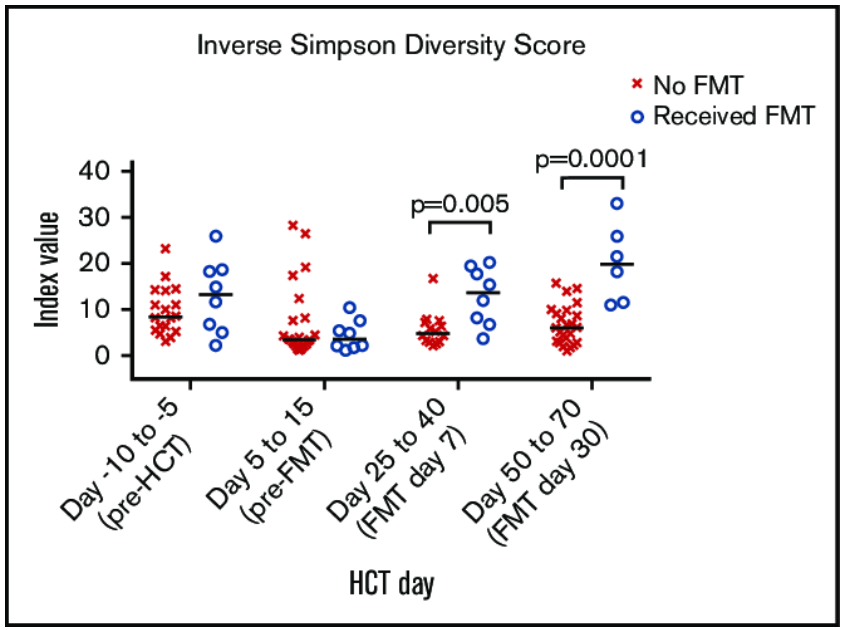


Application à la TMF



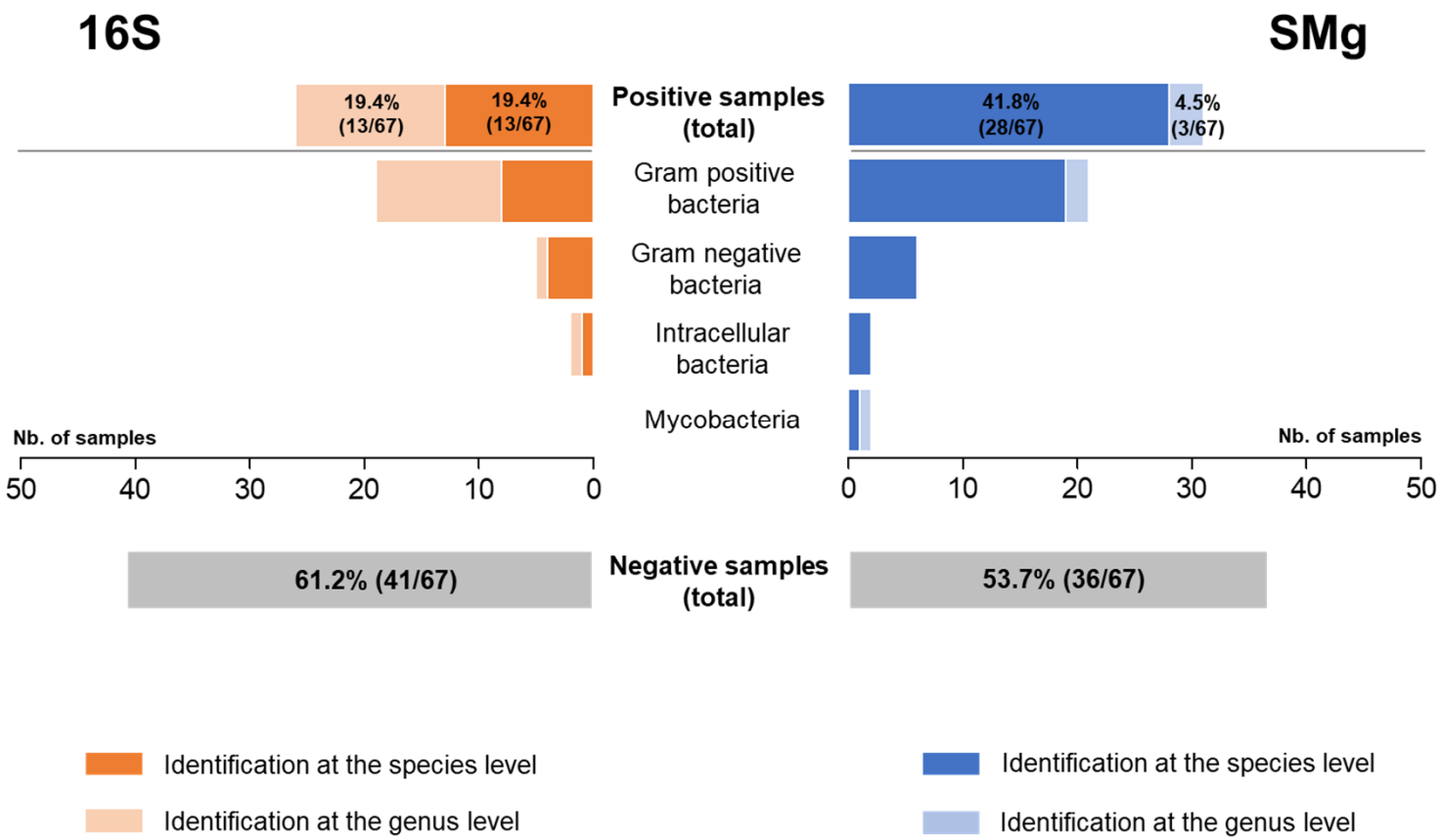


Hueso et al; gut microbes 2020



DeFilipp et al; blood advances 2018

Application 16S : documentation bactérienne



Etude prospective (N=67)

Polymicrobien
BK
+ 10 % de diagnostic à l'espèce

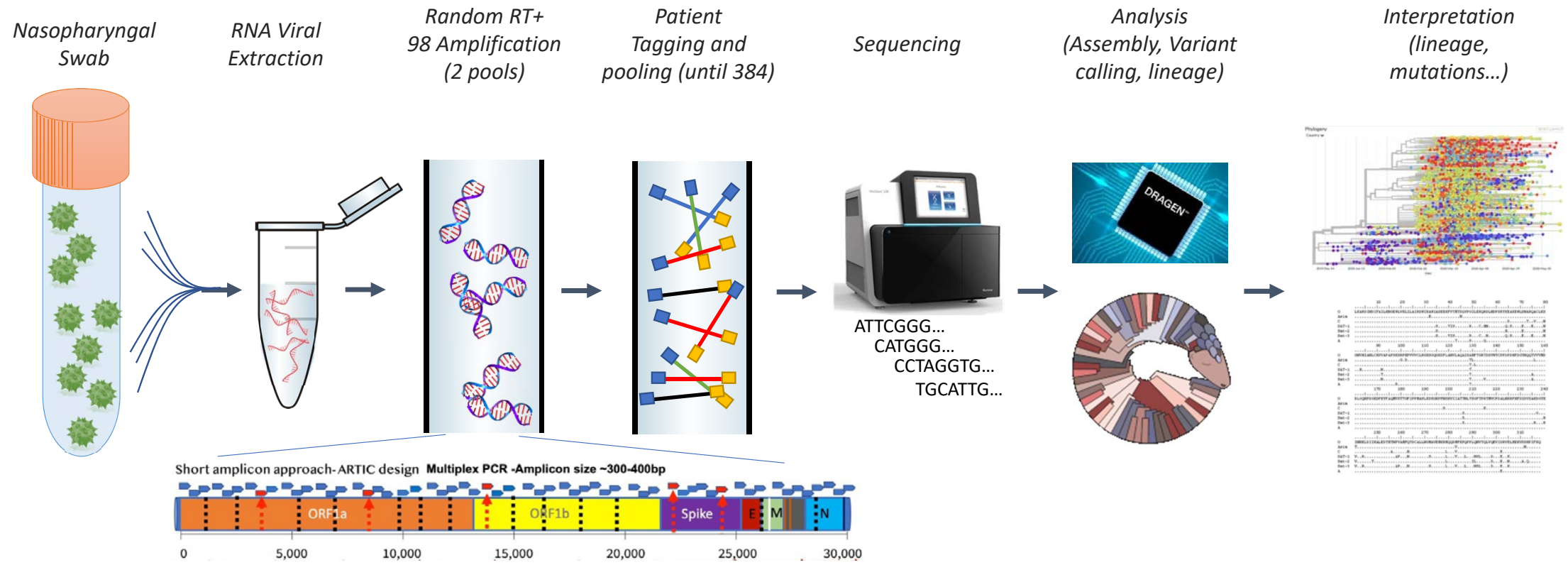
Lamoureux *et al.; Front Microbiol 2022*

Applications diagnostiques

- *Résistance*
- *Microbiote par approche 16S/ITS*
- *Surveillance*

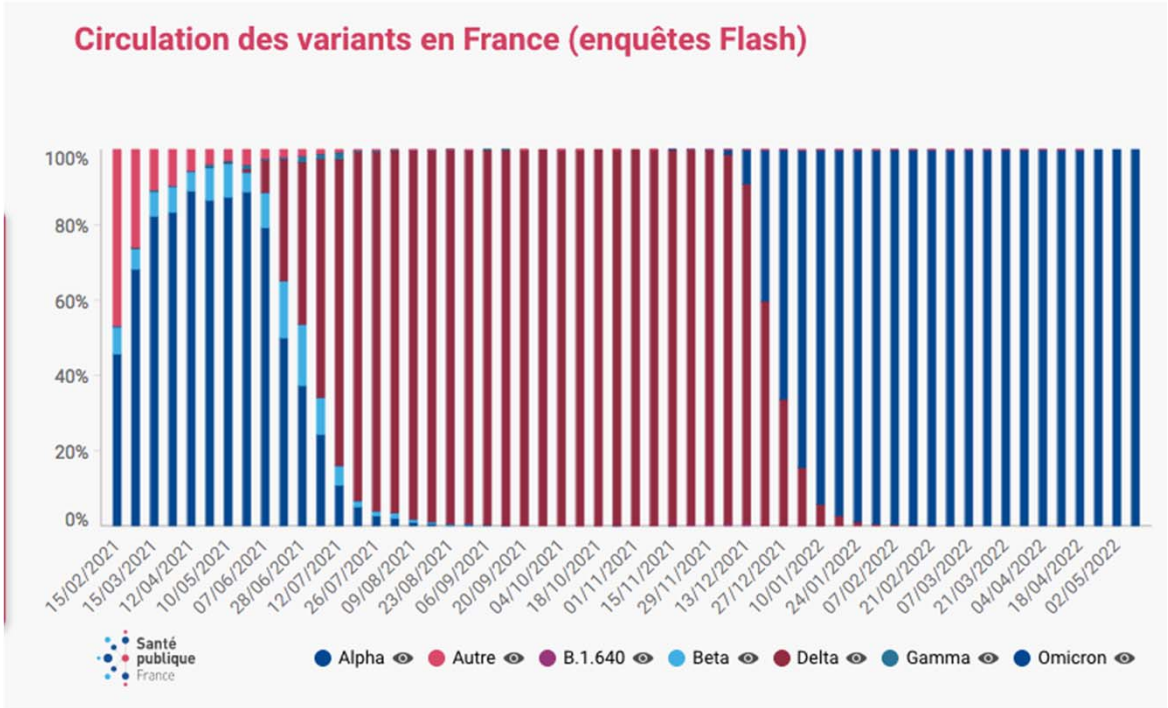
Surveillance Covid

- Protocole CovidSeq (Illumina)



EMERGEN

- https://www.santepubliquefrance.fr/dossiers/coronavirus-covid-19/coronavirus-chiffres-cles-et-evolution-de-la-covid-19-en-france-et-dans-le-monde



Activité de séquençage nationale : 563 045 séquences au total produites depuis 2021-S01

L'activité de dépôt dans GISAID place la France au 6ème rang mondial des contributeurs, et au 3ème rang des pays de l'Union Européenne, après l'Allemagne et le Danemark.

Applications du NGS Non ciblé

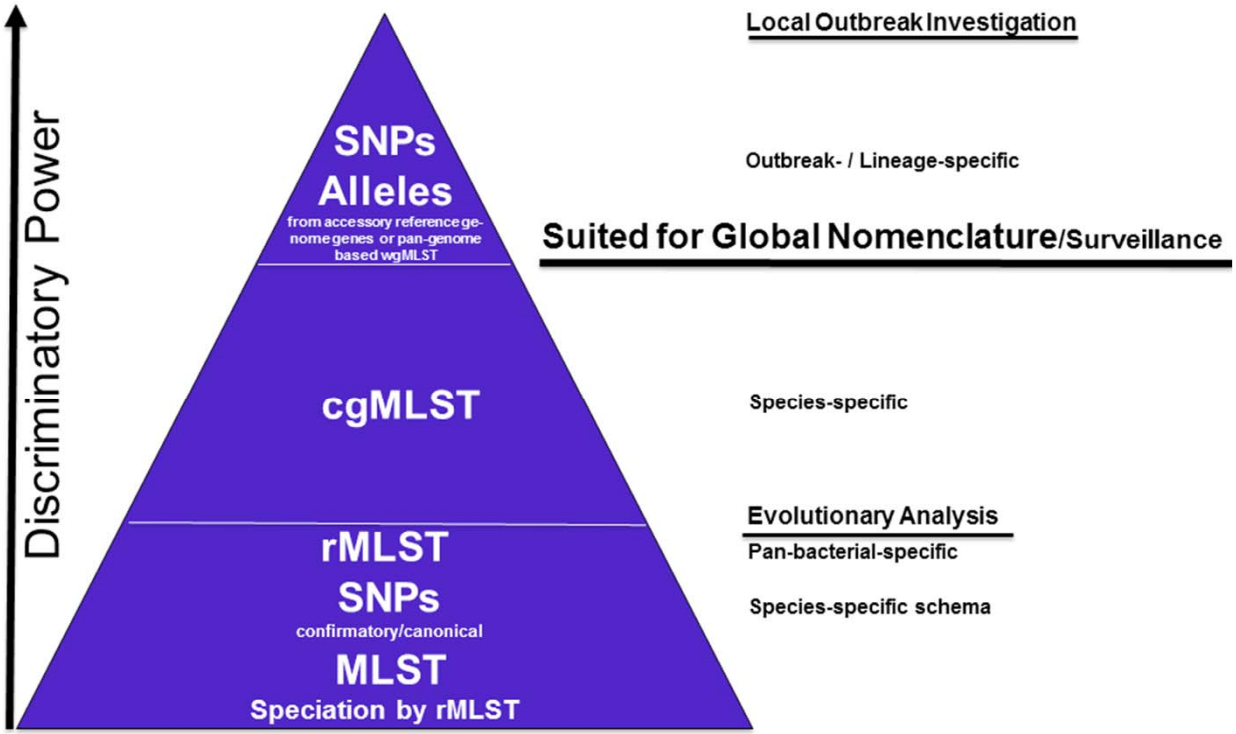
Applications diagnostiques

- *Hygiène hospitalière : Comparaison de souches*
- *Shotgun métagénomique*
- *Transcriptomique de l'hôte*

Applications diagnostiques

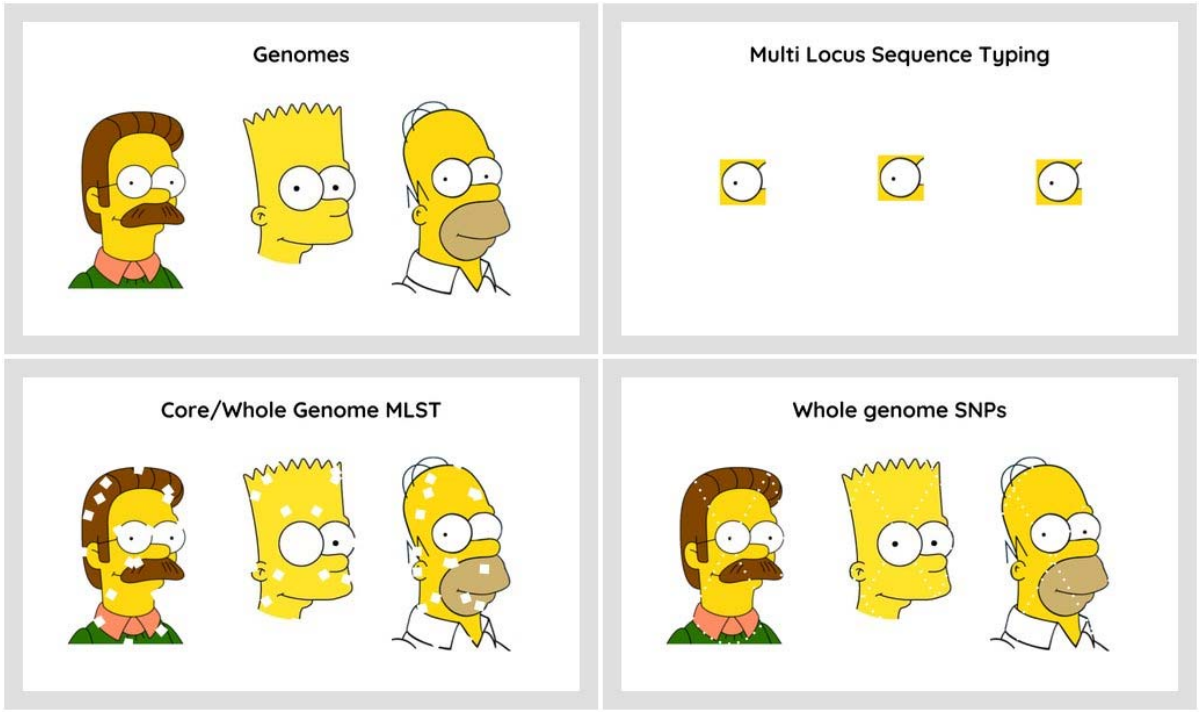
- *Hygiène hospitalière : Comparaison de souches*
- *Shotgun métagénomique*
- *Transcriptomique de l'hôte*

Epidémie ?

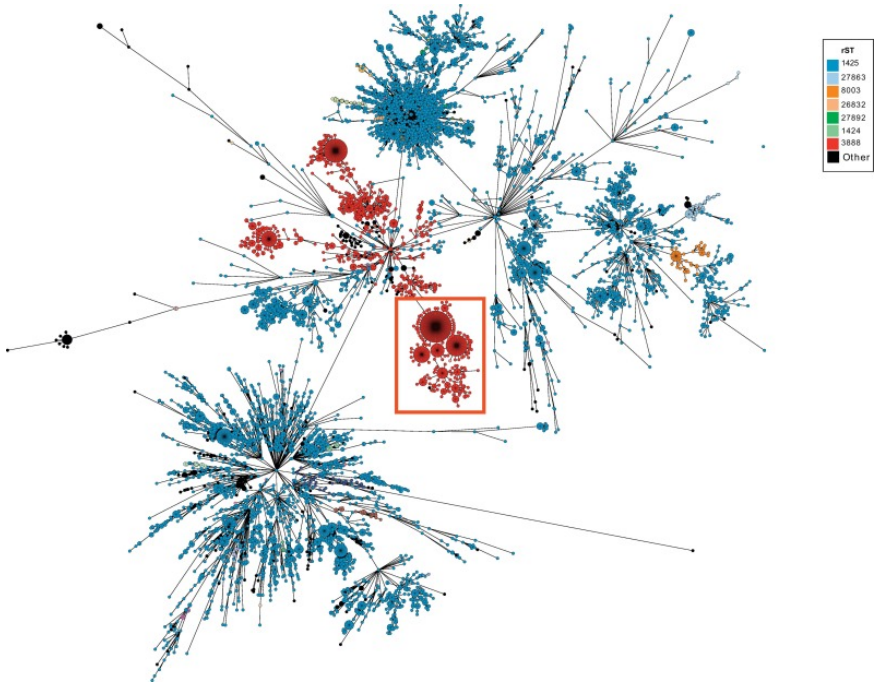


https://www.ridom.de/seqsphere/cgmlst/

Epidémie ?



Alexander Ensmiger (Twitter)



Pearce et al; International Journal of Food Microbiology 2018

Applications diagnostiques

- *Hygiène hospitalière : Comparaison de souches*
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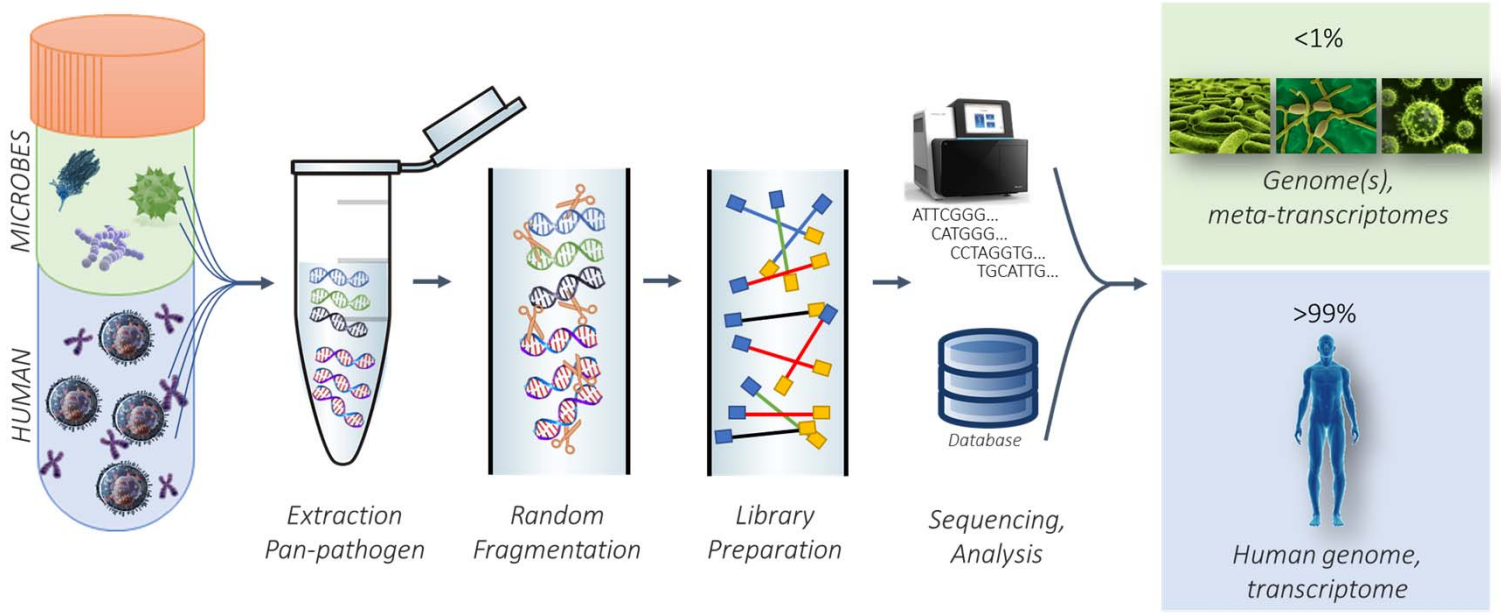
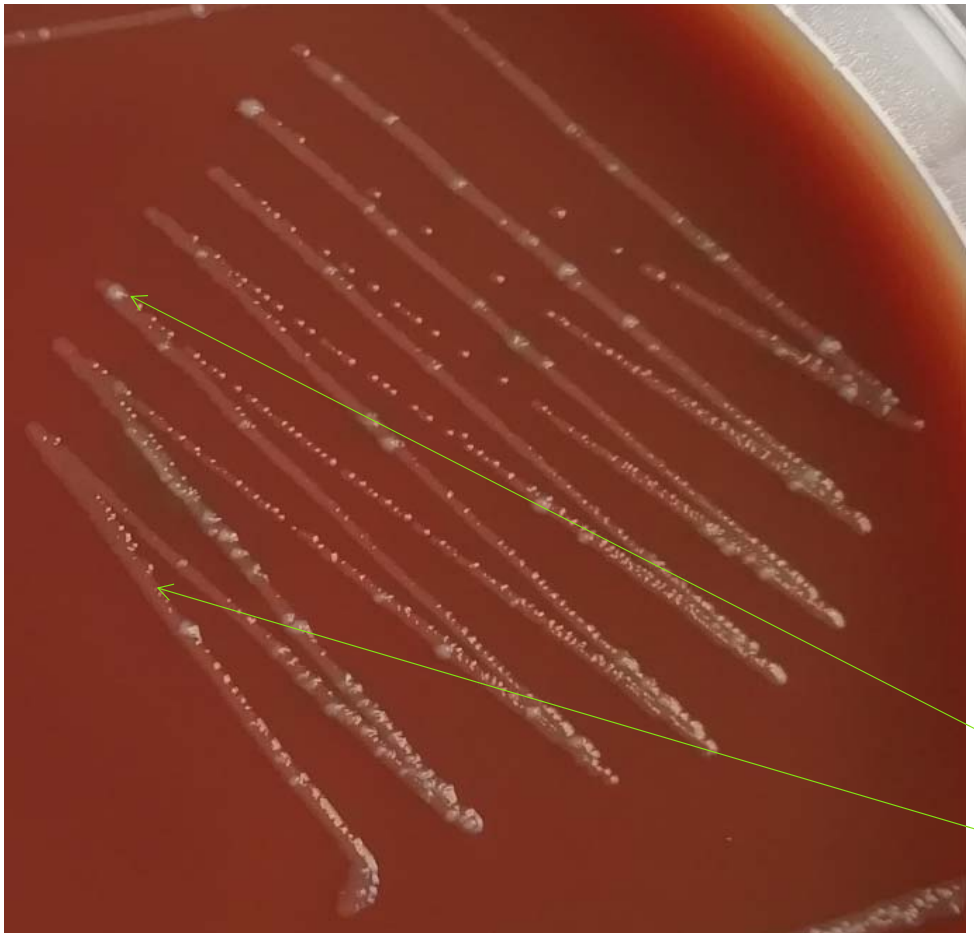
Approche métagénomique Shotgun (SMg)

- Métagénomique : étude de la totalité du contenu génétique d'un échantillon par séquençage à haut débit
- Dans le cadre d'une infection chez l'homme :



Reporting for clinicians

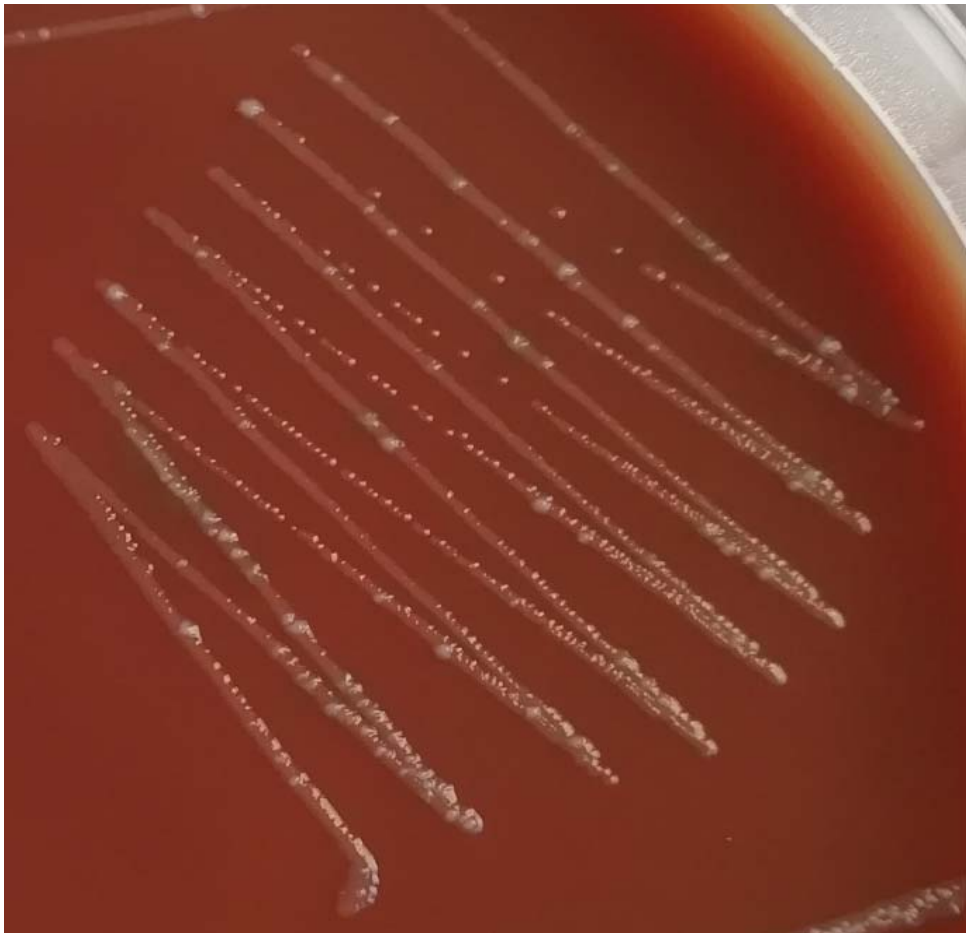


Résultats

Bactéries

Streptococcus sp. oral taxon 431	0.00000354
Streptococcus sp. A12	0.00000461
Haemophilus sp. oral taxon 036	0.00000481
Veillonella parvula	0.00000493
Neisseria meningitidis	0.00000797
Prevotella melaninogenica	0.00000933
Streptococcus pseudopneumoniae	0.00000975
Quantité intermédiaire	
Neisseria mucosa	0.00001054
Haemophilus parainfluenzae	0.00001373
Neisseria sicca	0.00002096
Streptococcus pneumoniae	0.00002158
Streptococcus mitis	0.00002703
Prevotella jejuni	0.00002841
Staphylococcus aureus	0.00006137
Haemophilus influenzae	0.00261808

Reporting for clinicians



Henri Mondor hospital
Mr XYZ

Metagenomics Results

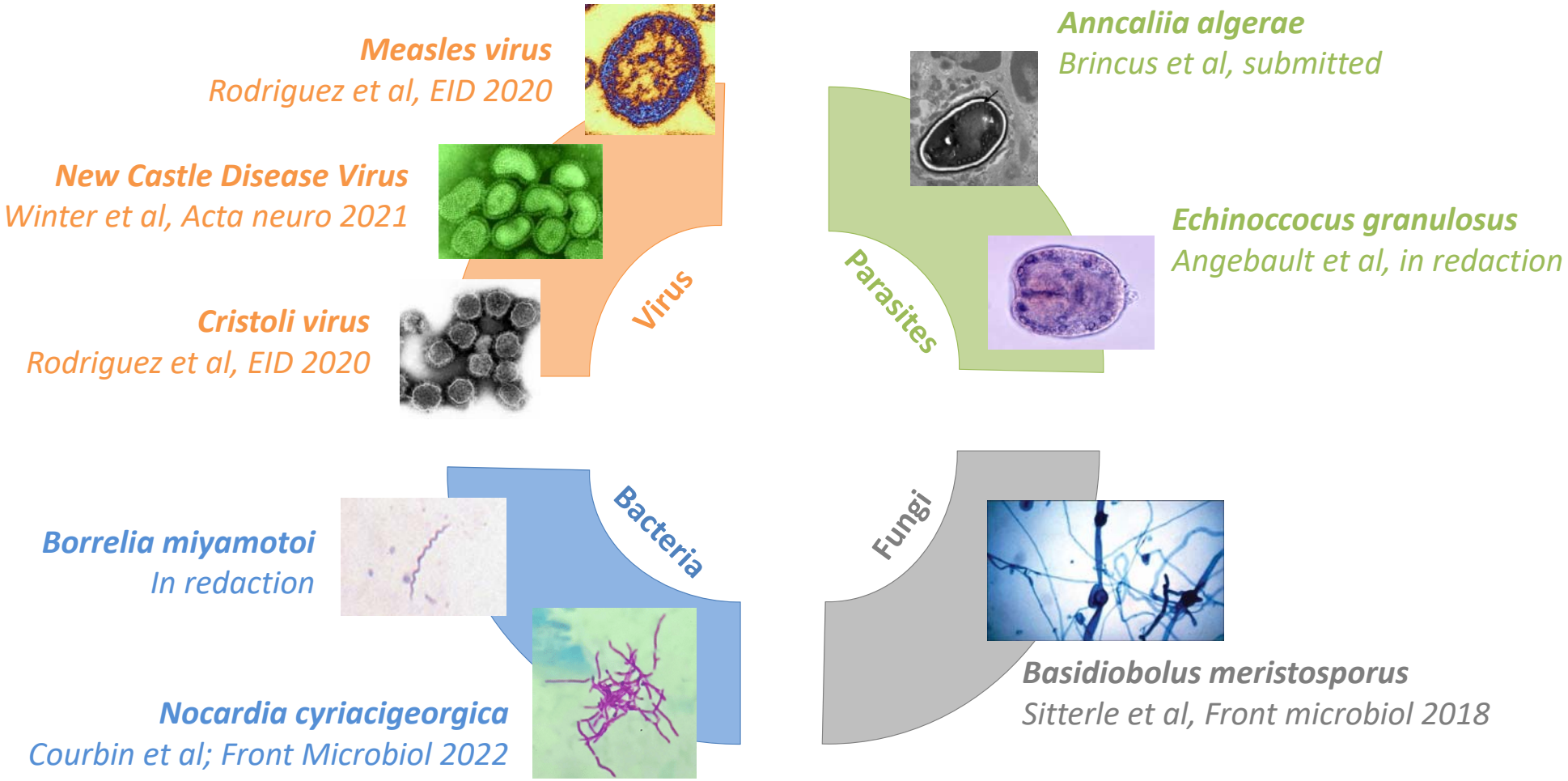
Bacteria
Predominance of Haemophilus influenzae and presence of S. aureus within an ENT flora

Virus
Absence

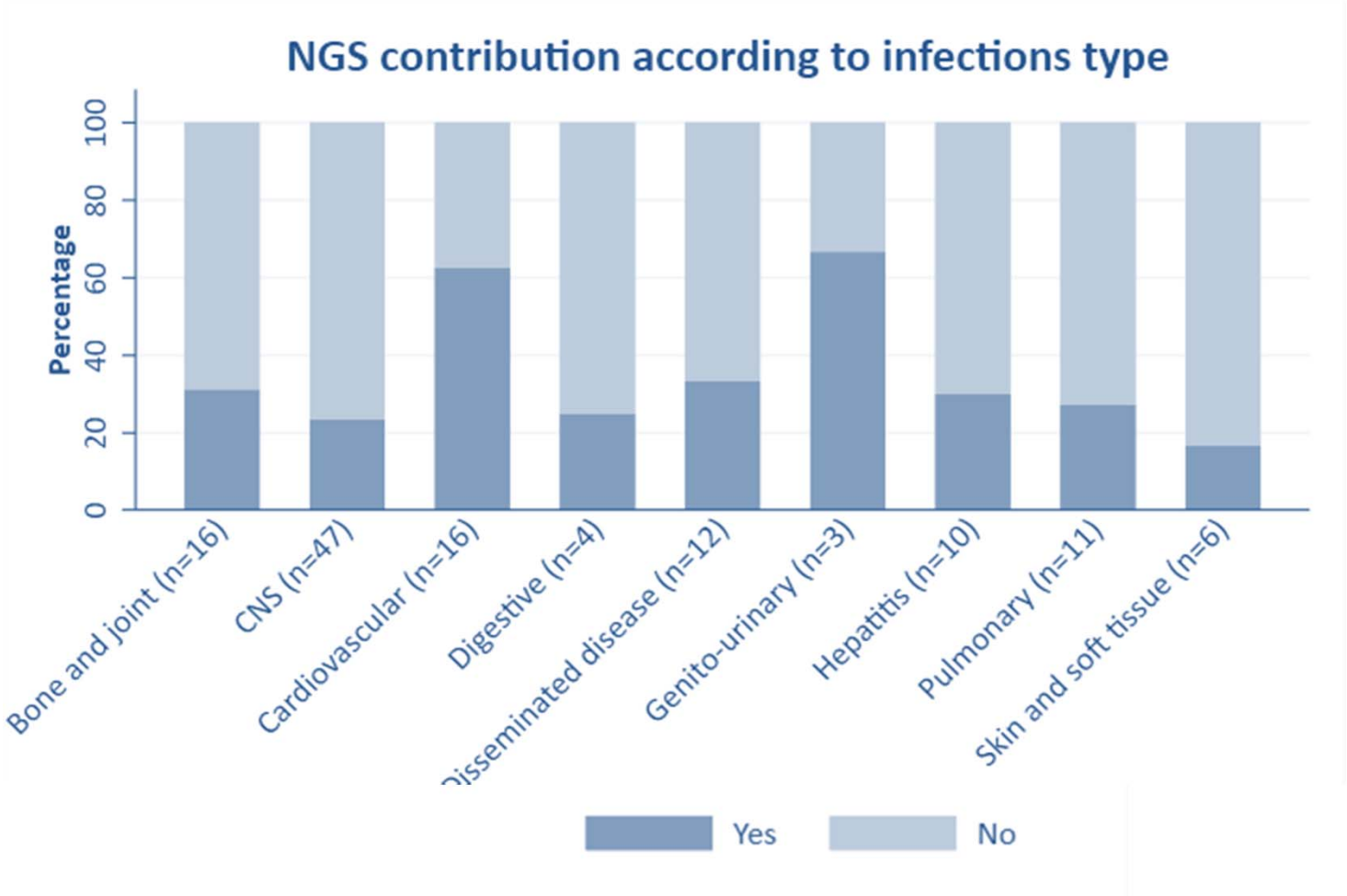
Fungi
Absence

Parasites
Absence

Complex infections : panpathogen documentation



Prospective clinical study : SMg vs rest of the world



- Contribution in 32% of cases in patients with high suspicion
- Contribution whatever the type of infection

Surgers *et al.; in redaction*

Applications diagnostiques

- *Hygiène hospitalière : Comparaison de souches*
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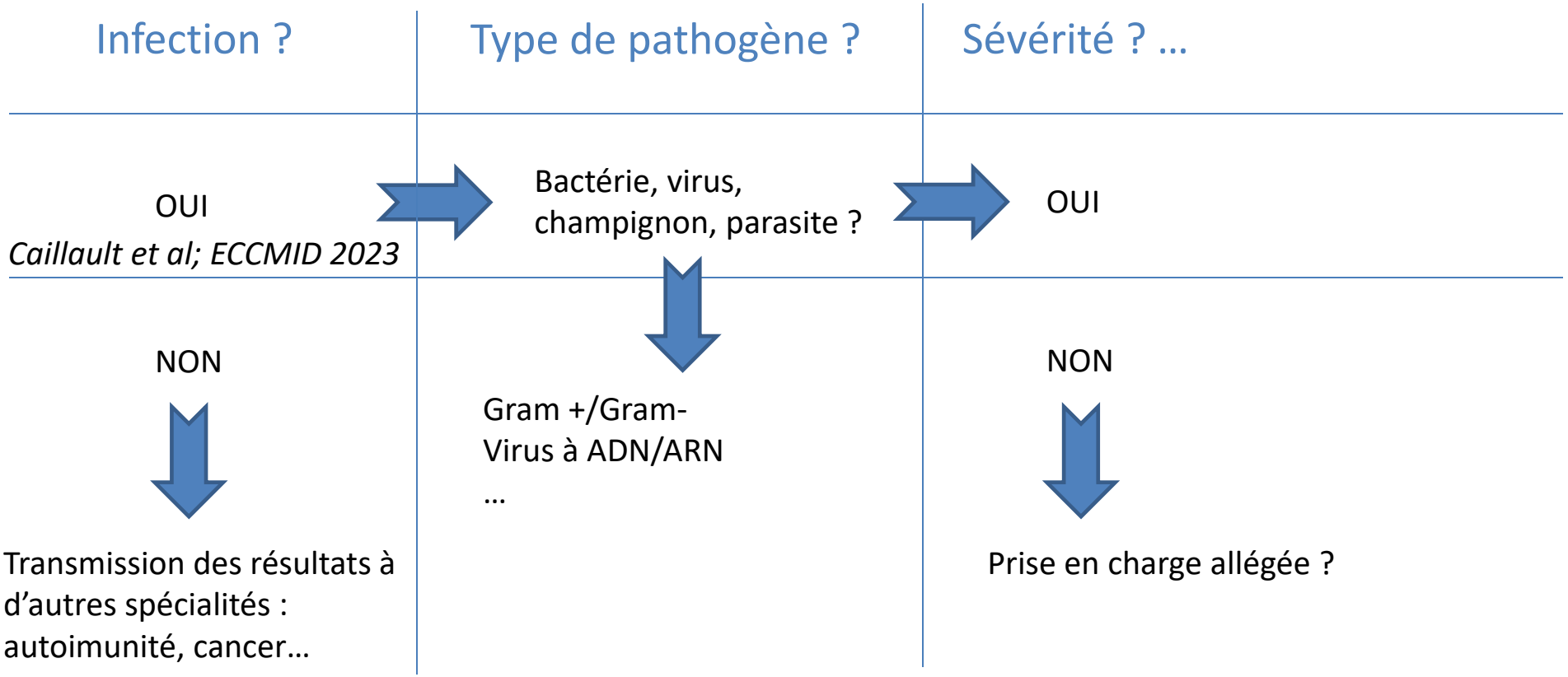
Applications potentielles (en cours d'évaluation)



Caillault et al; ECCMID 2023 *Caillault et al; ECCMID 2023* *Rodriguez et al; Plos Pathogens 2021*

Conclusion

- Deux grands types d'applications : Surveillance et diagnostic
- Deux grands types d'information : Documentation et caractérisation
- Deux grands types de techniques NGS : Ciblées et Non ciblées
- Les techniques ciblées permettent :
  - La recherche de résistance
  - La surveillance de souches (Covid)
  - La mesure de diversité bactérienne
- Les techniques non ciblées permettent EN PLUS :
  - La caractérisation complète du génome et sa comparaison (hygiène)
  - Une documentation pan-pathogène
  - La découverte de nouveaux pathogènes
  - L'exploration de l'hôte

Many thanks to…

MetaMIC Project

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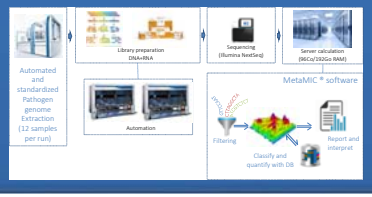
<u>Coordination</u>
Jean-Michel Pawlotsky



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