

# SARS-COV-2 AND INFLUENZA SEQUENCING

## GIHSN WEBINAR

LAURENCE JOSSET – HCL

ANTONIN BAL – HCL

QUENTIN SEMANAS - HCL

HADRIEN REGUE – HCL

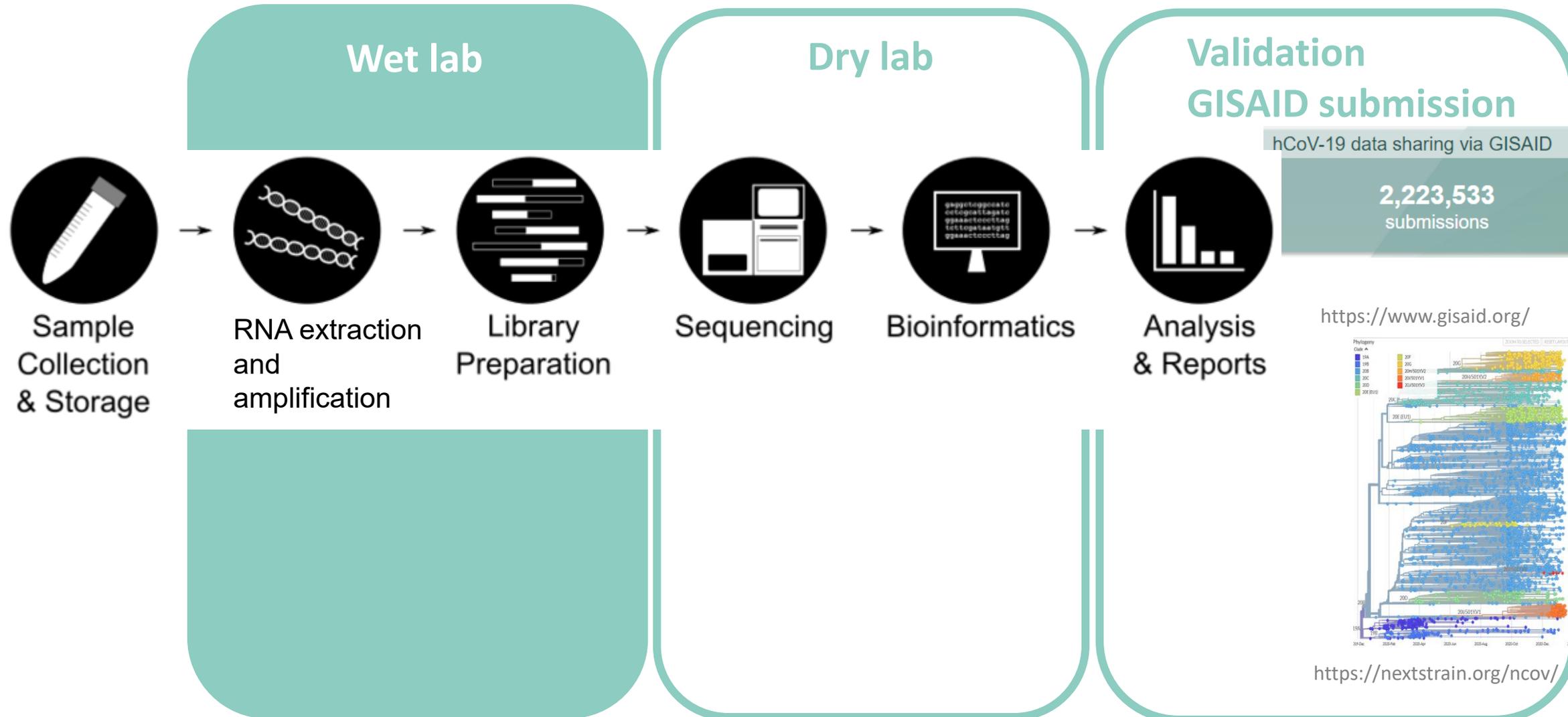
NATHALIE BERGAUD - HCL

CATHERINE COMMAILLE – IMPACT HEALTHCARE

2025/12/11    NGS TEAM- CNR VIRUS RESPIRATOIRES FRANCE SUD

**HCL**  
HOSPICES CIVILS  
DE LYON

# SARS-COV-2 AND INFLUENZA SEQUENCING

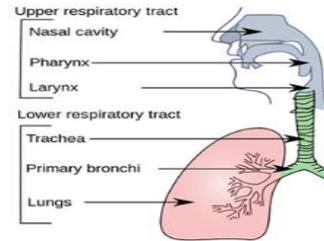


# METAGENOMICS-NGS

# METAGENOMICS-NGS

Detection of **known** and **unknown viruses**

**Universal method for viral detection and whole genome sequencing**



Influenza A & B, AdV, CMV, HHV-6, EBV, HBoV, HRV, RSV, PIV, MPV, CoV, Enterovirus, Measles, SARS-CoV-2

Bal et al., BMC Inf Dis, 2018

Filtration

DNase

Extraction

Nucleic acid precipitation

cDNA Amplification (WTA)

Lib Prep (COVIDSeq)

Illumina Sequencing

February 2020

Molecular characterization of SARS-CoV-2 in the first COVID-19 cluster in France reveals an amino acid deletion in nsp2 (Asp268del)

A. Bal <sup>1,2,3,4,\*</sup>, G. Destras <sup>1,2,3,\*</sup>, A. Gaymard <sup>1,2,3</sup>, M. Bouscambert-Duchamp <sup>1,2</sup>, M. Valette <sup>1,2</sup>, V. Escuret <sup>1,2,3</sup>, E. Frobert <sup>1,2,3</sup>, G. Billaud <sup>1,2</sup>, S. Trouillet-Assant <sup>3,4</sup>, V. Cheynet <sup>4</sup>, K. Brengel-Pesce <sup>4</sup>, F. Morfin <sup>1,2,3</sup>, B. Lina <sup>1,2,3</sup>, L. Josset <sup>1,2,3,\*</sup>

# PROS AND CONS FOR mNGS

## REFERENCE METHOD

- Non-targeted method adapted for new emerging variant detection

- Low sensitivity: Ct<20
- Expensive
- Low throughput
- Time-consuming process

Charre, *Virus Evol*, 2020

# AMPLICON-BASED TECHNICS

# AMPLICON-BASED APPROACH – SARS-COV-2

## ARTIC V5.4.2 98 AMPLICONS ~400 BP

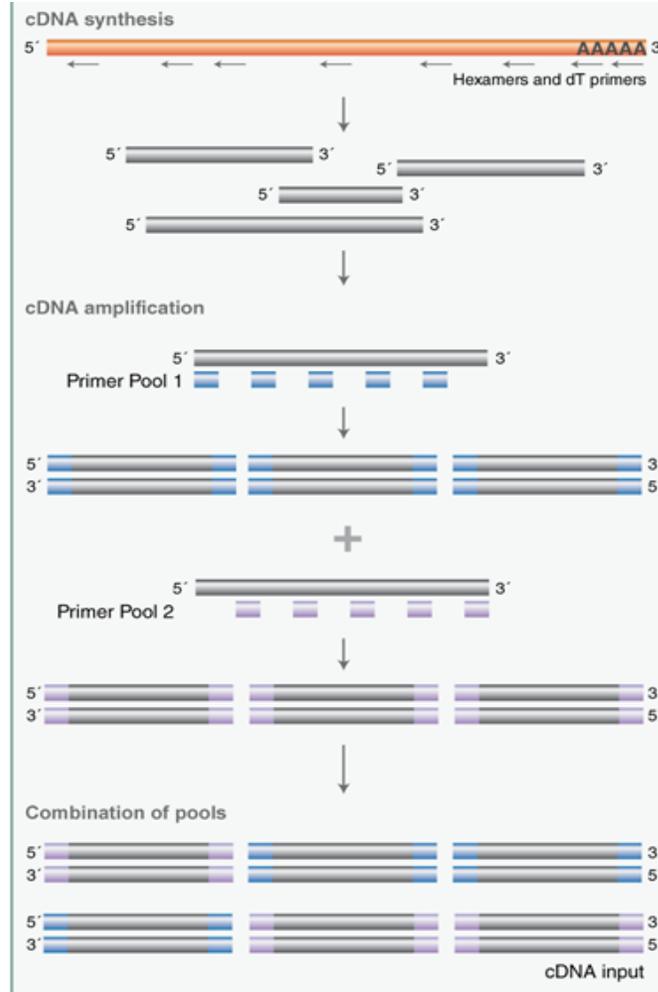
1) Nucleic acid extraction

96-Well plate



2) Miniaturized and automatized cDNA synthesis and amplification

384-Well plate



3) Miniaturized and automatized Lib preparation

- Illumina : COVIDSeq
- ONT: sequencing of 400nt amplicons

4) Illumina / ONT Sequencing

# AMPLICON-BASED APPROACH – INFLUENZA

## UNIVERSAL PRIMERS

1) Nucleic acid extraction

96-Well plate



2) Miniaturized and automatized cDNA synthesis and amplification

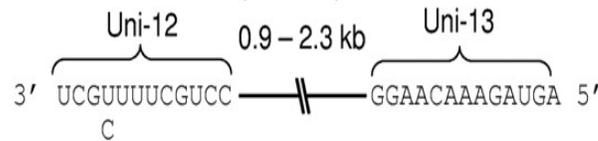
384-Well plate



> [J Virol.](#) 2009 Oct;83(19):10309-13. doi: 10.1128/JVI.01109-09. Epub 2009 Jul 15.

**Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza A viruses**

Bin Zhou<sup>1</sup>, Matthew E Donnelly, Derek T Scholes, Kirsten St George, Masato Hatta, Yoshihiro Kawaoka, David E Wentworth



[J Clin Microbiol.](#) 2014 May; 52(5): 1330–1337.  
doi: [10.1128/JCM.03265-13](#)

PMCID: PMC399  
PMID: [2450](#)

**Universal Influenza B Virus Genomic Amplification Facilitates Sequencing, Diagnostics, and Reverse Genetics**

Bin Zhou,<sup>a</sup> Xudong Lin,<sup>a</sup> Wei Wang,<sup>a</sup> Rebecca A. Halpin,<sup>a</sup> Jayati Bera,<sup>a</sup> Timothy B. Stockwell,<sup>a</sup> Ian G. Barr,<sup>b</sup> and David E. Wentworth<sup>1a</sup>

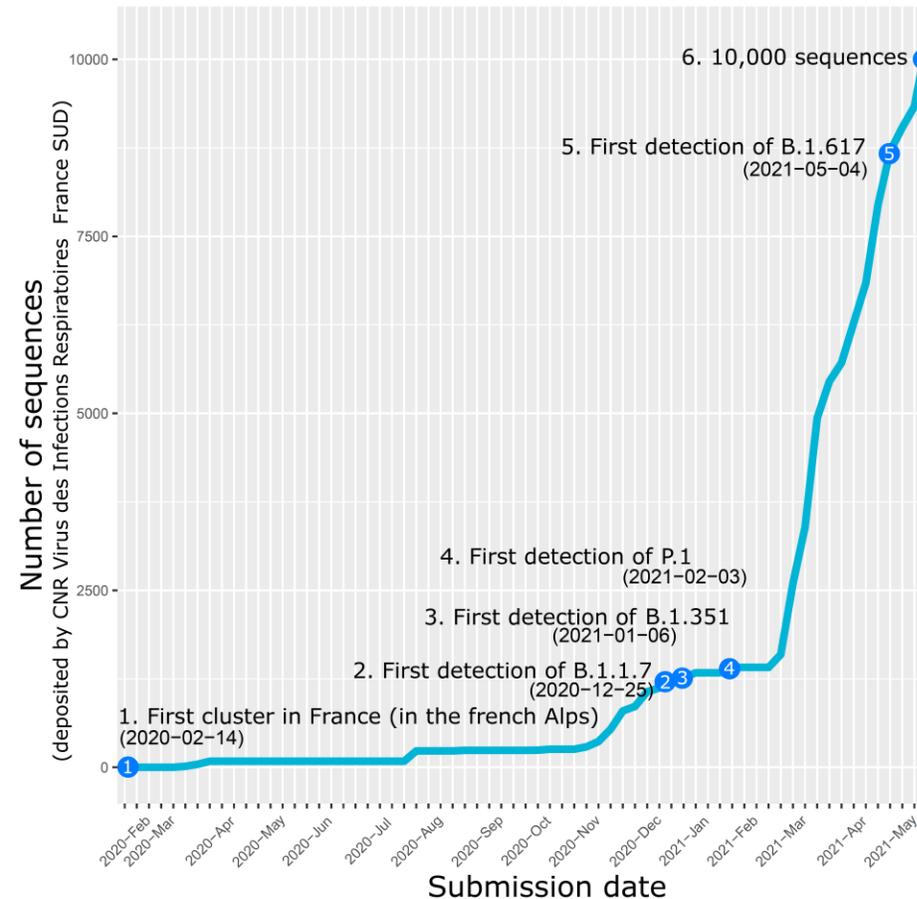
3) Miniaturized and automatized Lib preparation

- Illumina : COVIDSeq
- ONT: sequencing of 400nt amplicons

4) Illumina / ONT Sequencing

# PROS AND CONS FOR AMPLICONS APPROACHES

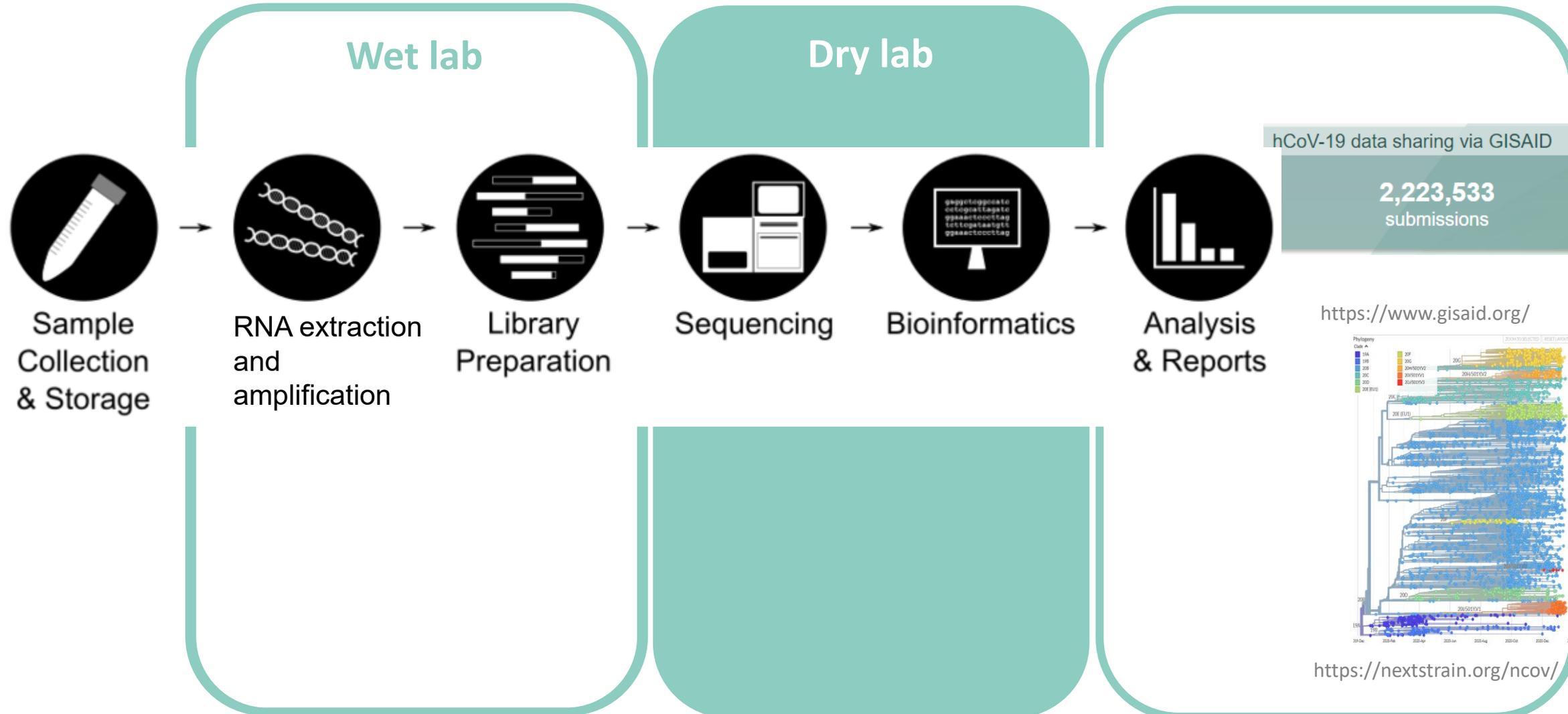
- Sensitivity
- Cost-effective
- High throughput
- Short-time process



- Impacted by little genome variations (SNPs or indels)

# SARS-COV-2 AND INFLUENZA SEQUENCING

## 3 STEPS



# BIOINFORMATICS

## FROM RAW DATA TO THE CONSENSUS SEQUENCE: ILLUMINA SEQUENCING



# BIOINFORMATICS

## OPEN SOURCE AND COMMERCIAL SOLUTIONS

For Illumina users...



« SEQMET », our « in house » pipeline  
freely available on request:

Input viruses:

-SARS-CoV-2

-Influenza A/B (avian analysis are in  
reworking)

-Respiratory Syncytial Viruses A/B

... and nanopore adepts

We haven't pipeline dedicated to ONT data analysis  
to address, however we can provide help with your  
data as well!

Several analysis workflows are proposed by ONT:

Influenza workflow :

<https://github.com/epi2me-labs/wf-flu>

SARS-CoV-2 workflow:

<https://github.com/epi2me-labs/wf-artic>



EPI2ME

<https://labs.epi2me.io/> provide a large  
analysis panel (and tutorials) to process  
online your data

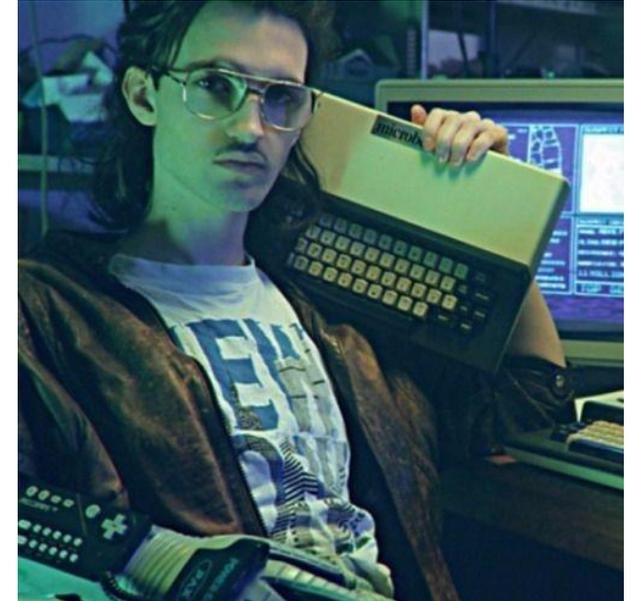
# BIOINFORMATICS

## CONTACTS

- We can provide help in your setup process
- Feel free to contact us

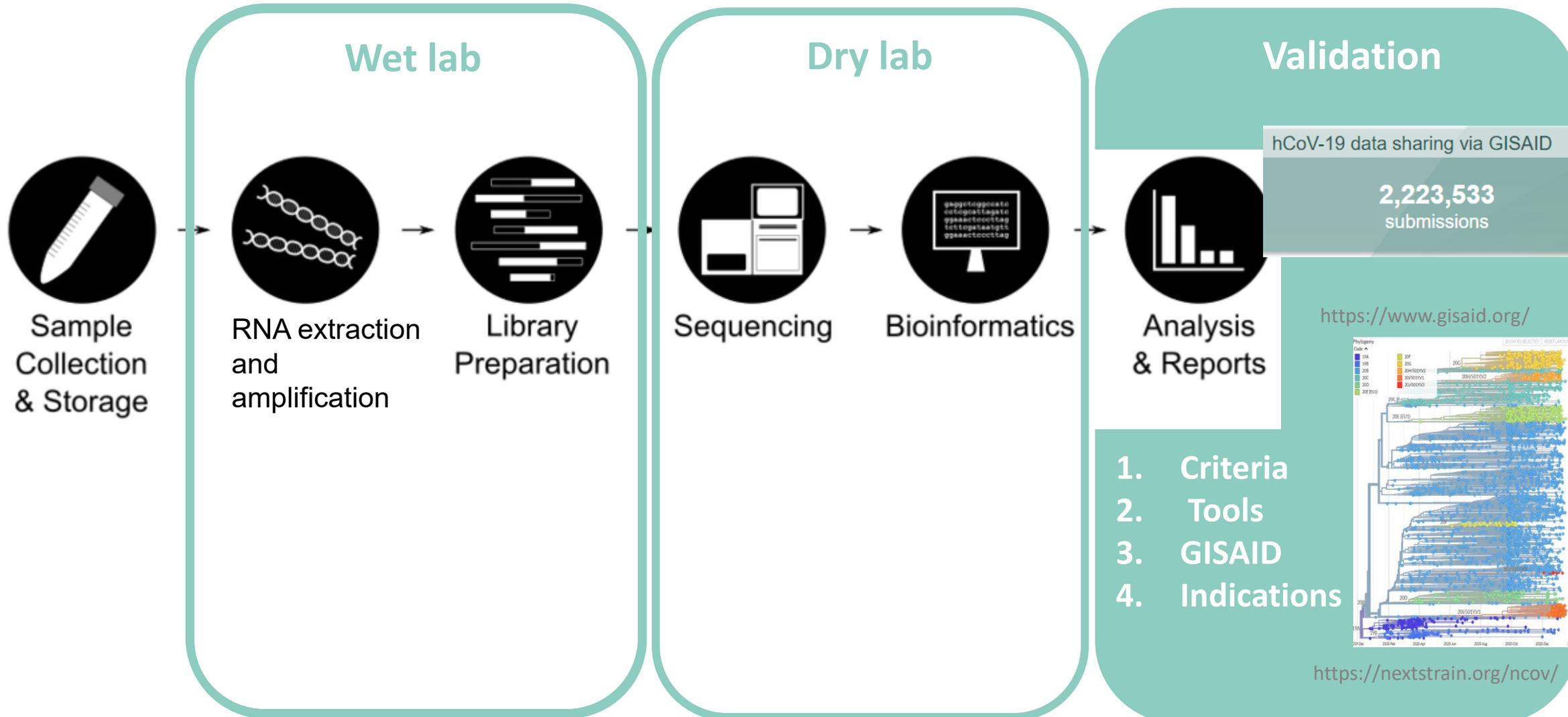
NRC's Lyon Bioinformatician:

-Hadrien Regue: [hadrien.regue@chu-lyon.fr](mailto:hadrien.regue@chu-lyon.fr)



# SARS-COV-2 AND INFLUENZA SEQUENCING

## 3 STEPS



# VALIDATION CRITERIA

# VALIDATION CRITERIA

## NEGATIVE CONTROLS - CONTAMINATION

- **3 No-Template-Controls (NTC)** per 96-well plate
- Nuclease free-water **processed through the whole** workflow (including extraction step)

Viral genome coverage for NTCs < 5%

# VALIDATION CRITERIA

## POSITIVE CONTROLS - CONTAMINATION

- Viral isolate belonging **to an historical clade**
- **Known mutations**
- **One positive control / 96-well plate**

Viral genome coverage for positive control > 95%

# VALIDATION CRITERIA

## COVERAGE

- Reminder: minimal depth of coverage / base for consensus sequence generation: 10X for Illumina sequencing
- Otherwise : N

The sequence is validated if the genome coverage is > 95 % with a mean depth of coverage > 200X

- SARS-CoV-2 : WGS with >95% coverage
- Influenza virus : HA and NA segments with >95% coverage

# VALIDATION CRITERIA

## NUMBER AND TYPE OF MUTATIONS

- Divergence (number and type of mutations):
  - Nextclade Mut and phylogenetic tree
  - Check unknown indel
  - compare mutation profile with outbreak.info (for SARS-CoV-2)
- Frameshift mutation: QC nextclade FrameShift
- Atypical set of mutations : QC nextclade Private Mutation
- Minor variants / ambiguous bases : QC Mixed sites

Repeat extraction  
+/- mNGS

ID	Sequence name	QC	Clade	Mut.	non-ACGTN	Ns	Gaps
23	✓ Switzerland/AG-ETHZ-430474/2020	N M P C F S					
42	⚠ USA/CA-CDC-LC0027675/2021	N M P C F S					
29	✓ BHR/30005866/2021	N M P C F S					
35	✓ USA/CA-CDC-LC0024684/2021	N M P C F S					
34	⚠ USA/NJ-CDC-LC0019972/2021	N M P C F S					
22	✓ Switzerland/un-ETHZ-420516/2020	N M P C F S					
9	✓ HongKong/VM20006541/2020	N M P C F S					
67	✓ USA/FL-CDC-ASC210057011/2021	N M P C F S					
28	⚠ USA/VA-DCLS-3814/2021	N M P C F S					
84	✓ Switzerland/100064/2020	N M P C F S					
30	⚠ USA/TX-CDC-STM-000011246/2021	N M P C F S					
50	✓ USA/GA-CDC-ASC210019884/2021	N M P C F S					
73	✓ USA/IL-CDC-LC0051439/2021	N M P C F S					
57	✓ USA/VA-DCLS-5091/2021	N M P C F S					
21	✓ USA/JGGF/2020	N M P C F S					

Overall QC score: 158  
Overall QC status: bad  
Detailed QC assessment:

- N Missing Data: mediocre**  
Missing data found. Total Ns: 2942 (3000 allowed). QC score: 98
- M Mixed Sites: good**  
No issues
- P Private Mutations: good**  
No issues
- C Mutation Clusters: good**  
No issues
- F Frame shifts: good**  
No issues
- S Stop codons: mediocre**  
1 misplaced stop codon(s) detected. Affected gene(s): ORF8. QC score: 75





Color By

Clade

Filter Data

Type filter query here...

Currently selected filter categories:

1 x Node type

Tree

Layout

RECTANGULAR

RADIAL

UNROOTED

SCATTER

Branch Labels

clade

Show all labels

Tip Labels

Filtered to New (3084)

Phylogeny

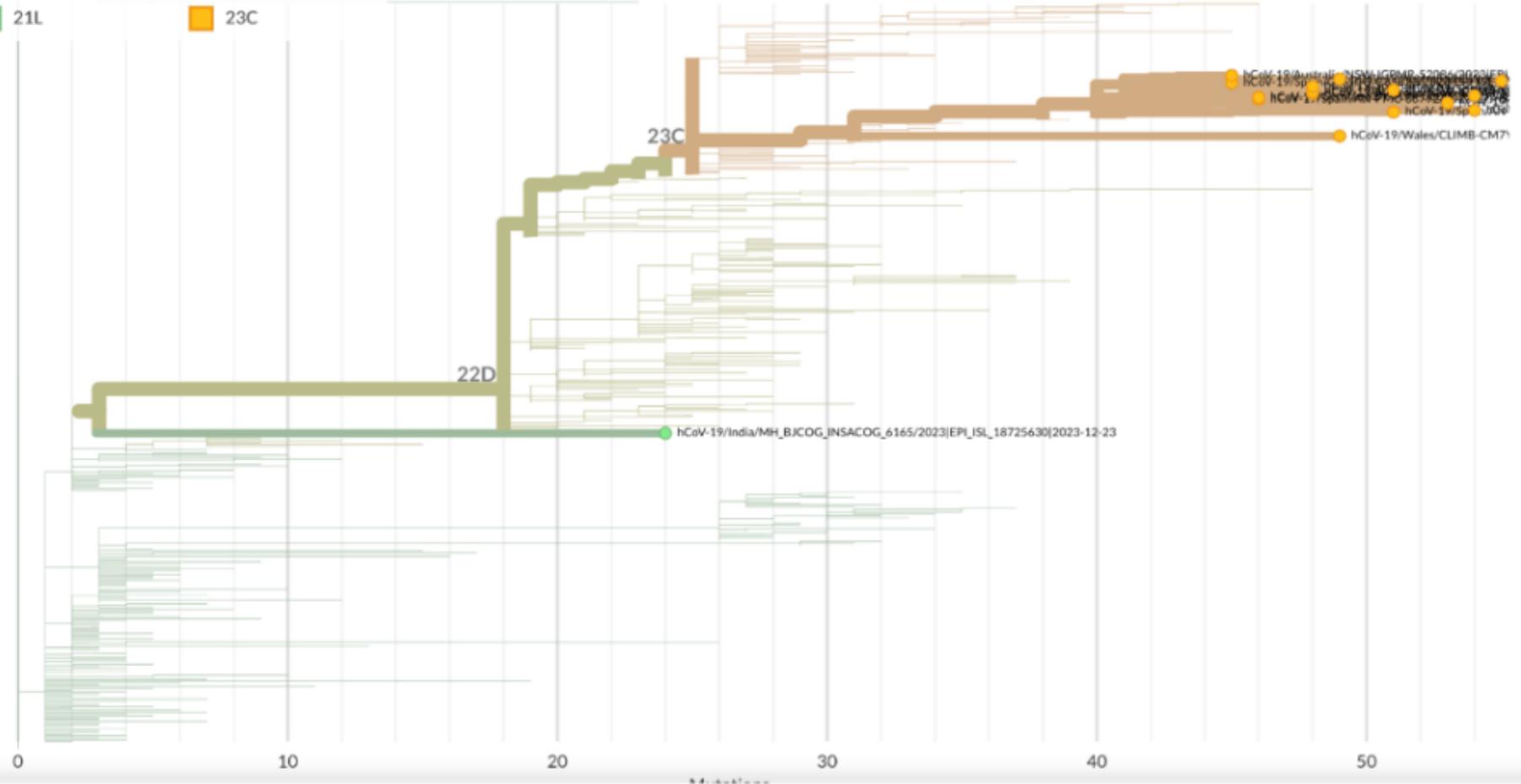
Clade

21L

23C

Click "Tree" tab to load this tree view

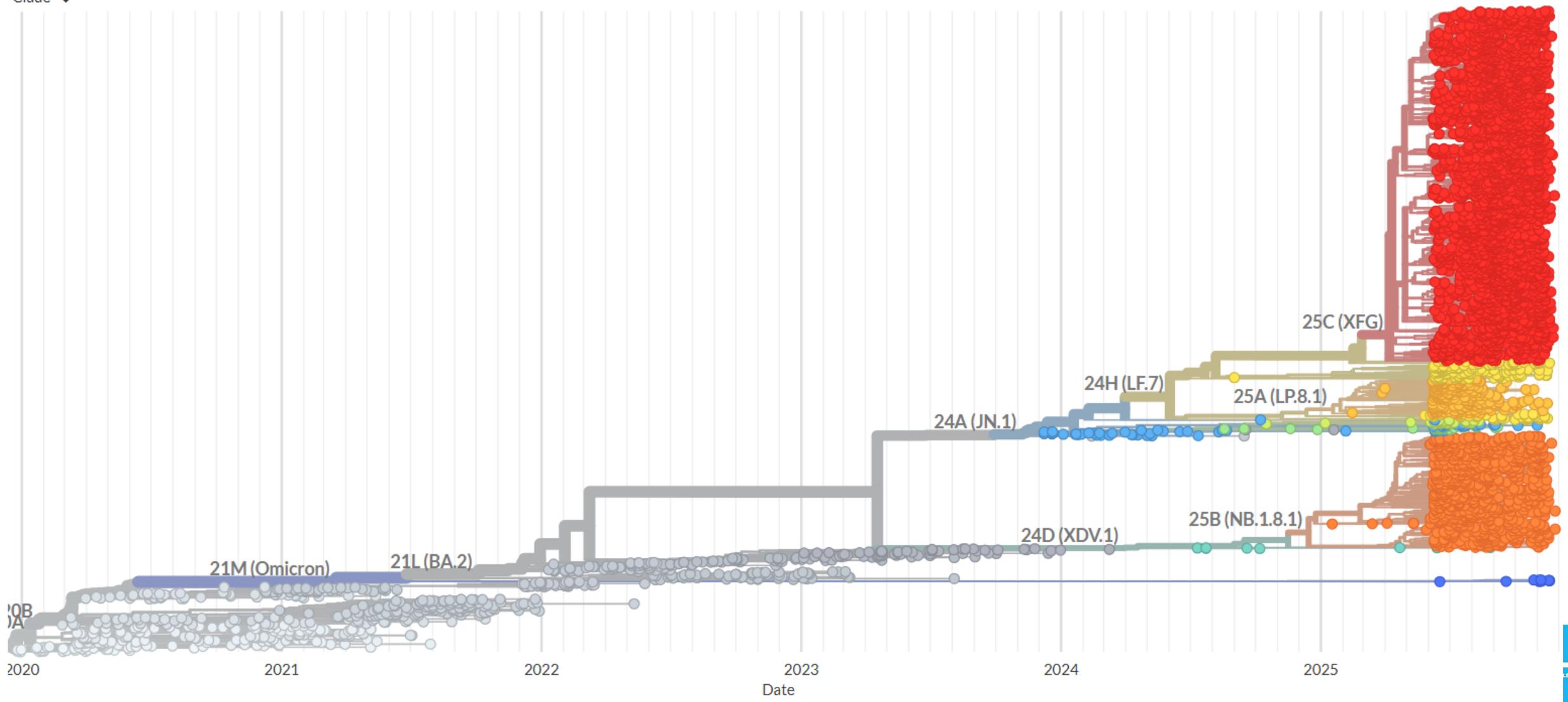
ZOOM TO SELECTED RESET LAYOUT



Phylogeny

Clade ▾

🔍 ZOOM TO SELECTED ZOOM TO ROOT



# VALIDATION CRITERIA

## MOLECULAR EPIDEMIOLOGY

- Different clades within the same cluster
- Clade different from the background
- Clade not circulating in a given area (return from travel)
- Former clades no longer circulating

Repeat  
extraction

# TOOLS FOR QUALITY CONTROL AND VISUALIZATION

[HTTPS://CLADES.NEXTSTRAIN.ORG/](https://clades.nextstrain.org/)

The screenshot shows the Nextclade website interface. At the top, there is a navigation bar with the Nextclade logo, a 'What's new' link, a language dropdown set to 'English', and social media icons for Twitter, GitHub, and a user profile. The main heading is 'Nextclade beta v0.12.0' with the tagline 'Clade assignment, mutation calling, and sequence quality checks'. Below this, there are two main sections: 'Simple' and 'Private'. The 'Simple' section states 'No installation or setup - drop a file and see the results'. The 'Private' section states 'No remote processing - sequence data never leaves your computer'. To the right, there is a large form for uploading sequences. It includes a dropdown menu for 'SARS-CoV-2', a toggle for 'Simple mode' (which is currently selected) and 'Advanced mode'. Below the dropdown are three buttons: '\* Sequences required', 'From file', 'From URL', and 'Paste'. A large dashed box contains the text 'Drag & Drop a file here' and 'or' above a blue 'Select a file' button. A 'FASTA' file icon is also visible. At the bottom of the form, there is a link 'Show me an Example'.

 Nextclade is currently under active development. Implementation details and data formats are subjects to change. The app may contain bugs. Please report any issues and leave feedback at [github.com/nextstrain/nextclade](https://github.com/nextstrain/nextclade)

# NEXTCLADE



Settings

What's new

English



Back

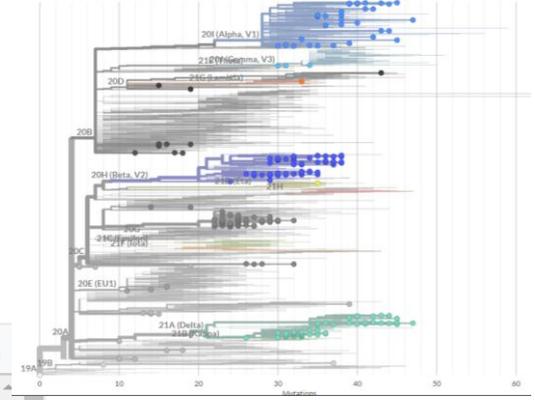
Done. Total sequences: 384. Succeeded: 276. Failed: 108



ID	Sequence name	QC	Clade	Mut.	non-ACGTN	Ns	Gaps	Ins.	Nucleotide sequence
29	PI241-021115521401_S84	N M P C F S	20I (Alpha, V1)	33	0	71	19	0	
32	PI241-021115525101_S67	N M P C F S	20I (Alpha, V1)	33	0	121	13	0	
48	PI241-021115548301_S94	N M P C F S	20I (Alpha, V1)	32	0	121	13	0	
50	PI241-021115552601_S92	N M P C F S	20I (Alpha, V1)	33	0	121	13	0	
51	PI241-021115554001_S91	N M P C F S	20I (Alpha, V1)	33	0	121	13	0	
53	PI241-021115556001_S89	N M P C F S	20I (Alpha, V1)	32	0	121	13	0	
97	PI242-021116063302_S119	N M P C F S	20I (Alpha, V1)	29	0	124	18	0	
101	PI242-021116366101_S186	N M P C F S	20I (Alpha, V1)	35	0	121	16	0	
102	PI242-021116366601_S187	N M P C F S	20I (Alpha, V1)	34	0	126	13	0	
108	PI242-021116371501_S180	N M P C F S	20I (Alpha, V1)	37	0	121	13	0	
133	PI242-021116388701_S98	N M P C F S	20I (Alpha, V1)	30	0	100	43	6	
150	PI242-021117223901_S132	N M P C F S	20I (Alpha, V1)	29	0	177	47	0	
158	PI242-021117246301_S137	N M P C F S	20I (Alpha, V1)	32	0	289	47	0	
164	PI242-021117263001_S128	N M P C F S	20I (Alpha, V1)	32	0	295	47	0	
170	PI242-021117281701_S125	N M P C F S	20H (Beta, V2)	30	0	294	47	0	
193	PI243-021117197701_S198	N M P C F S	20H (Beta, V2)	33	0	237	47	0	
195	PI243-021117201701_S197	N M P C F S	20H (Beta, V2)	32	0	101	47	0	
198	PI243-021117205601_S196	N M P C F S	20H (Beta, V2)	32	0	291	47	0	
211	PI243-021117239601_S207	N M P C F S	20H (Beta, V2)	30	0	278	47	0	
214	PI243-021117247301_S206	N M P C F S	20H (Beta, V2)	32	0	281	47	0	

Overall QC score: 0  
 Overall QC status: good  
 Detailed QC assessment:

- N** Missing Data: good  
No issues
- M** Mixed Sites: good  
No issues
- P** Private Mutations: good  
No issues
- C** Mutation Clusters: good  
No issues
- F** Frame shifts: good  
No issues
- S** Stop codons: good  
No issues



# PANGOLIN

[HTTPS://PANGOLIN.CO-UK.IO/](https://pangolin.cog-uk.io/)

Drag and drop fasta file

Select fasta file to upload

## Pangolin COVID-19 Lineage Assigner



Phylogenetic Assignment of Named  
Global Outbreak LINEages

You can upload one or more sequences by dragging and dropping a (multi)fasta file or clicking "Select fasta file to upload" and selecting a (multi)fasta file.

This Web Application assigns lineages to COVID-19 sequences based on the methodology described in this [article](#)

The software to assign lineages based on the algorithm that was developed by [Áine O'Toole](#), [Verity Hill](#), [JT McCrone](#), [Emily Scher](#) and [Andrew Rambaut](#).

The source code can be found [here](#)

Recommended browsers   or 

# INSAFLU

Explore these "expand-and-collapse"

click here to download the consensus sequence; i.e., a multi-FASTA file containing a version of the reference sequence with all validated variants replaced.  
NOTE: consensus sequences are exclusively generated for locus with 100% of its length covered by ≥10-fold (GREEN circles)

Project "samples" list

EXPLORE THE "MORE INFO" ICON

Sample Name	Type And Subtype	Putative Mixed Infection	Dataset	Coverage	Consensus File	Alerts	Results
sample_1	A-H3N2	Yes	early_season	●●●●●●●●	Consens..._1.fasta	1	More info
sample_10	A-H3N2	No	early_season	●●●●●●●●			More info
sample_2	A-H3N2	Yes	early_season	●●●●●●●●			More info
sample_3	A-H3N2	No	early_season	●●●●●●●●	Not available.	8	More info

Sample result list (.csv): [Sample\\_list.csv](#) Sample result list (.tsv): [Sample\\_list.tsv](#)

Locus: 7  
Mean depth of coverage: 1751.1  
% of size covered by at least 1-fold: 100.0%  
% of size covered by at least 10-fold: 100.0%

Check the influenza type and subtype/lineage

Check which samples represent "putative mixed infections"

- by directing the mouse pointer on each circle you can check the overall coverage report  
- by clicking on each circle you can check the coverage plot

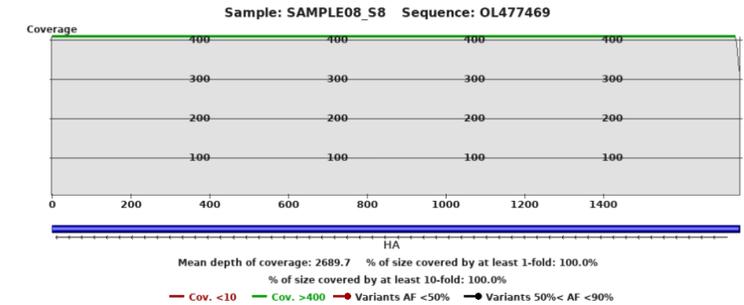
# INSAFLU

## EXEMPLE WITH AVIAN SAMPLES

Sample Name	Uploaded Date	#Fastq Files	Technology	Classification	Data Set	Alerts	#Quality Seq. (Fastq1)-(Fastq2)
SAMPLE08_S8	09-12-2025 10:24	2	Illumina/IonTorrent	A-H5N1	Generic	0	<b>i</b> (874676/147.6)-(874676/144.7) (1749352)
SAMPLE09_S9	09-12-2025 10:21	2	Illumina/IonTorrent	A-H5N1	Generic	0	<b>i</b> (1169243/147.5)-(1169243/144.9) (2338486)
SAMPLE10_S10	09-12-2025 10:20	2	Illumina/IonTorrent	A-H5N1	Generic	0	<b>i</b> (998971/147.5)-(998971/144.8) (1997942)

*Quick subtyping when uploading your data*

Samples	OL477466	OL477467	OL477468	OL477469	OL477470	OL477471	OL477472	OL477473
SAMPLE10_S10	803.8 - 100.0% (10x)	517.0 - 100.0% (10x)	135.8 - 100.0% (10x)	5626.2 - 97.5% (10x)	229.6 - 100.0% (10x)	3726.9 - 87.9% (10x)	466.0 - 100.0% (10x)	311.5 - 100.0% (10x)
SAMPLE09_S9	1986.4 - 94.2% (10x)	439.3 - 100.0% (10x)	889.8 - 100.0% (10x)	732.0 - 100.0% (10x)	231.1 - 100.0% (10x)	5797.1 - 99.8% (10x)	315.8 - 100.0% (10x)	5480.2 - 99.1% (10x)
SAMPLE08_S8	120.1 - 100.0% (10x)	699.2 - 100.0% (10x)	372.3 - 100.0% (10x)	689.7 - 100.0% (10x)	749.5 - 100.0% (10x)	2904.7 - 99.8% (10x)	839.9 - 100.0% (10x)	391.4 - 100.0% (10x)



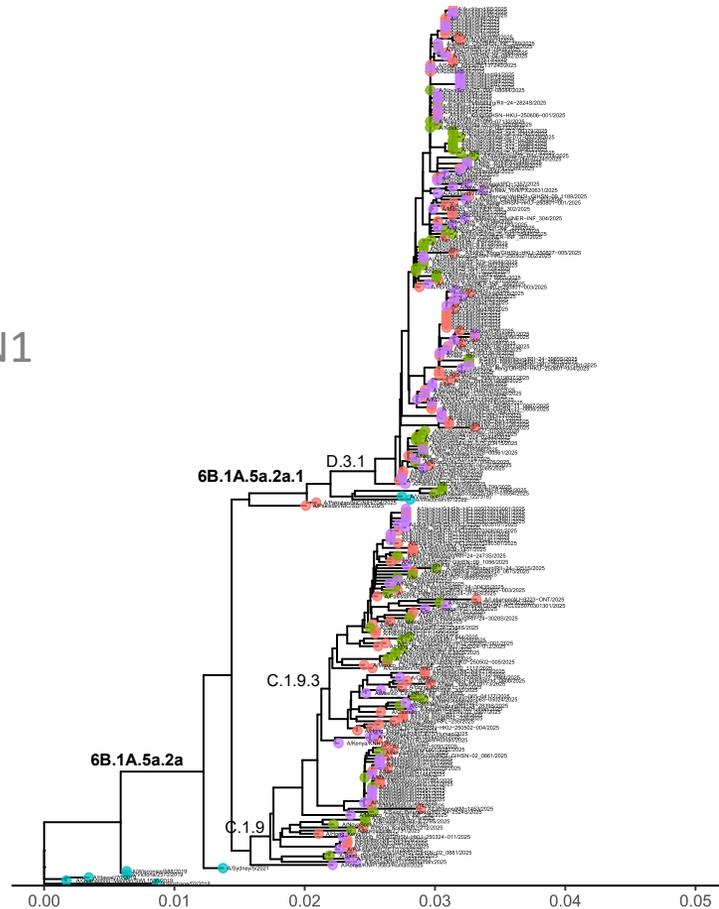
*Coverage plot for the HA segment*

*Get all needed results, including coverage of each segments and consensus sequences*

# VACCINE COMPOSITION MEETING

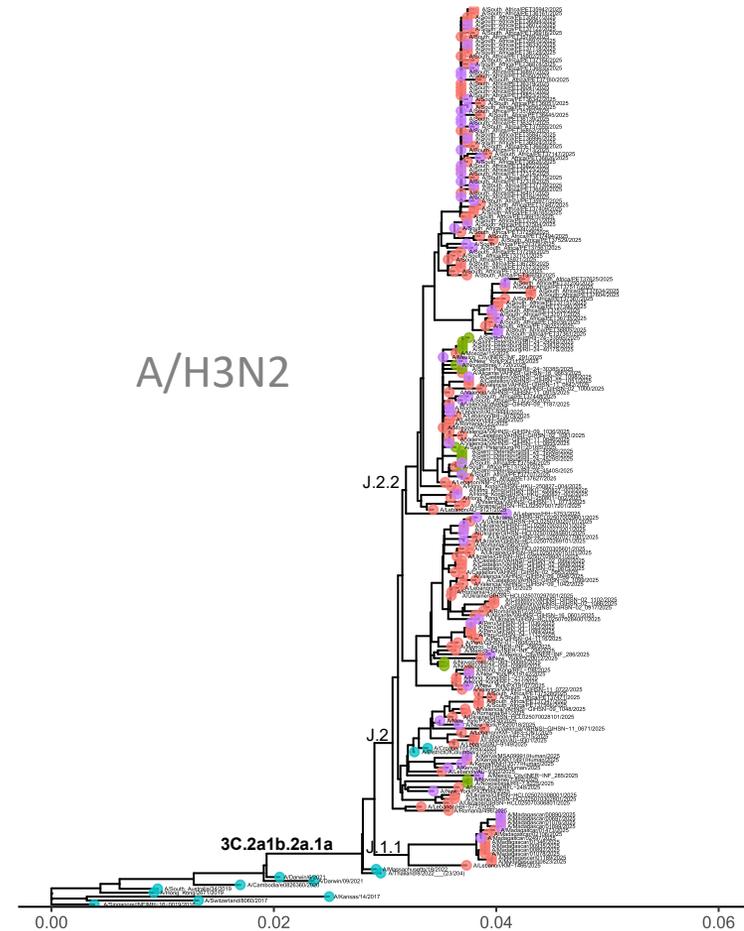
Timeliness sharing of relevant results for the WHO Vaccine Composition Meeting (scheduled in February for the Northern Hemisphere and in September for Southern Hemisphere).

A/H1N1



- O2
- No
  - unknown
  - Vaccine strain
  - Yes

A/H3N2



- O2
- No
  - unknown
  - Vaccine strain
  - Yes

**GISAID**

## CREATING AN ACCOUNT

The screenshot shows the GISAID website interface. At the top, there is a navigation bar with the GISAID logo and links for 'About us', 'Database Features', 'Events', 'Collaborations', 'References', 'Registration', and 'Help'. A search bar and a 'Login' button are also present.

The main content area is divided into several sections:

- In Focus:** A featured article titled 'Comment on recent spike protein changes' with a 3D molecular model of a protein. The text discusses mutations in the spike protein and their potential effects on viral fitness and clinical outcomes.
- Genomic epidemiology of hCoV-19:** A section with a world map and a bar chart showing genomic data.
- hCoV-19 Tracking of Variants:** A world map with colored dots indicating the geographic distribution of different hCoV-19 variants.
- Enabled by hCoV-19 data from GISAID:** A section highlighting the GESS (Genome position / Region / Area / Concurrence search and SNV birth query) tool and the status of detection systems.
- hCoV-19 Data Sharing via GISAID:** A bar chart showing the cumulative number of hCoV-19 submissions, reaching 419,828.
- Recent hCoV-19 Data Submissions:** A list of recent submissions, including hCoV-19/Hebei/IVDC-10-01/2021, hCoV-19/Australia/NSW3878/2021, and hCoV-19/Romania/GR-93715/2021.
- Recent Influenza Data Submissions:** A list of recent influenza submissions, including A/turkey/Poland/464/2020(H5N8), A/Guangdong/SF16348/2020, and A/chicken/Netherlands/20019879-001005/2020.

At the bottom, there is a footer section titled 'Public-Private Partnerships of the GISAID Initiative'. The browser's address bar shows 'gisaid.org' and the taskbar at the bottom displays various application icons and the system clock showing 14:28 on 26/01/2021.

# GISAID – INFLUENZA SEQUENCE SUBMISSION

## SINGLE UPLOAD

**Isolate detail**

Isolate ID

Isolate name\*   
*Example: A/Wisconsin/2145/2001 or A/chicken/Rostov/864/2007*

Passage details/history   
*Example: c1/c2*

Type\*  Subtype H  Subtype N  Lineage

*A/[country]/GIHSN-[your Sample\_ID]/2025*

**Institute information**

Select Originating lab provider of clinical specimen(s) and/or virus isolate(s)

Originating lab\*   
[add new institute](#)

Address

Sample ID given by the originating lab

Submitting lab\* generator of sequence data

Address   
CNR Virus des Infections Respiratoires, France SUD  
103 Grande Rue de la Croix-Rousse  
69004 Lyon  
France

Sample ID given by the submitting laboratory

Network  GISRS  GIHSN

Authors

## BATCH UPLOAD

	A	B	C	D	AS	AT
1	Isolate_Id	Segment_Ids	Isolate_Name	Subtype	Note	PMID
2			A[Country]/GIHSN-[SAMPLE ID]/2021	H1N1	GIHSN	

There is no clear GIHSN option to select using influenza batch upload template.

The « GIHSN » tag have to be present in the Isolate Name.

As we working with GISAID for this issue, you can adress your GISHN sequences using the « Note » column in the batch upload template file.

NB: before submitting batch upload, you need to use at least single upload once by your own to get a lab ID.

# GISAID – SARS-COV-2 SEQUENCE SUBMISSION

## SINGLE UPLOAD

Virus detail	
Virus name*	<input type="text" value="hCoV-19/Country/Identifier/2023"/>
Accession ID	<input type="text"/>
Type	<input type="text" value="betacoronavirus"/>
Passage details/history*	<input type="text" value="Example: Original, Vero"/>

*hCoV-19/[country]/GIHSN-[your Sample\_ID]/2025*

Institute information	
Originating lab*	<input type="text"/>
Address*	<input type="text" value="Where the clinical specimen or virus isolate was first obtained"/>
Sample ID given by the originating lab	<input type="text"/>
Submitting lab*	<input type="text" value="CNR Virus des Infections Respiratoires, France SUD"/>
Address*	<input type="text" value="Where sequence data have been generated and submitted to GISAID"/>
Sample ID given by the Submitting lab	<input type="text"/>
Consortium	<input type="text" value="Global Influenza Hospital Surveillance Network (GIHSN)"/>
Authors*	<input type="text" value="Jane Doe, John Doe"/>

# GISAID – SARS-COV-2 SEQUENCE SUBMISSION

## BATCH UPLOAD

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### GISAID hCoV-19 Batch Upload

**Upload genetic sequence as single FASTA-File and metadata, available clinical and epidemiological data, geographical as well as species-specific data as XLS or CSV. Data will be reviewed by a curator prior to release. An email confirmation will be issued upon release.**

Metadata as Excel or CSV\*

*max size: 5M*  Aucun fichier choisi

Sequences as FASTA\*

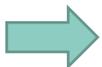
*max size: 32M*  Aucun fichier choisi

Report

# GISAID – SARS-COV-2 SEQUENCE SUBMISSION

## BATCH UPLOAD

Column information		
Submitter	mandatory	enter your GISAID-Username
FASTA filename	mandatory	the filename that contains the sequence without path (e.g. all_sequences.fasta not c:\users\meier\docs\all_sequences.fasta)
Virus name	mandatory	e.g. hCoV-19/Netherlands/Gelderland-01/2020 (Must be FASTA-Header from the FASTA file all_sequences.fasta)
Type	mandatory	default must remain "betacoronavirus"
Passage details/history	mandatory	e.g. Original, Vero
Collection date	mandatory	Date in the format YYYY or YYYY-MM or YYYY-MM-DD
Location	mandatory	e.g. Europe / Germany / Bavaria / Munich
Additional location information		e.g. Cruise Ship, Convention, Live animal market
Host	mandatory	e.g. Human, Environment, Canine, Manis javanica, Rhinolophus affinis, etc
Additional host information		e.g. Patient infected while traveling in ....
Sampling Strategy		e.g. Sentinel surveillance (ILI), Sentinel surveillance (ARI), Sentinel surveillance (SARI), Non-sentinel-surveillance (hospital), Non-sentinel-surveillance (GP network), Longitudinal sampling on same patient(s), S gene dropout
Gender	mandatory	Male, Female, or <i>unknown</i>
Patient age	mandatory	e.g. 65 or 7 months, or <i>unknown</i>
Patient status	mandatory	e.g. Hospitalized, Released, Live, Deceased, or <i>unknown</i>
Specimen source		e.g. Sputum, Alveolar lavage fluid, Oro-pharyngeal swab, Blood, Tracheal swab, Urine, Stool, Cloakal swab, Organ, Feces, Other
Outbreak		Date, Location e.g. type of gathering, Family cluster, etc.
Last vaccinated		provide details if applicable
Treatment		Include drug name, dosage
Sequencing technology	mandatory	e.g. Illumina Miseq, Sanger, Nanopore MinION, Ion Torrent, etc.
Assembly method		e.g. CLC Genomics Workbench 12, Geneious 10.2.4, SPAdes/MEGAHIT v1.2.9, UGENE v. 33, etc.
Coverage		e.g. 70x, 1,000x, 10,000x (average)
Originating lab	mandatory	Where the clinical specimen or virus isolate was first obtained
Address	mandatory	
Sample ID given by the originating laboratory		
Submitting lab	mandatory	Where sequence data have been generated and submitted to GISAID
Address	mandatory	
Sample ID given by the submitting laboratory		
Sequencing Consortium		Sequencing consortium the submitting lab is affiliated to
Authors	mandatory	a comma separated list of Authors with complete First followed by Last Name
Comment	leave empty	do not use this column
Comment Icon	leave empty	do not use this column



Fill this column with « Global Influenza Hospital Surveillance Network (GIHSN) »

# GISAID: WHICH INFORMATION TO ADDRESS TO THE GIHSN

When sending your sample for sequencing to Lyon, results are provided  
In a Excel sheet with your own sample number and the corresponding  
GISAID Isolate ID to address to the GIHSN form.

	A	Z
1	<b>Isolate_Id</b>	<b>Originating_Sample_Id</b>
2	EPI_ISL_19256954	01-1489
3	EPI_ISL_19256955	01-1494
4	EPI_ISL_19256956	01-1496
5	EPI_ISL_19256957	01-1500
6	EPI_ISL_19256958	01-1504
7	EPI_ISL_19256959	01-1505
8	EPI_ISL_19256960	01-1508
9	EPI_ISL_19256961	01-1509
10	EPI_ISL_19256962	01-1510
11	EPI_ISL_19256963	01-1513
12	EPI_ISL_19256964	01-1518
13	EPI_ISL_19256965	01-1527
14	EPI_ISL_19256966	01-1529
15	EPI_ISL_19256967	01-1530
16	EPI_ISL_19256968	04-0431
17	EPI_ISL_19256969	04-0434

*Influenza result table*

	A	W
1	Isolate ID	provider_sample_id
2	EPI_ISL_19257028	B-9 01-1497 MAI
3	EPI_ISL_19257029	B-1 04-0358 MAI
4	EPI_ISL_19257030	B-5 04-0415 MAI

*SARS-CoV-2 result table*

# GISAID: SEND THE SHIPMENT FORM CAREFULLY FILLED

When sending your sample for sequencing to Lyon, please fill information carefully. Sample registering and submission will be easier.

Thank you 😊

Name	Maiden Name	Surname	Gender	Date of birth	Age	Age_Unit	Collection number	type of samples	Collection_Date	Collection_Time	Country	Region	City	Host
------	-------------	---------	--------	---------------	-----	----------	-------------------	-----------------	-----------------	-----------------	---------	--------	------	------

Name PCR : covid	Ct PCR : COVID	Name PCR : Influenza	Ct PCR : Influenza	Name PCR : Influenza	Ct PCR : Influenza B	Influenza subtype	Ct subtype	02 Requireme	Health_Statu	Originating lab	Address Origin	Authors
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# PROCEDURES

# GIHSN OPERATIONAL PROCEDURES FOR WGS (1/2)

- ❖ All sites are expected to generate Whole genome sequencing (WGS) for **100 Influenza positive specimens (min. 50)\***
- ❖ All sites must submit WGS to the GISAID EpiFlu™ database **on a regular basis**, and in a timeframe so that results are **available for the WHO Vaccine Composition Meeting (VCM)**
  - ❖ In addition, WGS for SARS-CoV2 (if performed) are encouraged to be submitted to GISAID database to add to public knowledge and support WHO initiatives.
- ❖ **Each GISAID Accession Number (Isolate ID : “EPI\_ISL...”)** must be reported in the corresponding clinical questionnaire, allowing for the linkage of WGS data with clinical data.
- ❖ Storage (**preferably -70°C**, if not possible -20°C) of all influenza positive and negative study samples should be carried out for a minimum of one year. This will assure sample availability for additional retrospective investigations (e.g. SARS-CoV-2 or pathogen discovery initiatives) if necessary.

\*Some sites are in capacity to do more (Spain, NYC US, Pakistan & NZ)

# GIHSN OPERATIONAL PROCEDURES FOR WGS (2/2)

- ❖ If the site has no capacities to generate WGS data locally (on its own, or through the usual lab they cooperate with), the site may ship its specimens to the GIHSN sequencing platform at the NIC in Lyon, France. **This has to be discussed and agreed with the Foundation for Influenza Epidemiology before start of the season.**
- ❖ If specimens are to be sent to Lyon: Shipments are organized by the NIC in Lyon, but sites should be careful to group their shipments (2 shipments max per year).
  - ❖ Contact [nathalie.bergaud01@chu-lyon.fr](mailto:nathalie.bergaud01@chu-lyon.fr) with the following information : **address, 2 contacts** (mail+ tel), **nature and number of samples, number of tubes, volume, number and size of boxes**
  - ❖ The carrier World Courier will provide box + dry-ice + temperature probe
  - ❖ You will receive an **excel file to be completed comprehensively** with all the information regarding your samples. Please send it back asap to Lyon
  - ❖ Send preferably **P0 (400 µl minimum)**, if not possible, RNA (50 µl minimum stored at -70°C)

# GIHSN WGS REPORTS ARE PREPARED BY THE LAB IN LYON AND SENT TWICE A YEAR (FEBRUARY & SEPTEMBER) TO WHO PRIOR TO THE VACCINE COMPOSITION MEETING

Global Influenza Hospital Surveillance Network [www.gihsn.org](http://www.gihsn.org)

GIHSN report of activity prior to the WHO Consultation on the Composition of Influenza Virus Vaccines for use in the 2024-2025 Northern Hemisphere Influenza Season.

Report prepared the 13<sup>th</sup> of September 2024

**1 - Description of the network**

GIHSN is collecting clinical and virological information from hospitalized cases through a network of sites (20) located in different regions of the world (figure 1). This combined clinical and virological surveillance allows the identification of viruses responsible for severe influenza. This severity is assessed by the oxygen requirement of cases registered by the sites. In this report, viruses detected and sequenced from cases requiring oxygen supplementation are identified in the phylogenetic trees provided, to determine if specific lineages or clades are associated with more frequent severe presentation. It has been noted in the GIHSN report of the 2023 surveillance for the southern hemisphere VCM that O2 requirement seemed to be more frequently reported in the A(H3N2) 3C.2a1b.2a.3a HA lineage. This was not confirmed in the 2023-2024 Northern Hemisphere report.

For the SH 2024 surveillance in GIHSN, influenza activity was mainly due to Influenza A viruses in most countries, with co-circulation of A/H1N1 and A/H3N2 in different relative proportions.

Regarding the Influenza B viruses, as for the NH 2023-2024 surveillance, it has been reported a very limited number of detections of B/Victoria lineage viruses, and no B/Yamagata viruses have been detected by the network. This is the 3<sup>rd</sup> year of GIHSN surveillance with no detection of B/Yamagata viruses. Due to the very limited data available on influenza B viruses, no analysis regarding severity can be done at this stage.

This report is collating the available sequencing data from 12 sites collected on patients admitted in hospital between February 1<sup>st</sup>, 2024 and August 31<sup>st</sup>, 2024: Brazil (4), Kenya (25), Pakistan (50), Peru (21), Romania (63), Russia Moscow (12), Senegal (15), Spain (84), South Africa (175), Türkiye (9), Ukraine (80), USA (65). All 603 sequences, from hospitalized cases only, have been uploaded in the GISAID database with a GIHSN tag.

1

*viruses analyzed between February 1st, 2024 and August 31st, 2024, inferred using a Neighbor Joining approach (Seaview), and sub clade distribution; viruses sequenced by the laboratories of GIHSN, with vaccine reference strains displayed in black.*

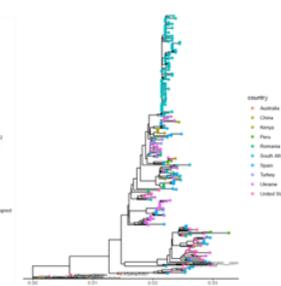
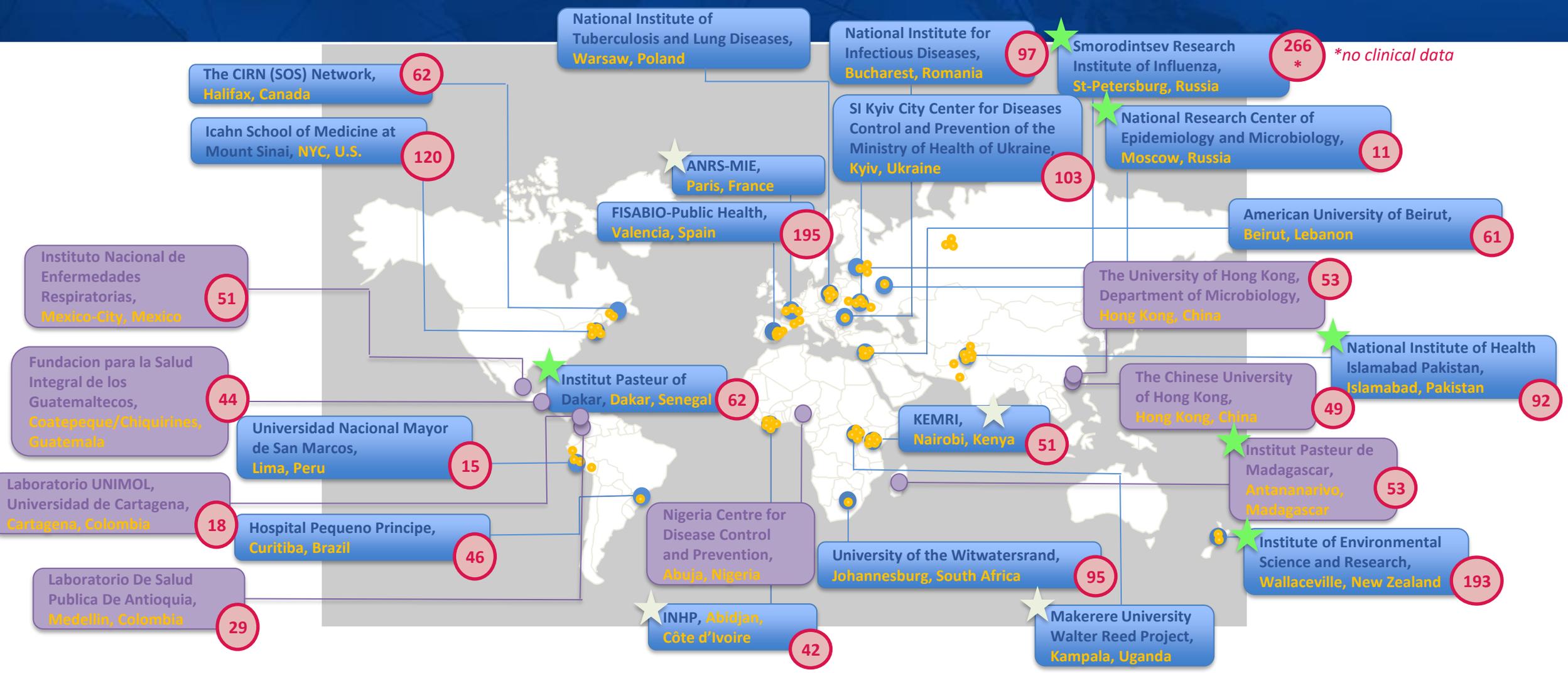


Fig 2b

- ❖ GIHSN sites which proceed with WGS locally are expected to share their WGS results **end of January at the latest** for the VCM of February and **end of August** for the VCM of September
- ❖ GIHSN sites which send their samples to Lyon for WGS are expected to send all relevant samples **by mid-January at the latest** and **mid-August**.

# WGS STATUS (INFLUENZA) TO DATE FOR 2024-2025 (1758 TO DATE)



- Sites already in GIHSN 2023-24
- New sites (2024-25)
- Hospital location
- XX Number of WGS Flu per site
- ★ National Influenza Center (NIC)
- ★ Close collaboration with NIC

# WGS REPORTING FOR 2025-2026

- ❖ WGS reporting **to start ASAP**, in view of the next VVCM (February 2026)
- ❖ WGS results to be shared end of January 2026 **at the latest** (exact dates to be circulated by email)

# THANK YOU!