

Utilité du NGS en infectiologie

Dr C. Rodriguez

CNR LE séquençage Covid

LBMR Métagénomique

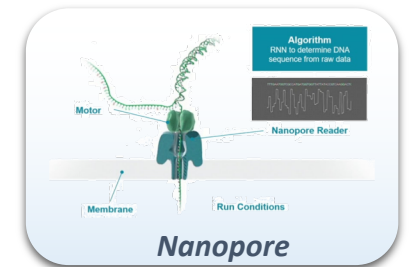
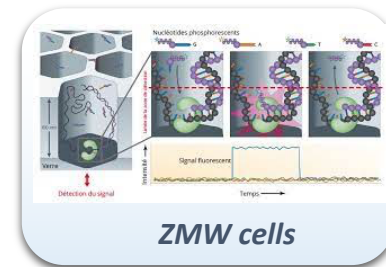
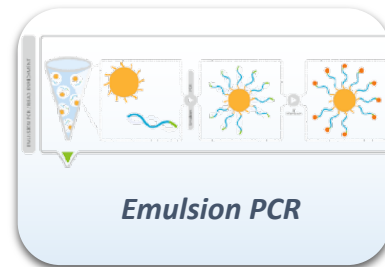
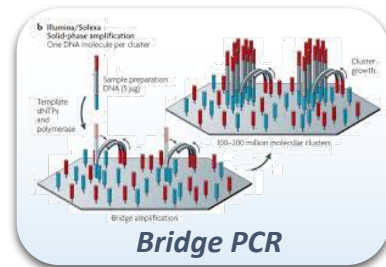
*Microbiology Dpt, INSERM U955 Team 18,
University hospital Henri Mondor, APHP, Créteil, France*

C'est quoi le NGS ?

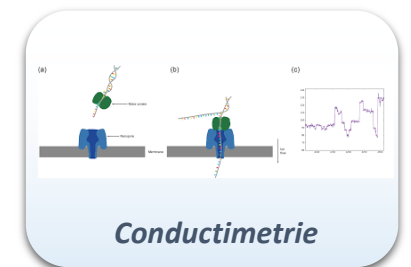
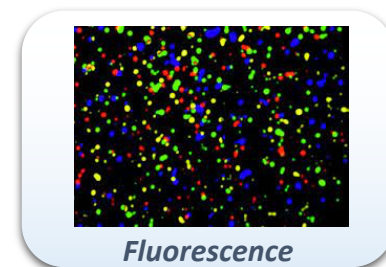
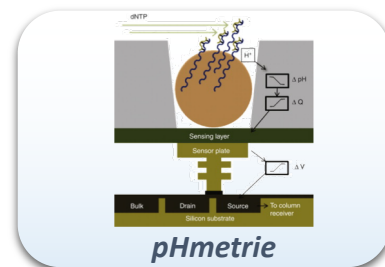
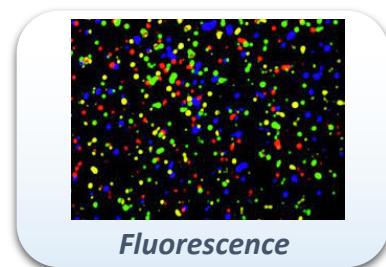
Plateforme



Isolement des séquences, amplification du signal

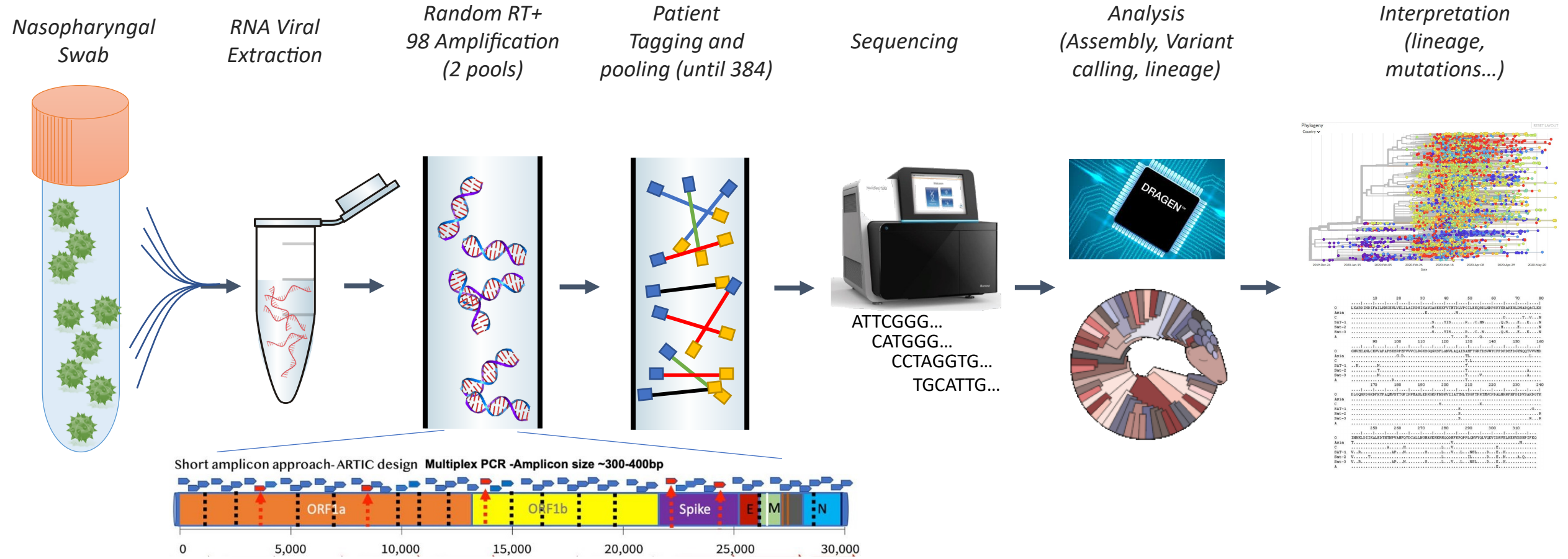


Acquisition du signal



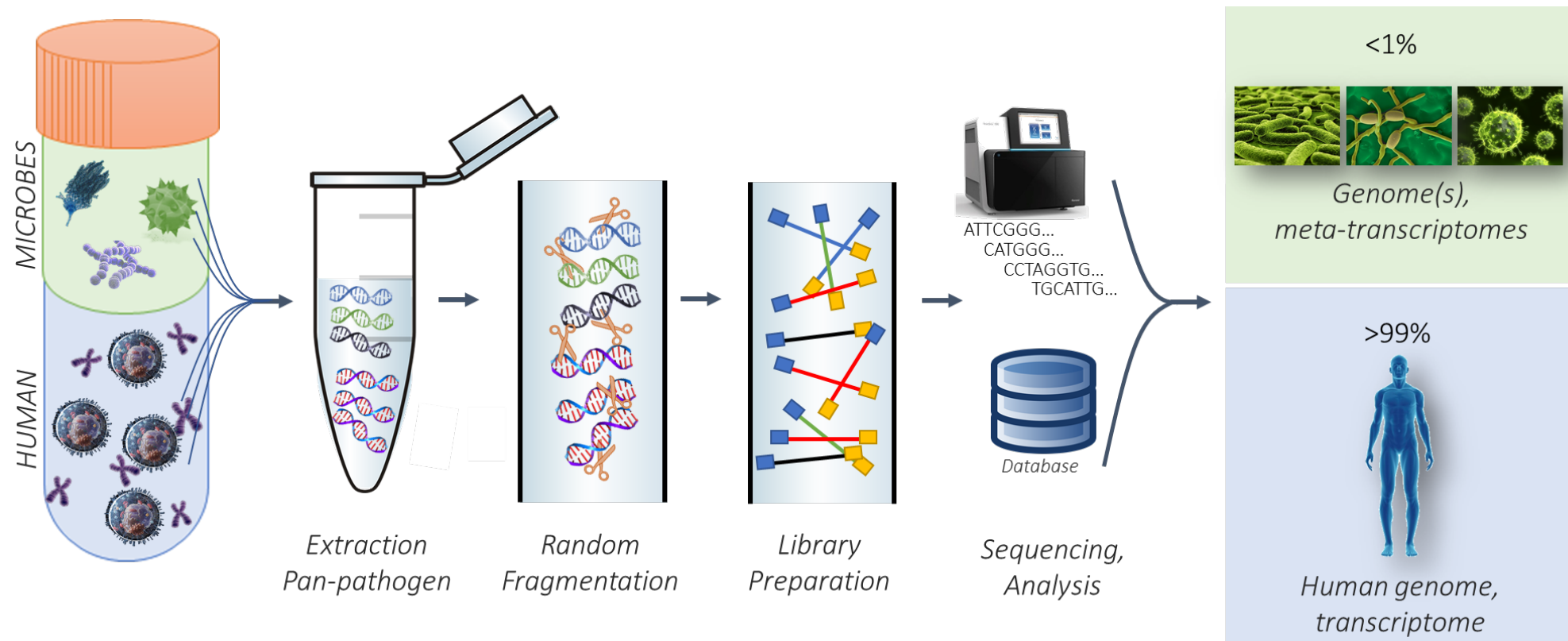
Approche Amplicon

- Protocole CovidSeq (Illumina)



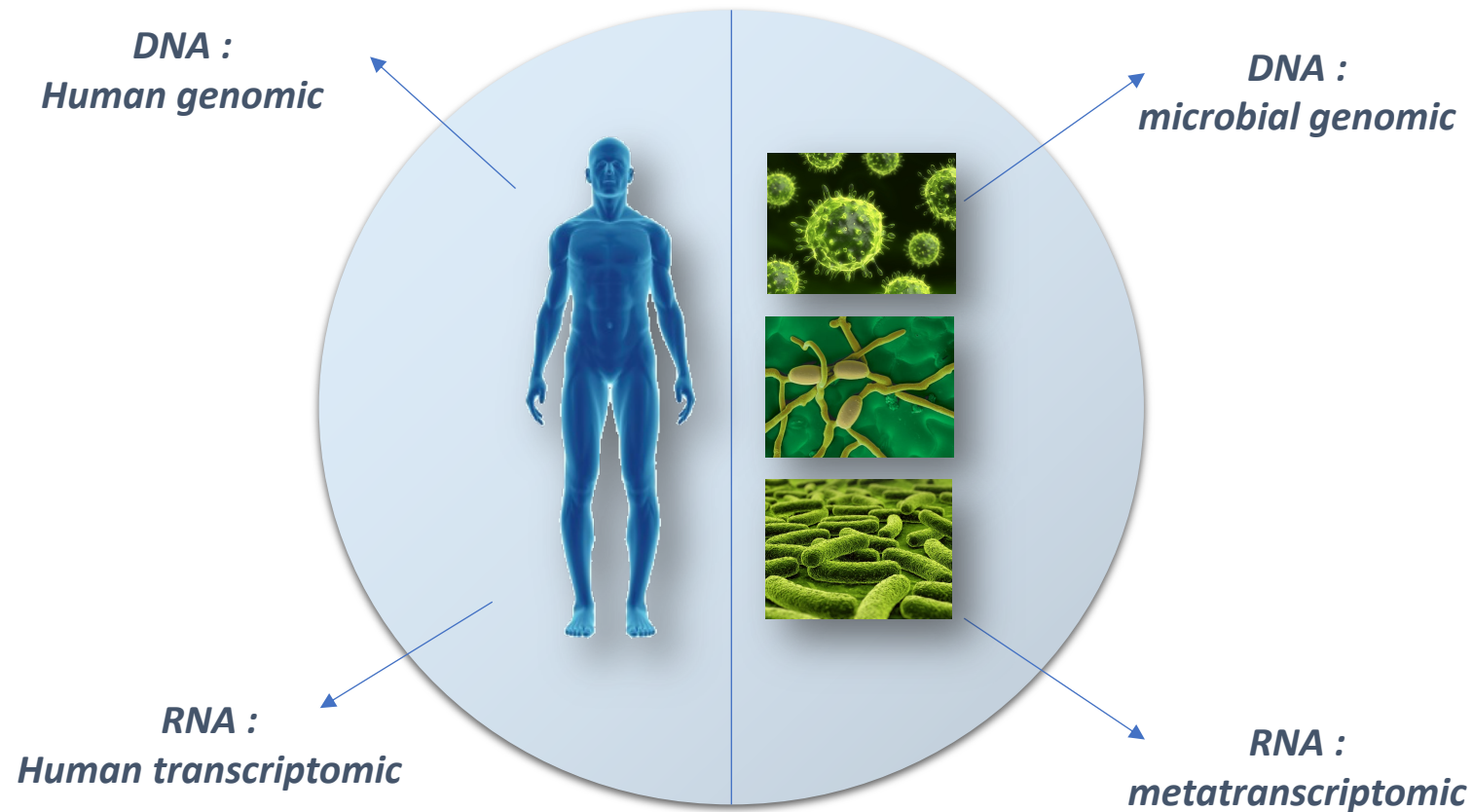
Approche métagénomique Shotgun (SMg)

- 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 :

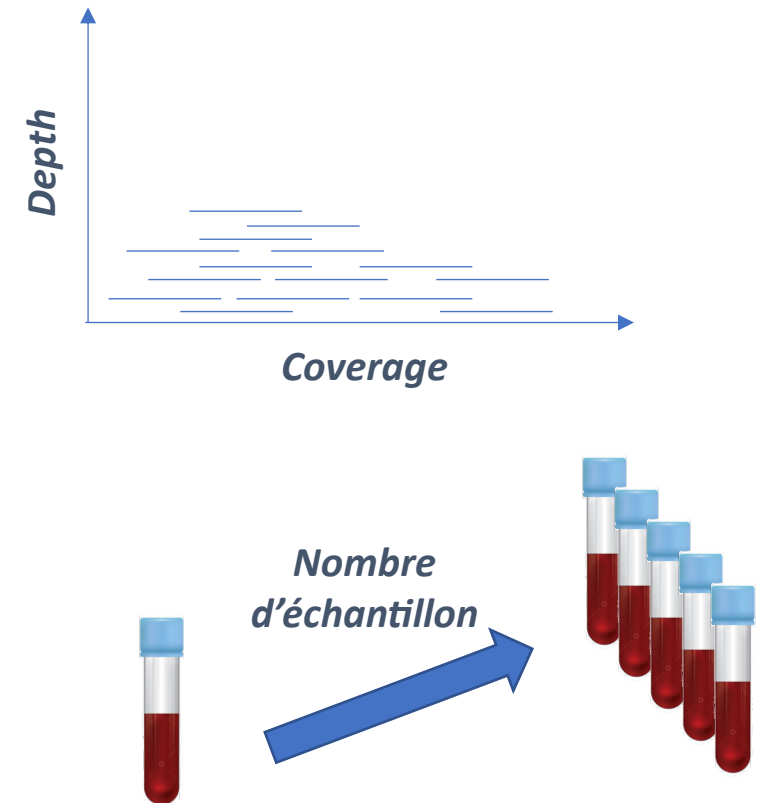


Qu'est ce que ça apporte de plus ?

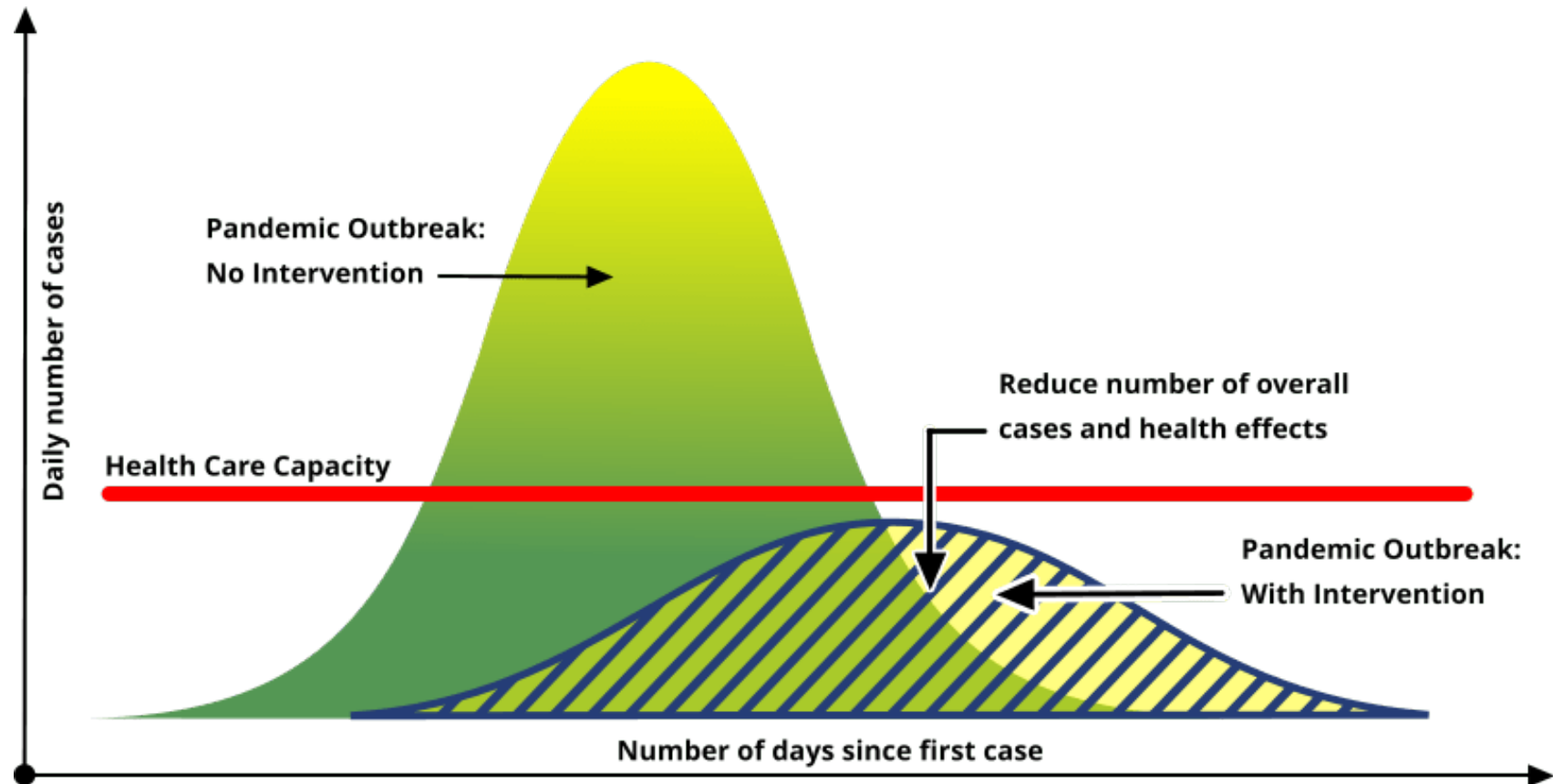
Qualité : Approche sans a priori



Quantité : Le débit



En infectiologie ?



Adapted from: CDC. Interim pre-pandemic planning guidance: community strategy for pandemic influenza mitigation in the United States—early, targeted, layered use of nonpharmaceutical interventions. Atlanta, GA: US Department of Health and Human Services, CDC; 2007. <https://stacks.cdc.gov/view/cdc/11425>.

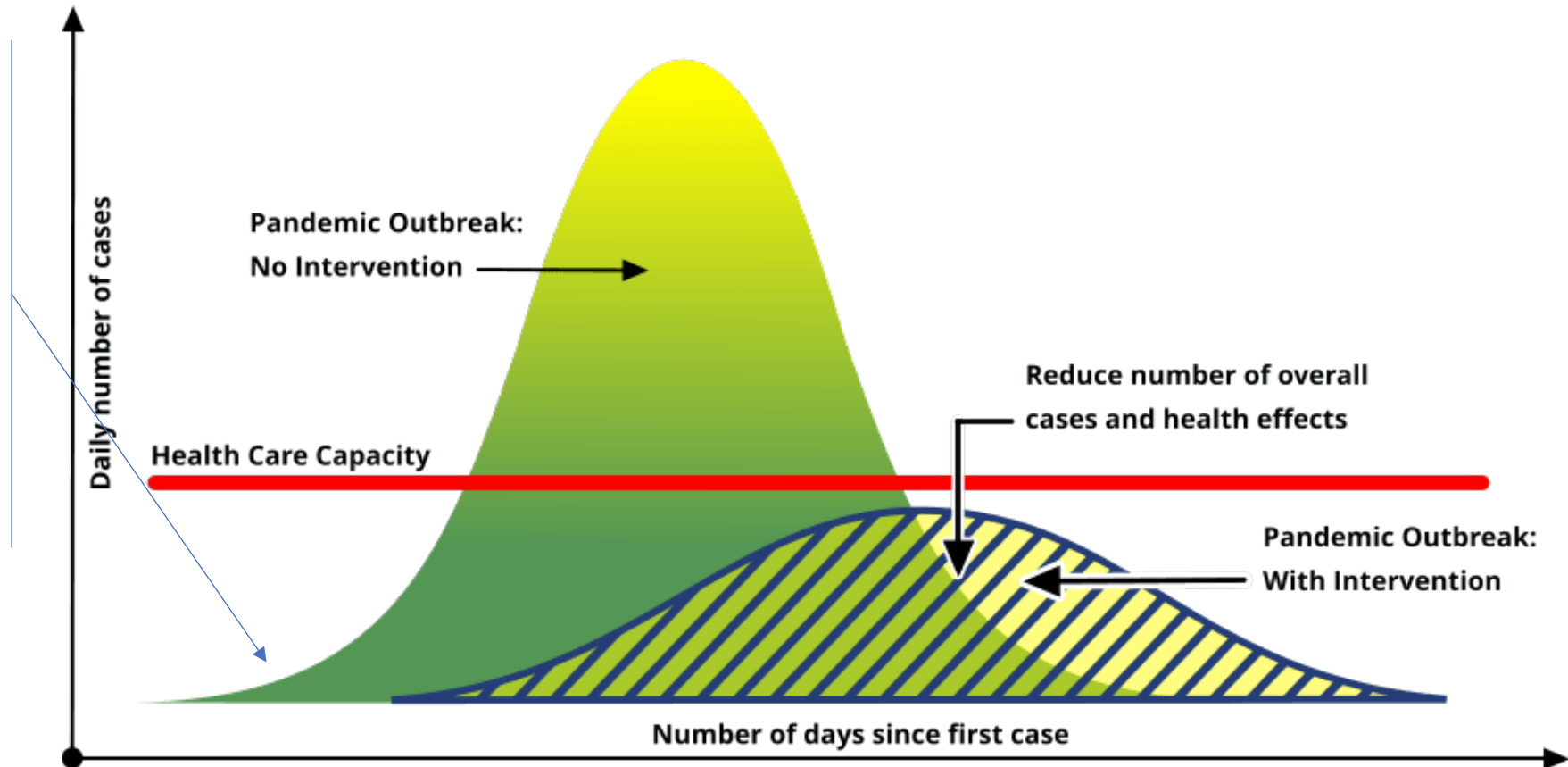
En infectiologie ?

Techniques Shotgun

Surveillance
environnementale

Surveillance
animale

Surveillance
humaine



Adapted from: CDC. Interim pre-pandemic planning guidance: community strategy for pandemic influenza mitigation in the United States—early, targeted, layered use of nonpharmaceutical interventions. Atlanta, GA: US Department of Health and Human Services, CDC; 2007. <https://stacks.cdc.gov/view/cdc/11425>.

Phase pré-pandémique : Environnement

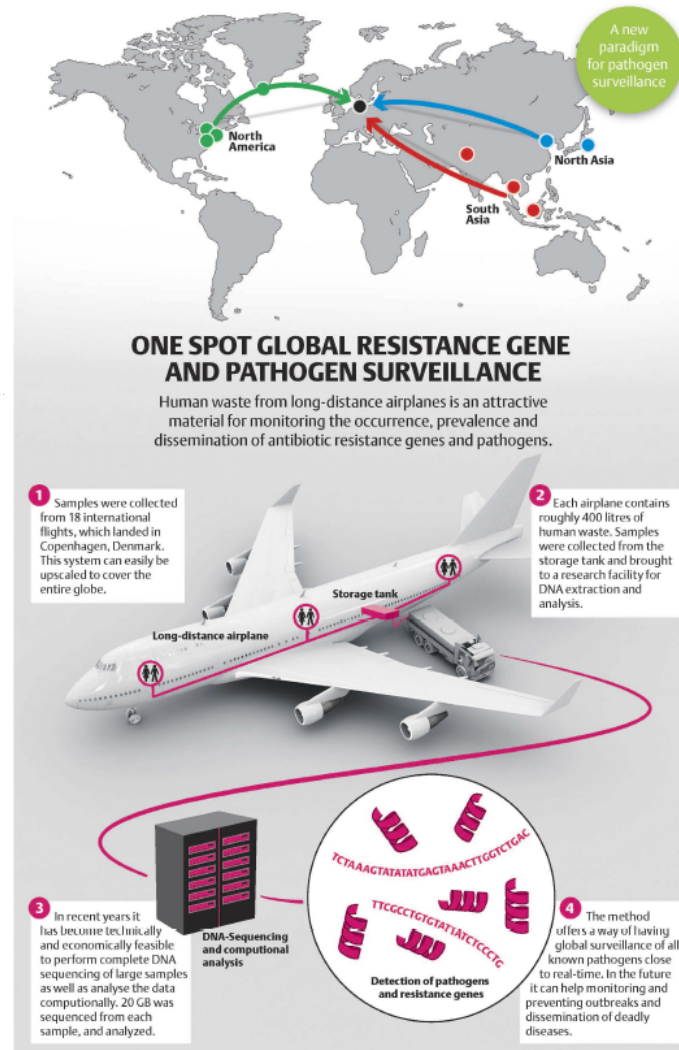


Figure 1. Origin of the 18 long-distance flights with destination being the international airport in Copenhagen, Denmark, as well as the analytic procedure applied. Figure created in Adobe Illustrator.

OPEN **Meta-genomic analysis of toilet waste from long distance flights; a step towards global surveillance of infectious diseases and antimicrobial resistance**

Thomas Nordahl Petersen¹, Simon Rasmussen¹, Henrik Hasman², Christian Caroe³, Jacob Bælum⁴, Anna Charlotte Schultz⁵, Lasse Bergmark³, Christina A. Svendsen⁶, Ole Lund⁴, Thomas Sicheritz-Pontén⁴ & Frank M. Aarestrup³

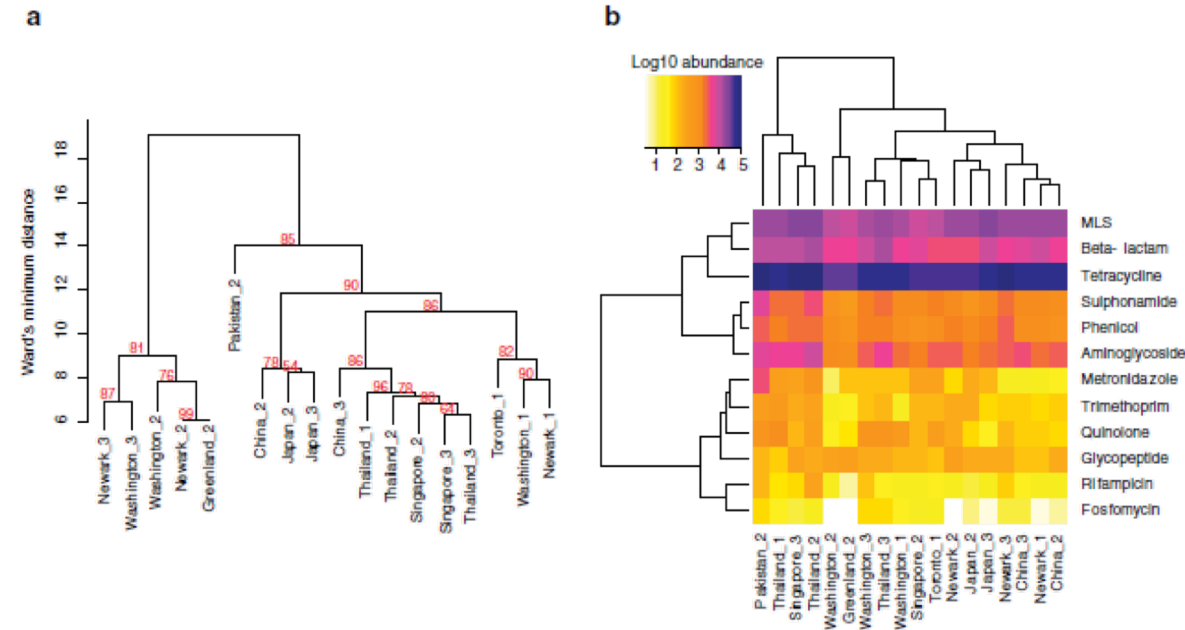


Figure 2. Geographical clustering of flight samples based on adjusted read abundance. a. Hierarchical clustering of flight samples based on normalized abundance of complete bacterial genomes and genomes from the human gut, bootstrap values are indicated in red and calculated using pvclust. Branch distance in the tree is Ward's minimum distance calculated using the Lance-Williams formula. b. Heatmap showing hierarchical clustering based on the normalized abundance of resistance gene classes. The abundance is in log₁₀ scale from white (low), yellow, orange (intermediate), magenta, blue (high). MLS: macrolide, lincosamide, streptogramin.

Phase pré-pandémique : Vecteurs



RNA shotgun metagenomic sequencing of northern California (USA) mosquitoes uncovers viruses, bacteria, and fungi

James Angus Chandler*, Rachel M. Liu* and Shannon N. Bennett*

Department of Microbiology, California Academy of Sciences, San Francisco, CA, USA

« Sequences related to single stranded RNA viruses of the *Bunyaviridae* and *Rhabdoviridae* were uncovered, along with an unclassified genus of double-stranded RNA viruses. Phylogenetic analysis finds that in all three cases, the closest relatives of the identified viral sequences are other mosquito-associated viruses, suggesting widespread host-group specificity among disparate viral taxa. »

FIGURE 1. Collection locations and a summary of the microbial sequences identified in each sample.

Phase pré-pandémique : Hommes



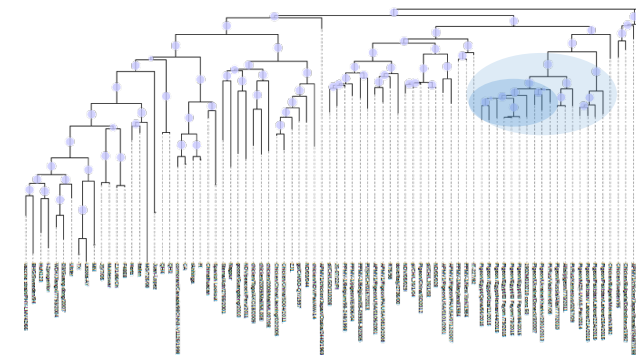
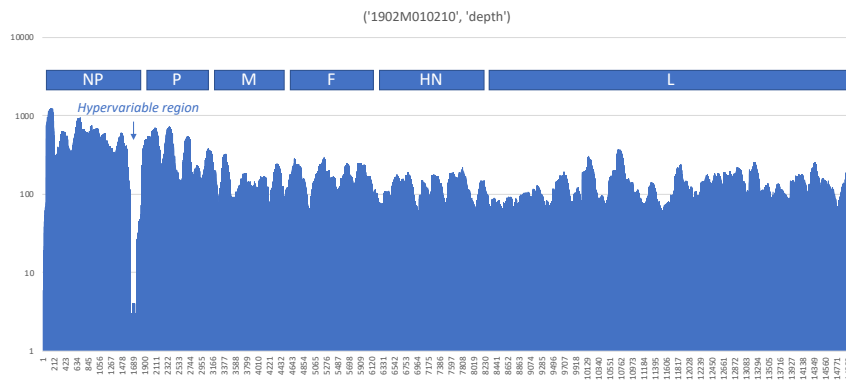
Patiente de 5 ans avec déficit immunitaire congénital, transplantée de moelle osseuse, développant une encéphalite grave puis un coma inexplicable au retour d'un voyage familial au Moyen-Orient

Diagnostic d'infection par **Avulavirus**

- Maladie grave connue chez l'oiseau
- Un seul cas mortel humain répertorié, pas de caractérisation du virus

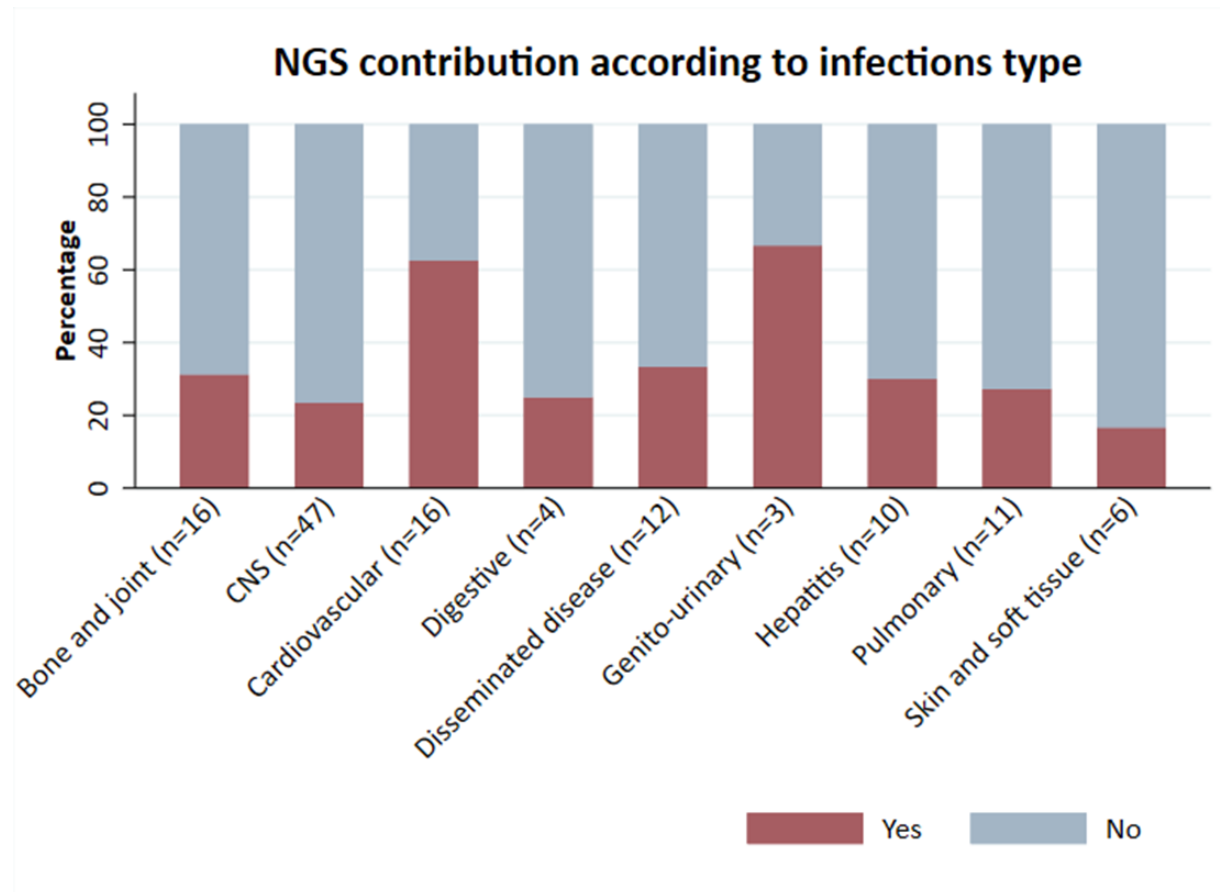


Séquence du génome complet et analyse phylogénique ont montré que la souche virale circule au moyen-orient et qu'elle comporte de nombreux facteurs de virulence



Phase pré-pandémique : Hommes

- Documentation des infections complexes non diagnostiquées (N=201)

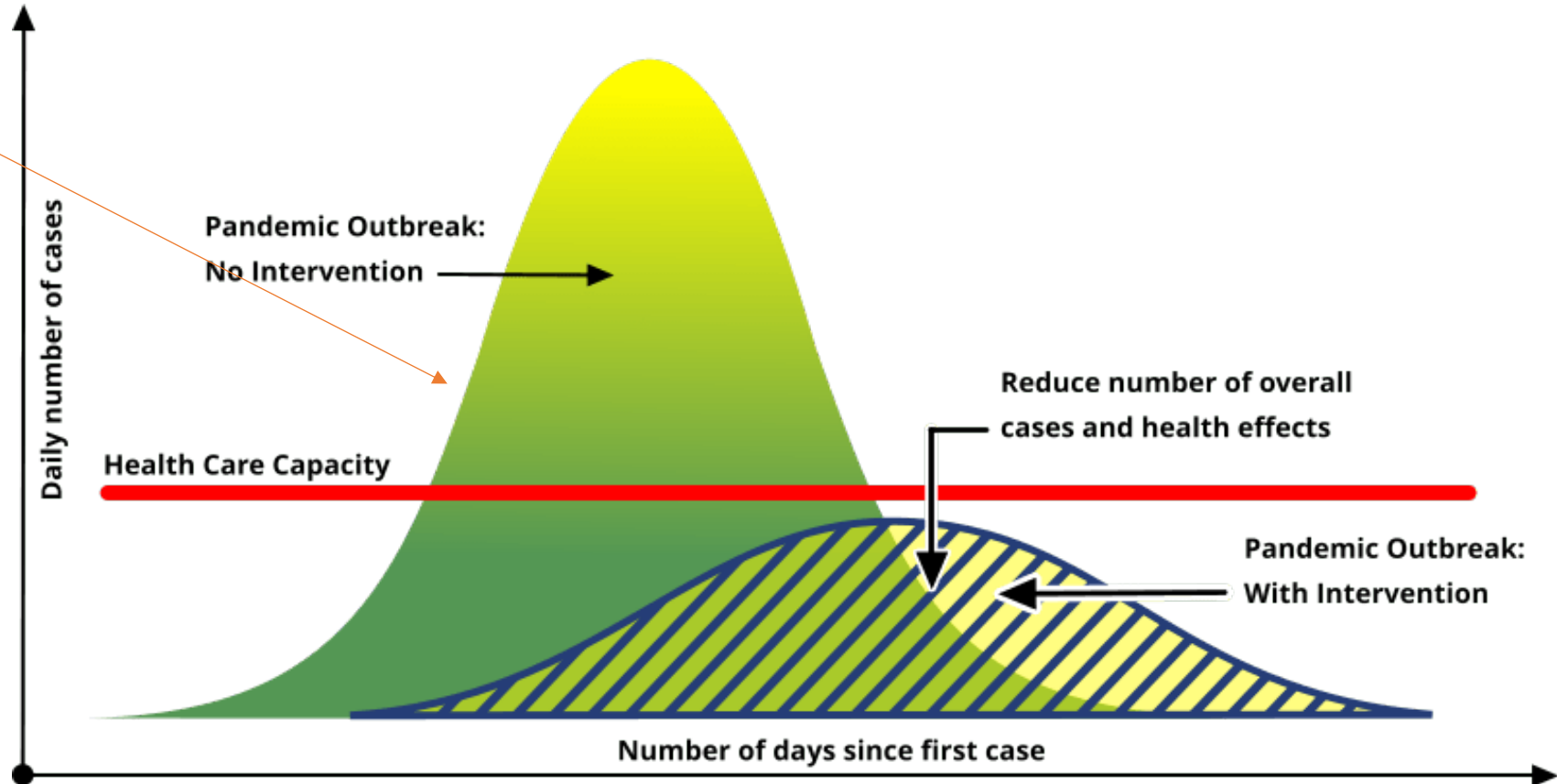


Moyenne : 32%
(+11% par rapport à l'ensemble des techniques diagnostiques réunies)

En infectiologie ?

Techniques Amplicons

Dépistage/diagnostic massif

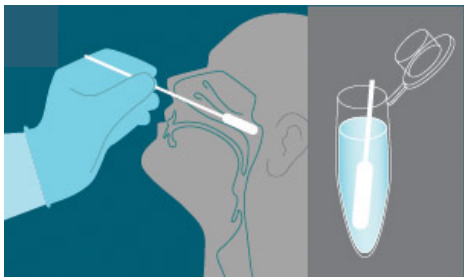


Adapted from: CDC. Interim pre-pandemic planning guidance: community strategy for pandemic influenza mitigation in the United States—early, targeted, layered use of nonpharmaceutical interventions. Atlanta, GA: US Department of Health and Human Services, CDC; 2007. <https://stacks.cdc.gov/view/cdc/11425>.

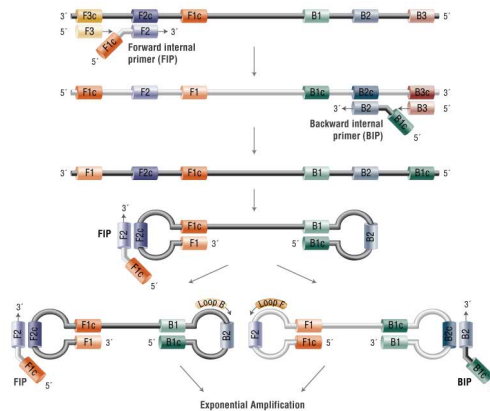
Phase d'Expansion pandémique

- LAMP-Seq

Collection sample

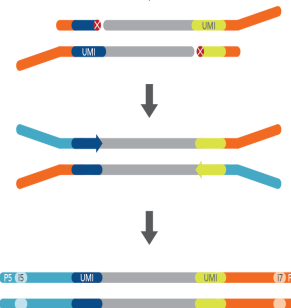


Neutralization, LAMP amplification, tagging



<1h

Library preparation, tagging



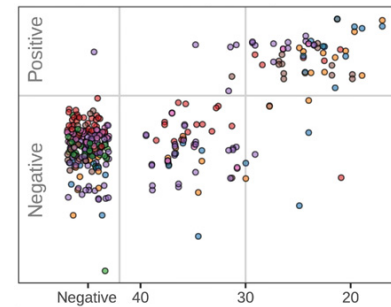
<3h

Sequencing



11h

Analysis



<1h

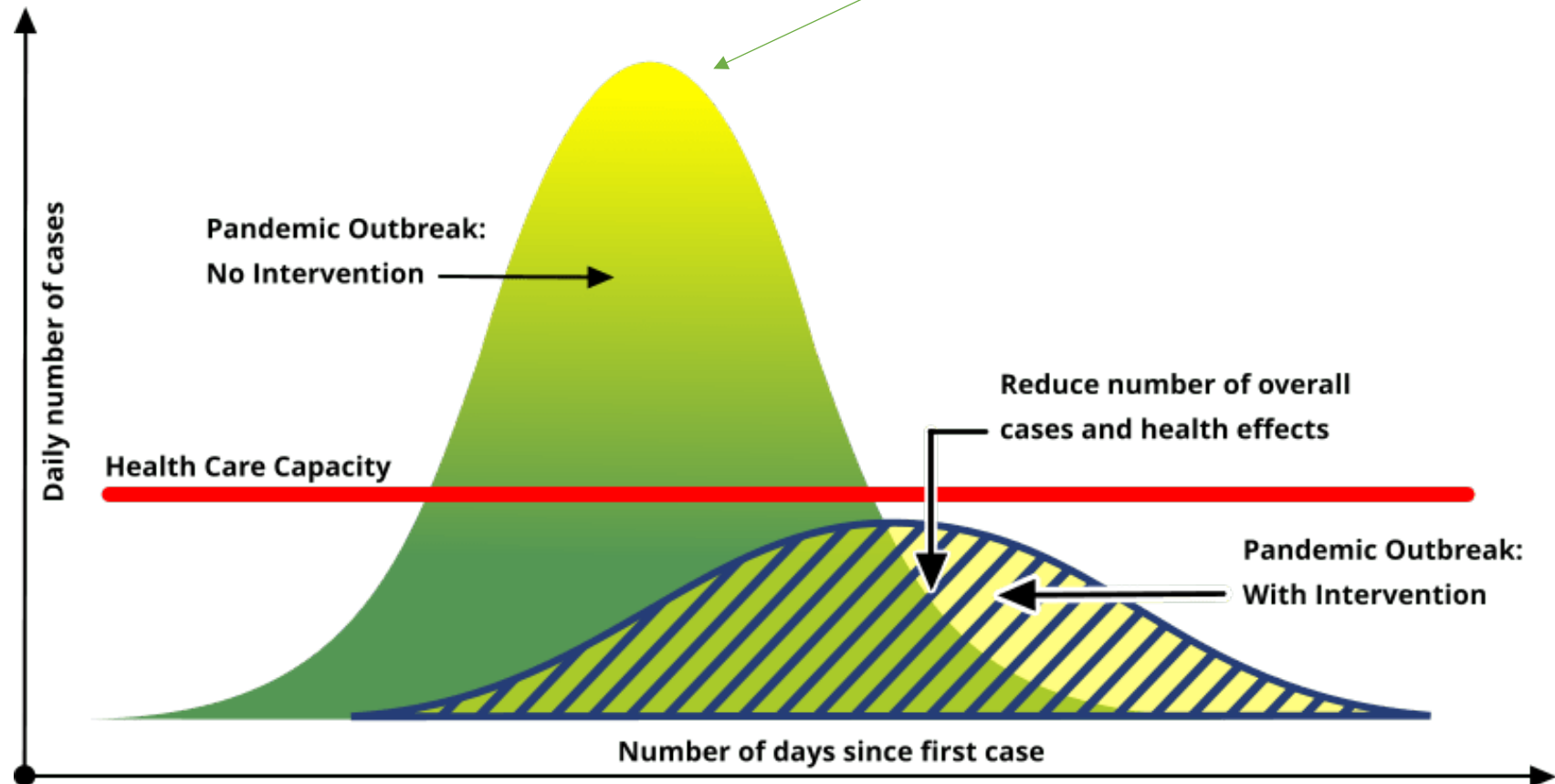
=> From 40 000 to up to 1 million samples in the same run

=> From 3€ to <1€/sample

En infectiologie ?

Techniques Amplicons

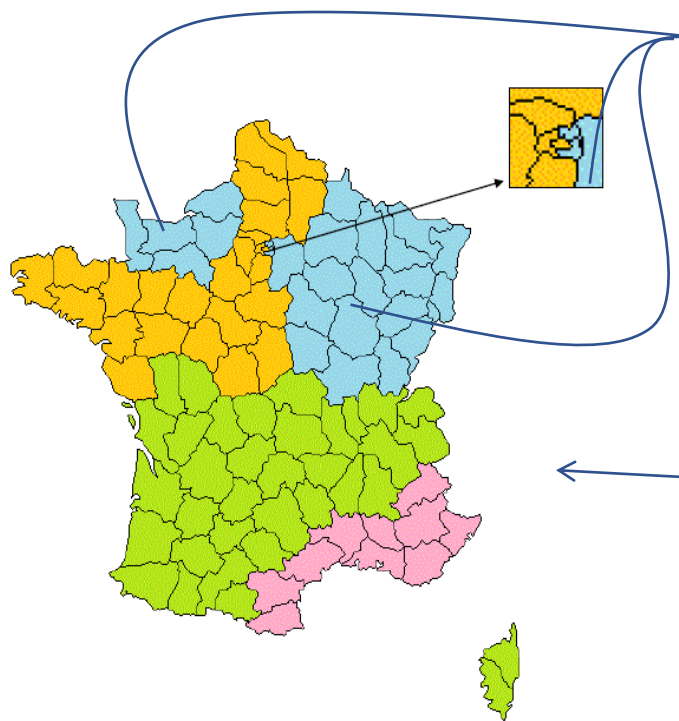
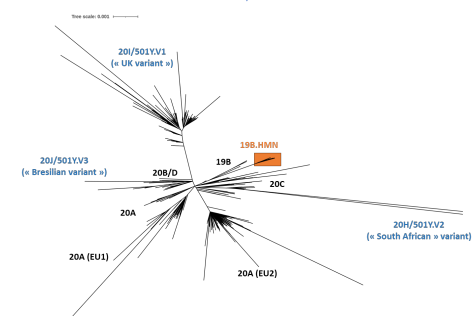
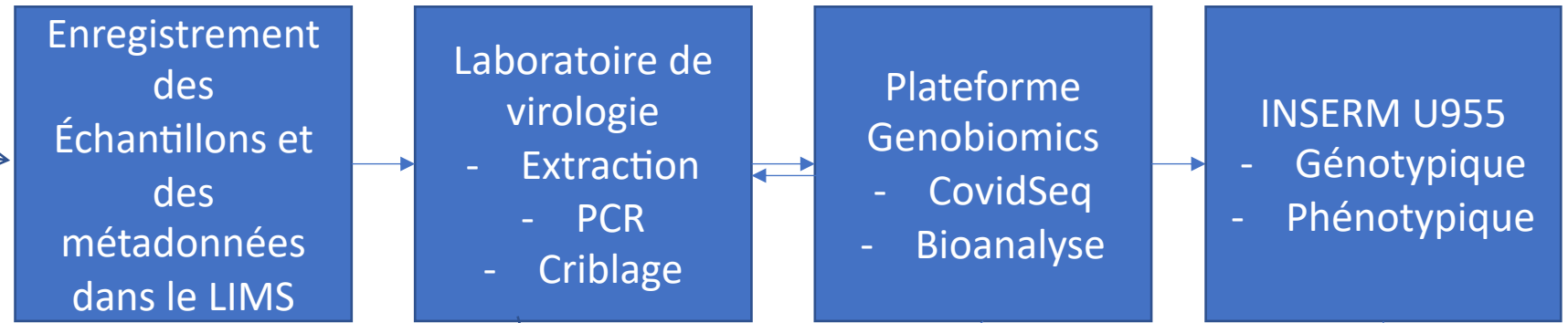
Surveillance/diagnostic
évolution du virus



Adapted from: CDC. Interim pre-pandemic planning guidance: community strategy for pandemic influenza mitigation in the United States—early, targeted, layered use of nonpharmaceutical interventions. Atlanta, GA: US Department of Health and Human Services, CDC; 2007. <https://stacks.cdc.gov/view/cdc/11425>.

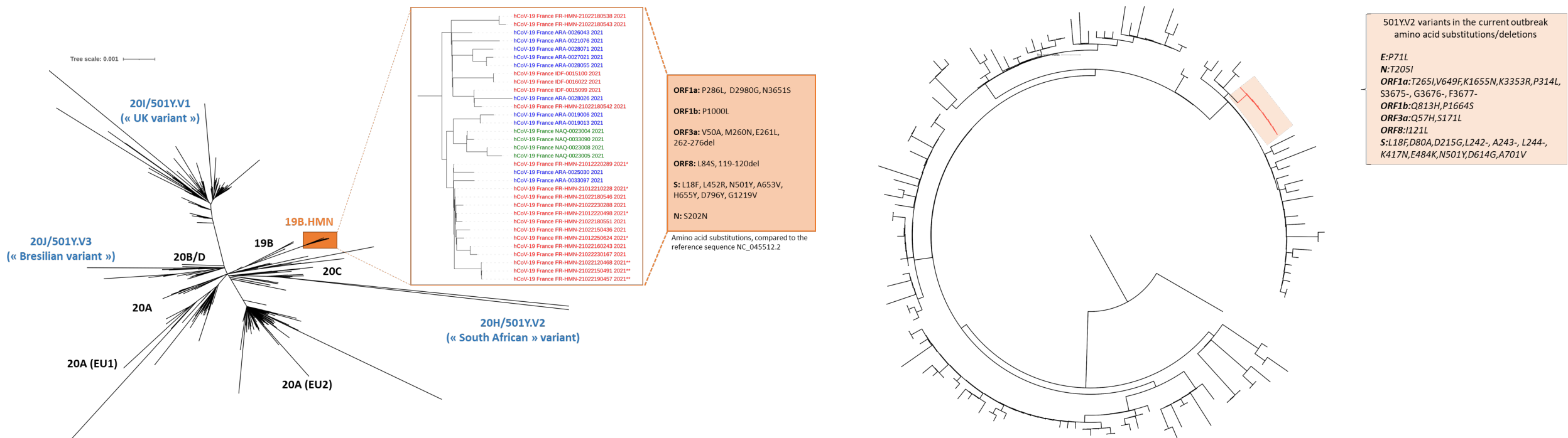
Phase pandémique : Emergence de variants

- Emergen

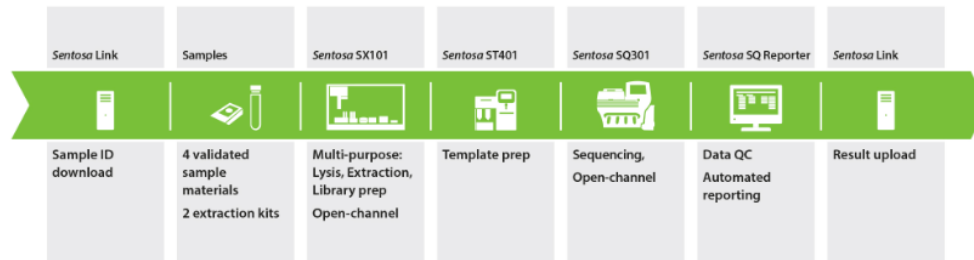


Phase pandémique : Emergence de variants

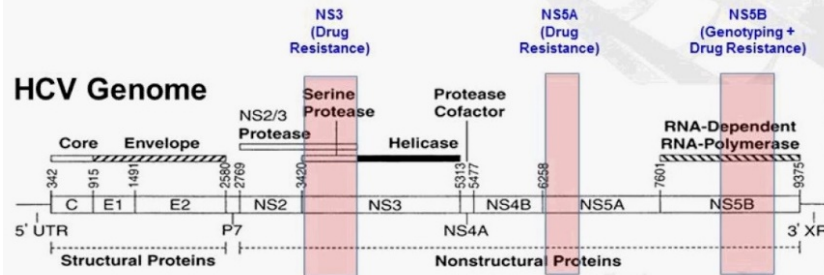
- Emergence de nouveaux variants, surveillance des cas graves



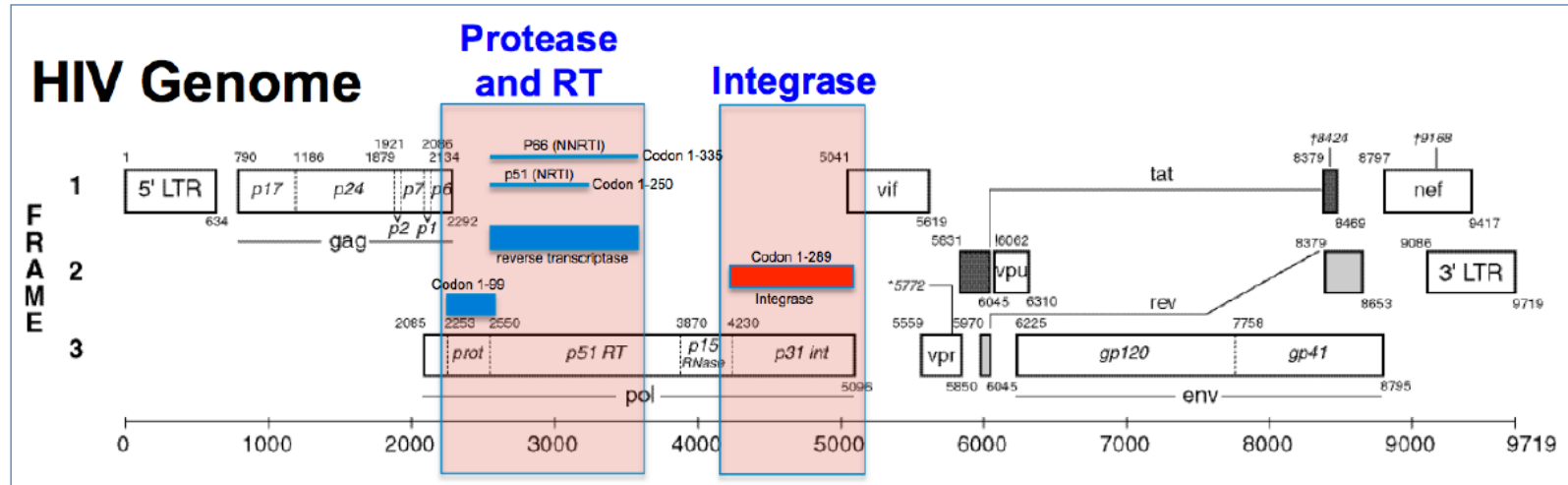
Phase pandémique : recherche de résistance



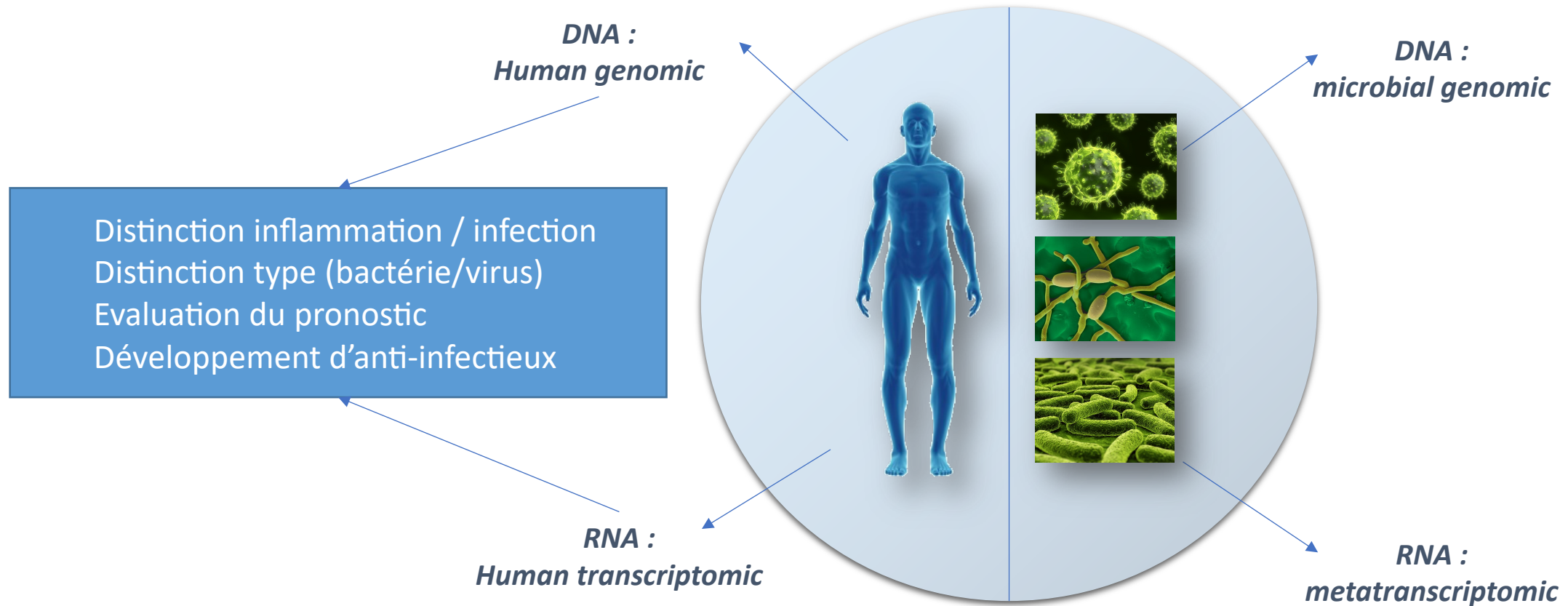
Design concept



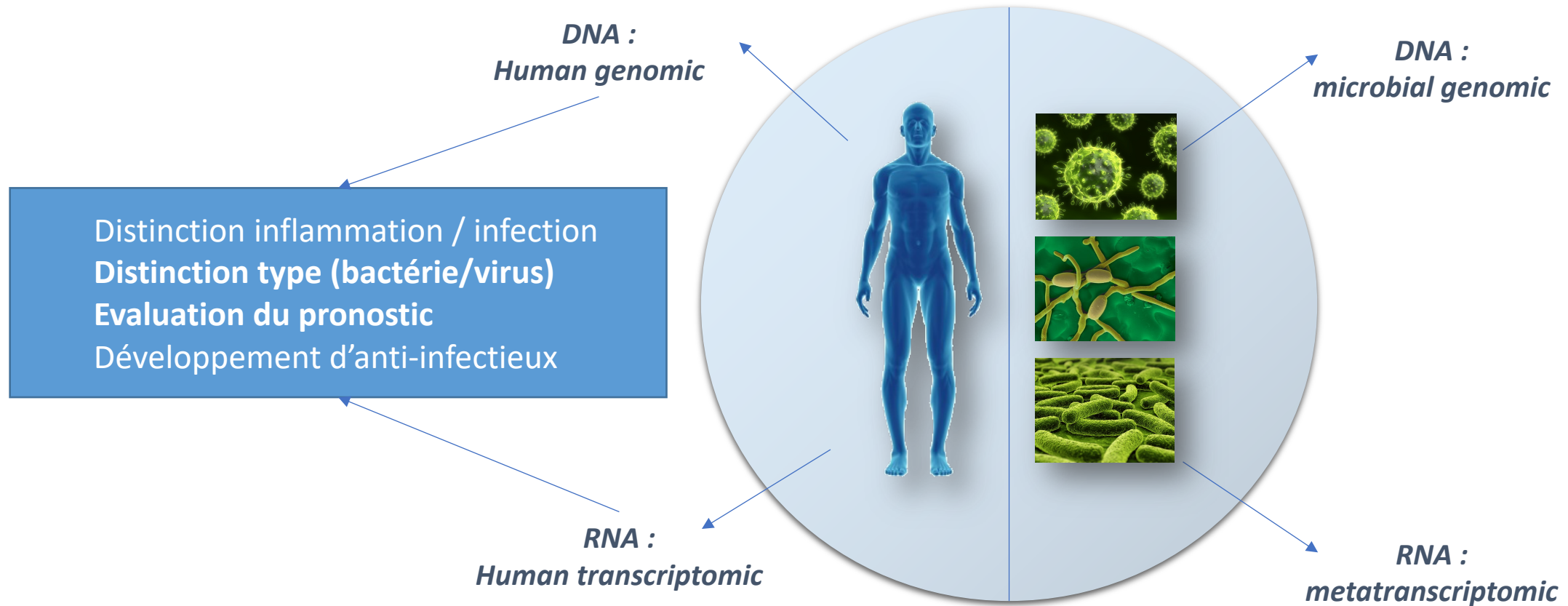
Target list includes NS3, NS5A and NS5B genes



Et demain ?

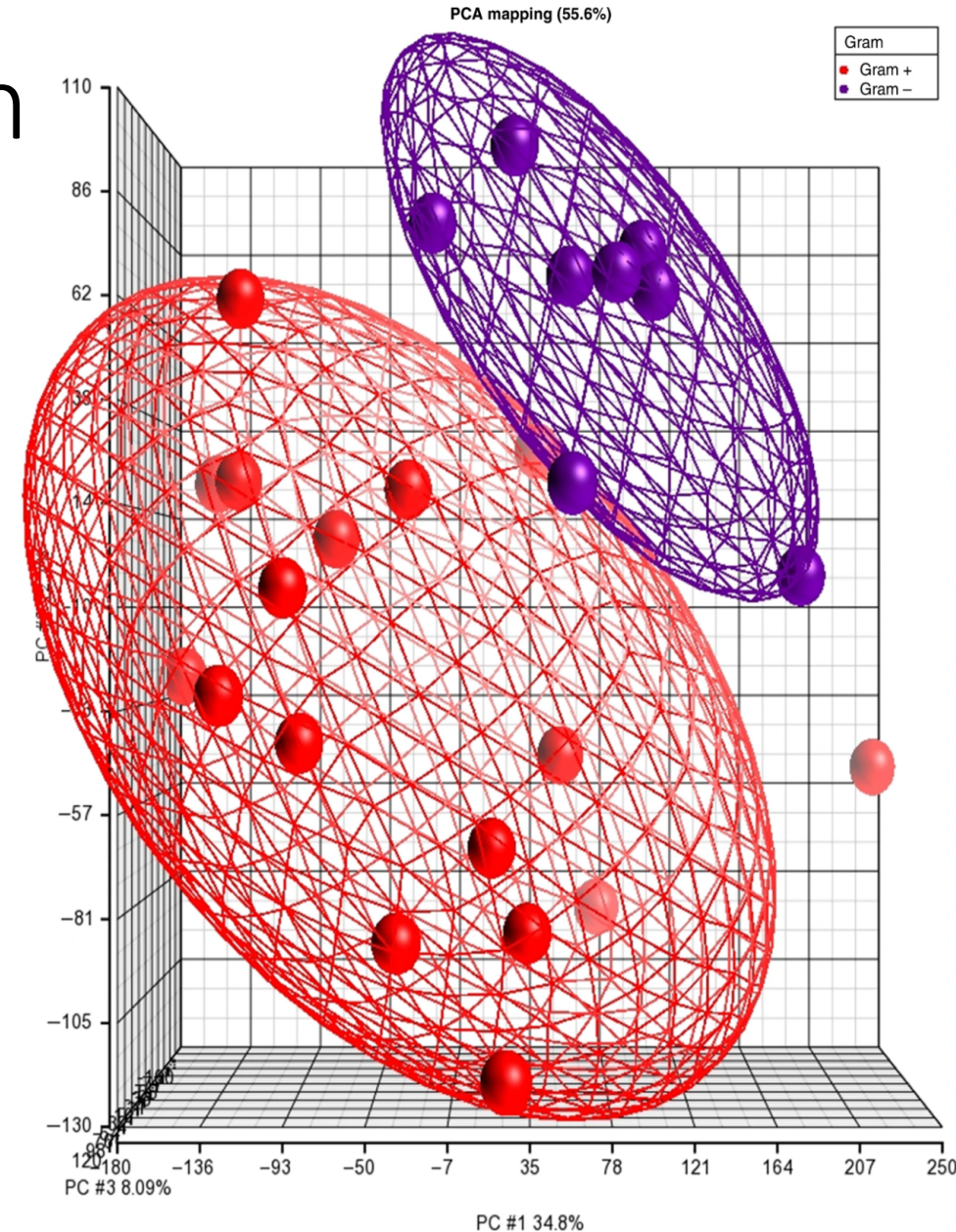


Et demain ?



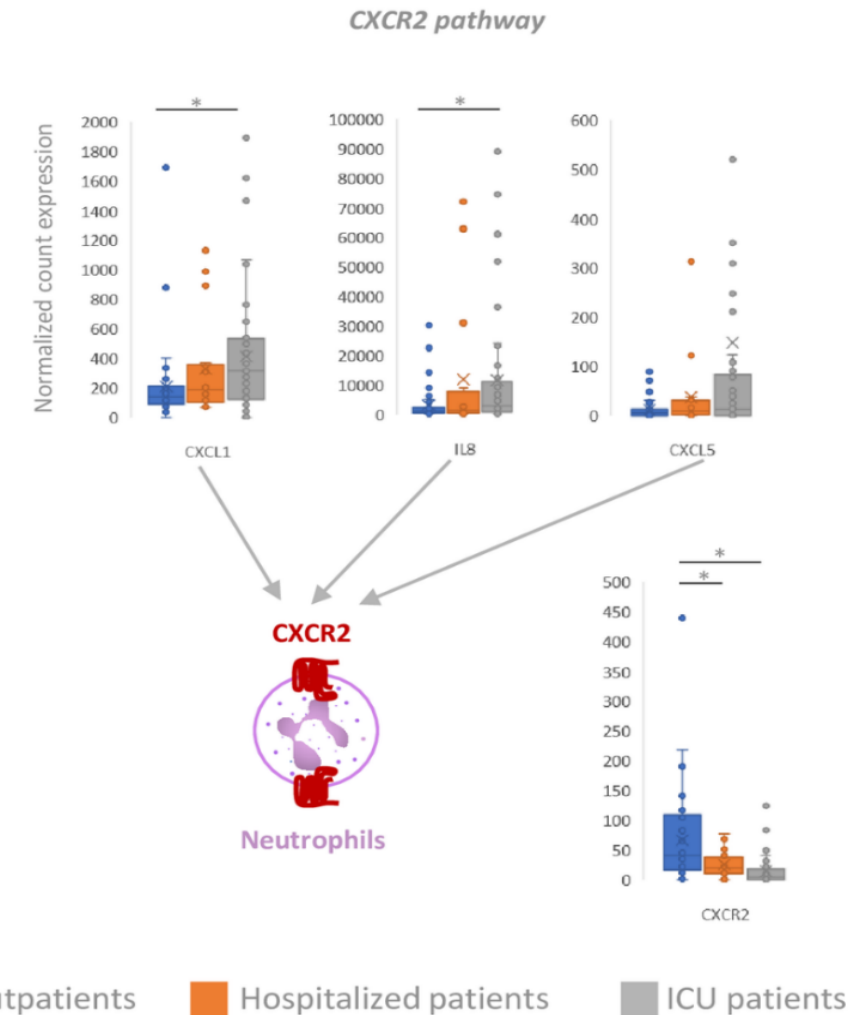
Distinction du type d'infection

- 25 nouveaux nés prématurés et septiques
- Recherche de profil transcriptomique prédictif du type d'infection
- L'analyse en composante principale montre des signatures moléculaires distinctes entre infection Gram+ et Gram-



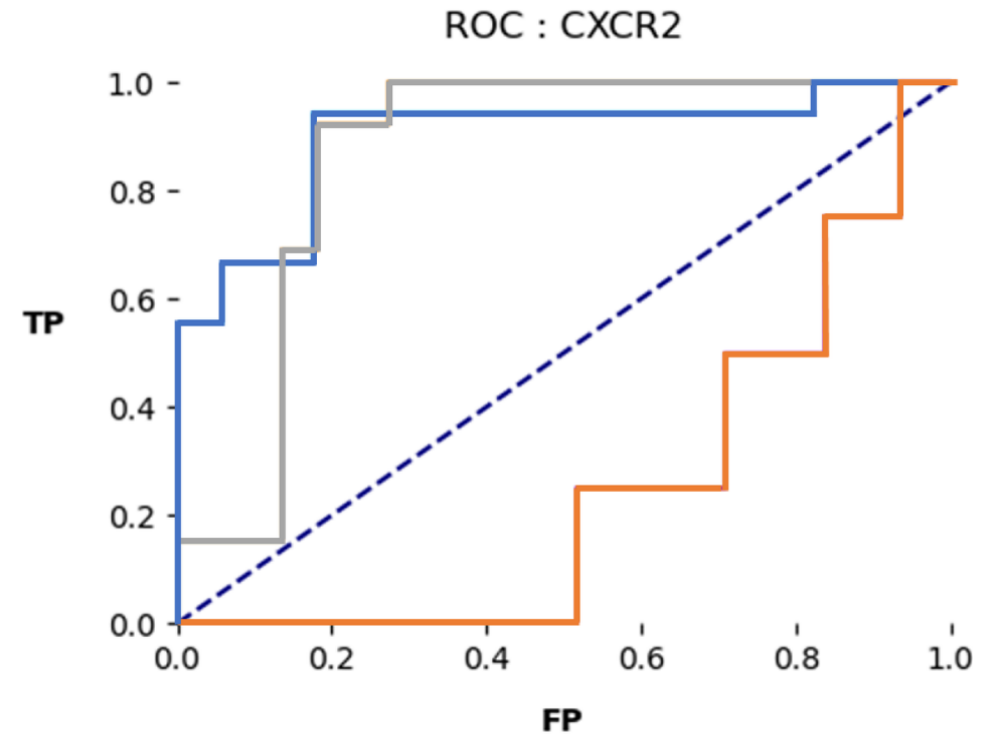
Evaluation du pronostic

- 113 patients positifs au SARS-CoV-2, 3 groupes (ambu, mal inf, réa)
- Caractériser la génomique, métagénomique et transcriptomique de l'hôte
- Plus forte expression de cytokines pro-inflammatoires chez les patients en réanimation
- Aucun marqueur viral associé à la gravité

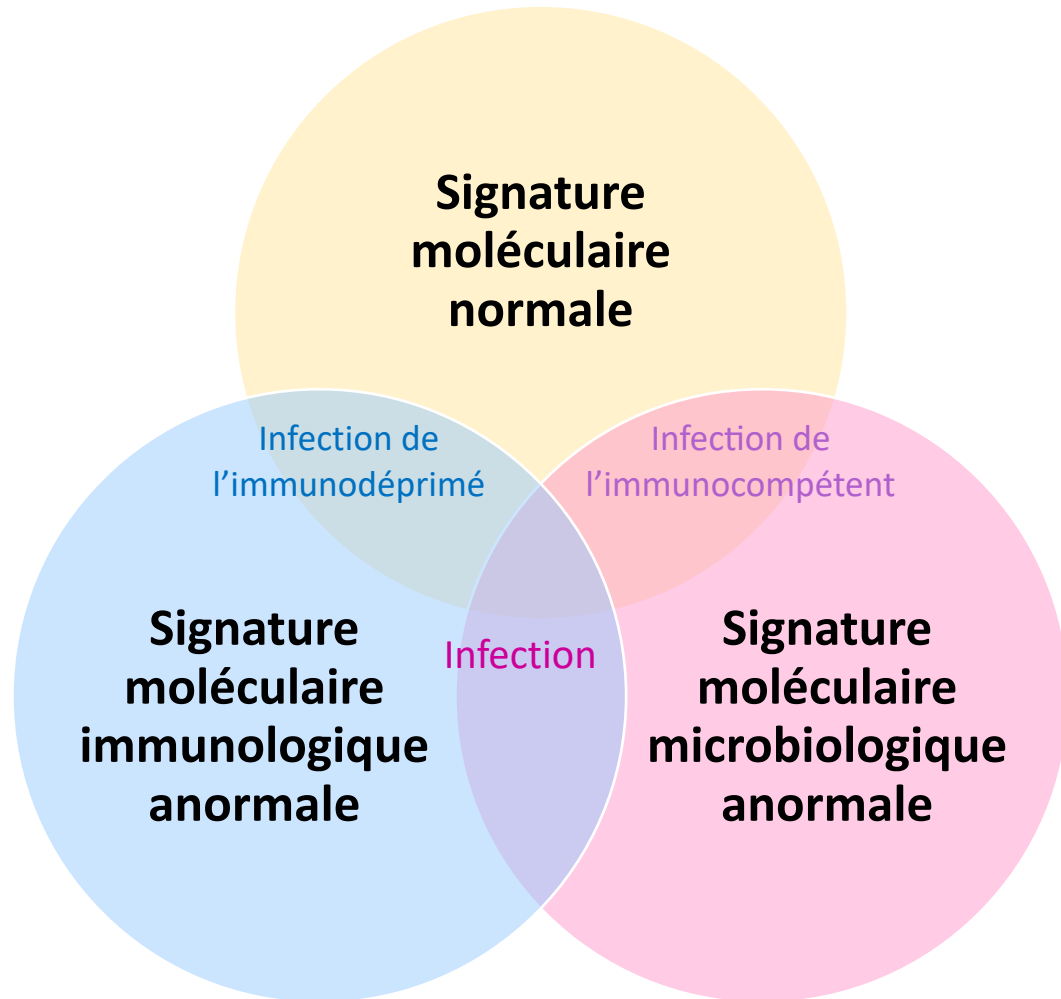


Evaluation du pronostic

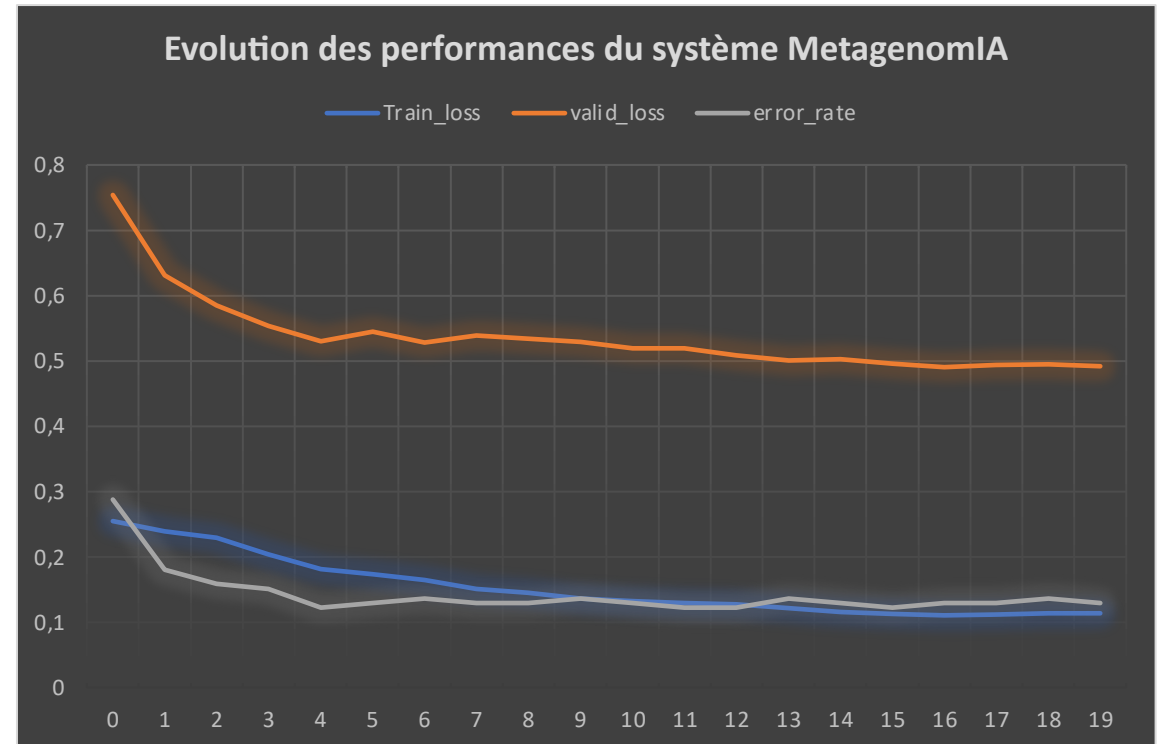
- Panel de transcrits prédictifs basé sur les données de transcriptomique
- AUC des ICU patients : 0,86
- AUC des Outpatients : 0,90



Le NGS demain ?



Thèse d'exercice Dr Amandine Caillault



Mémoire de DES Dr Pierre-Edouard Saint Antonin

Conclusion

- Le NGS apporte des solutions à chaque étape des épidémies pour :
 - Détecter précocement des émergences (pathogène, virulence, résistance...)
 - Apporter du diagnostic massif lorsque
 - Surveiller l'évolution des microorganismes (nouveaux variants, résistance, mutation d'échappement immunitaire...) à l'échelon global et individuel dans le cadre du diagnostic
- L'intégration de l'ensemble des données en un seul diagnostic est un pas important vers une médecine personnalisée, adaptée à la réponse spécifique de l'hôte à une infection par un pathogène spécifique
- Dans le futur, les signatures moléculaires globales pourront peut-être permettre une analyse complète du set de données ais sa complexité nécessitera sûrement la mise en place d'une IA dont les résultats sont déjà prometteurs.

Remerciements

MetaMIC Project

Technique

Vanessa Demontant,
Anais Nguyen

Bioinformatique

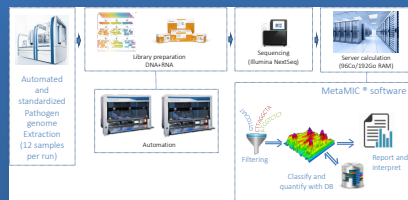
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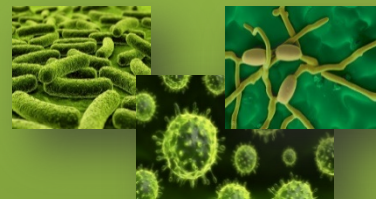
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French National Reference Center for
hepatitis

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Hospital, Créteil, France

U2TI (infectious diseases unit), Henri
Mondor, Créteil, France

All Clinicians and their patients



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