Genotyping and phenotypic testing of RSV

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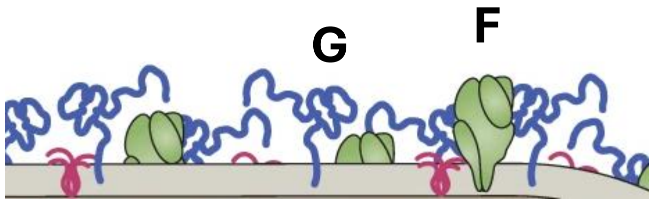
Objectives of virological surveillance ?

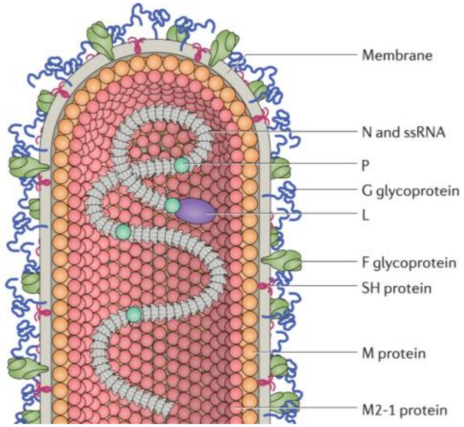
- **Description of RSV clades circulation**

  ➡ Identification of clades of interest

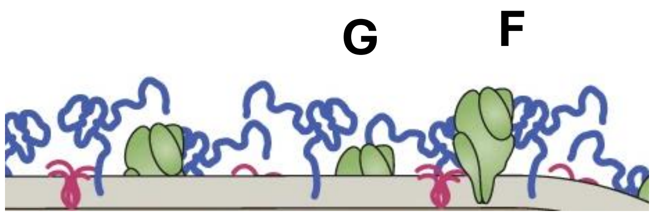
- **Assessing and reporting susceptibility to**
  - **Monoclonal antibodies**
  - Vaccines
  - Antiviral drugs

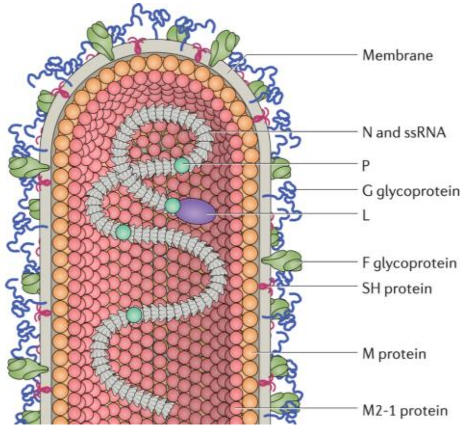
RSV has two major surface glycoproteins



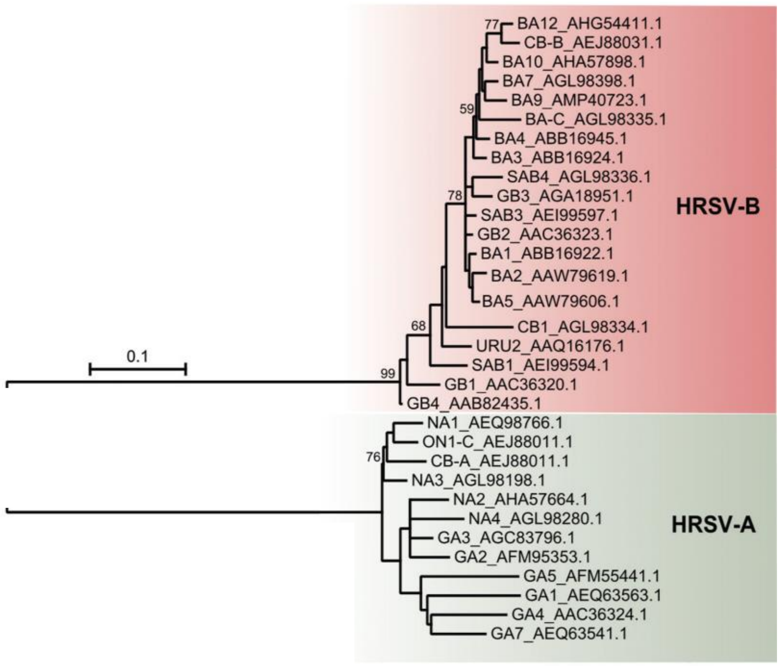


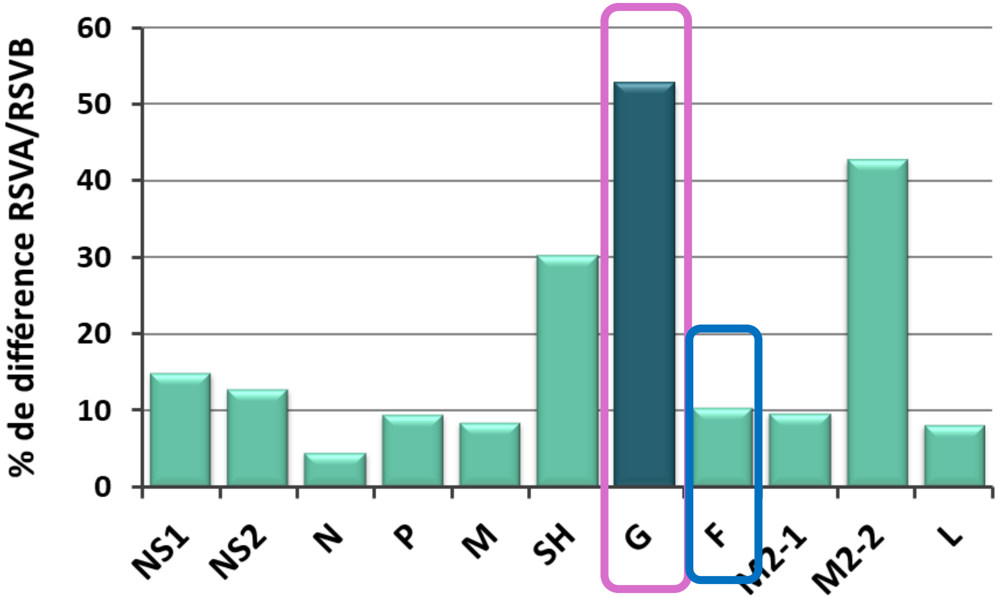
RSV variability concentrated on G protein





2 groups : RSV-A and RSV-B





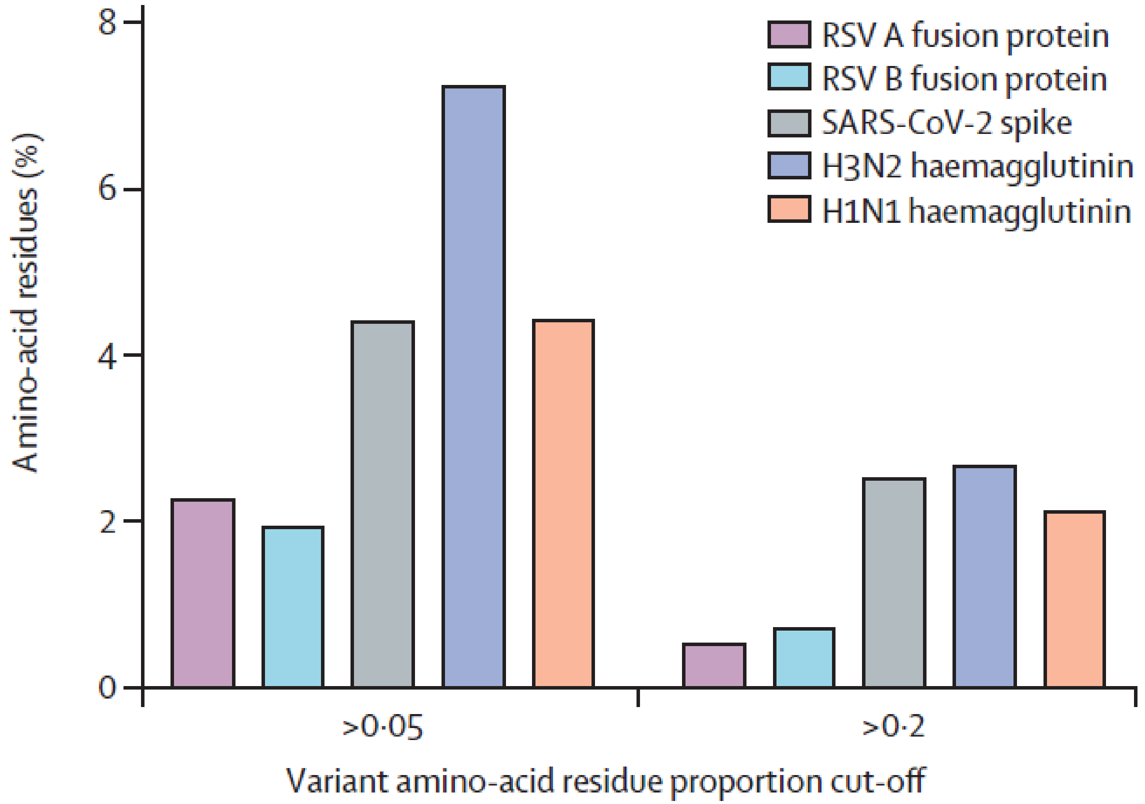
From data by Bose et al, 2015 (27 RSV-A and 19 RSV-B from virus origins over 10 years).

F protein is the main target for monoclonal Ab, vaccine
(and antivirals)

F : highly conserved

structural constraints ?

limited selection pressure ?



Wilkins et al, *Lancet Infect Dis* 2023 https://doi.org/10.1016/ S1473-3099(23)00062-2

Description of RSV clades: Recent definition of RSV lineages

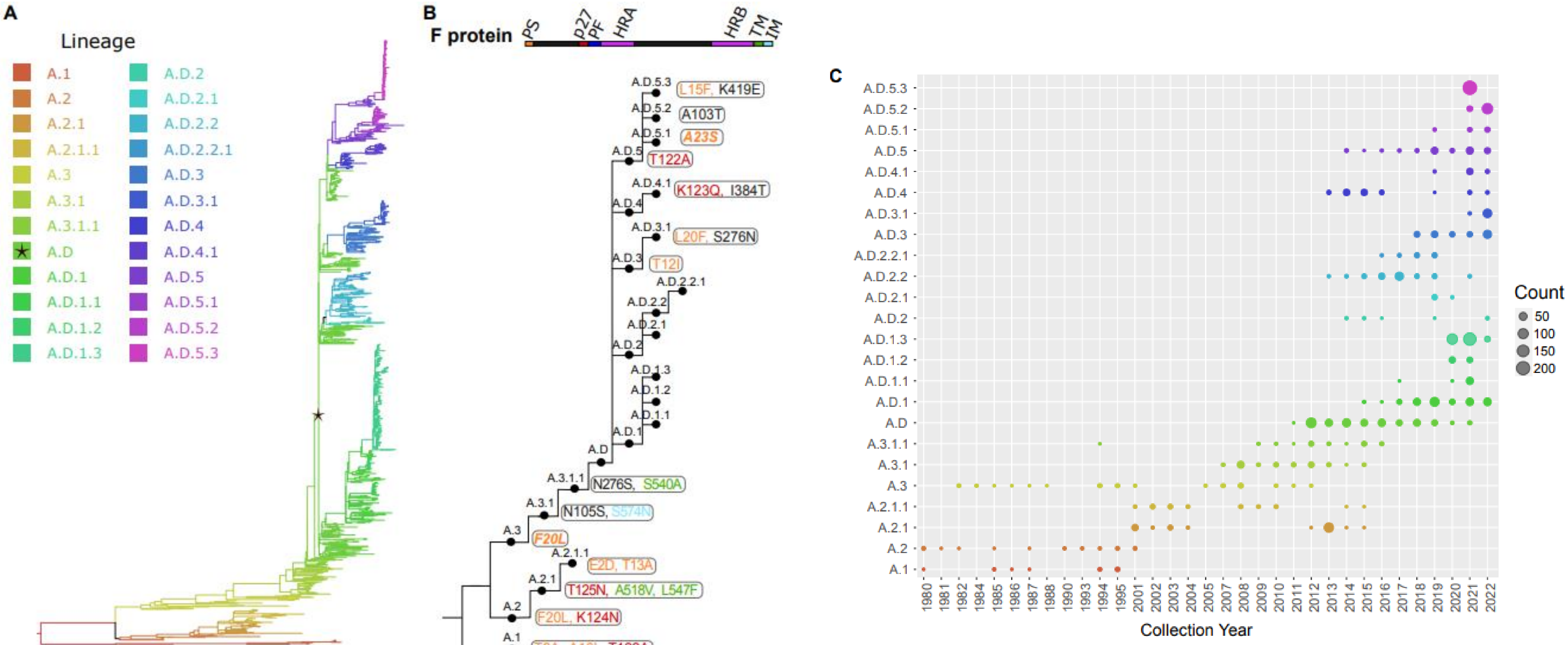
Based on whole genome sequences from the first NS1 codon to the last L codon

Minimal misclassification (1.2% error) was found in RSV-A and none in RSV-B when using only G sequence

New lineage definition: **>10 seq** with **more than 5 coding mutations**

Goya 2024 medRxiv preprint doi: https://doi.org/10.1101/2024.02.13.24302237

Recent definition of RSV lineages



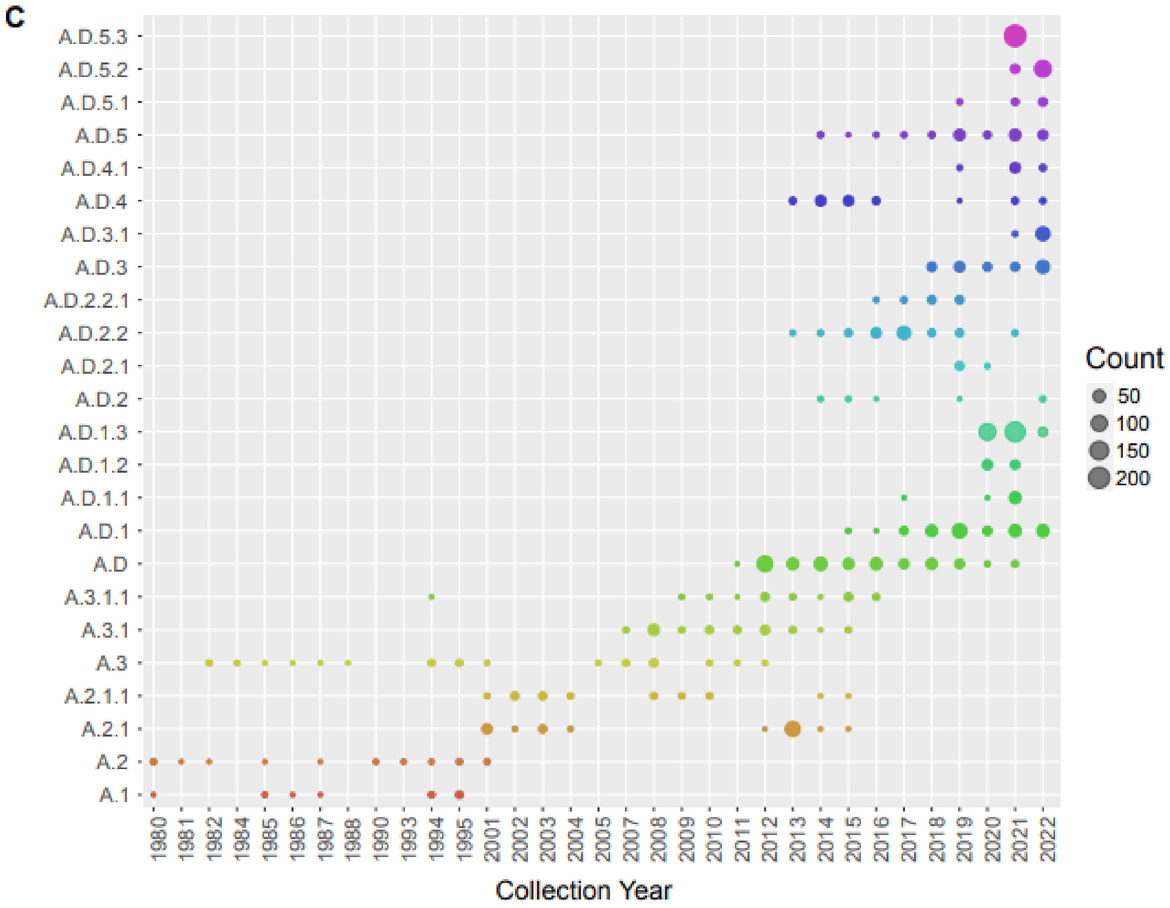
Goya 2024 medRxiv preprint doi: https://doi.org/10.1101/2024.02.13.24302237

Recent definition of RSV lineages

Co-circulation of different lineages

Multiple introductions

Persistance of some lineages



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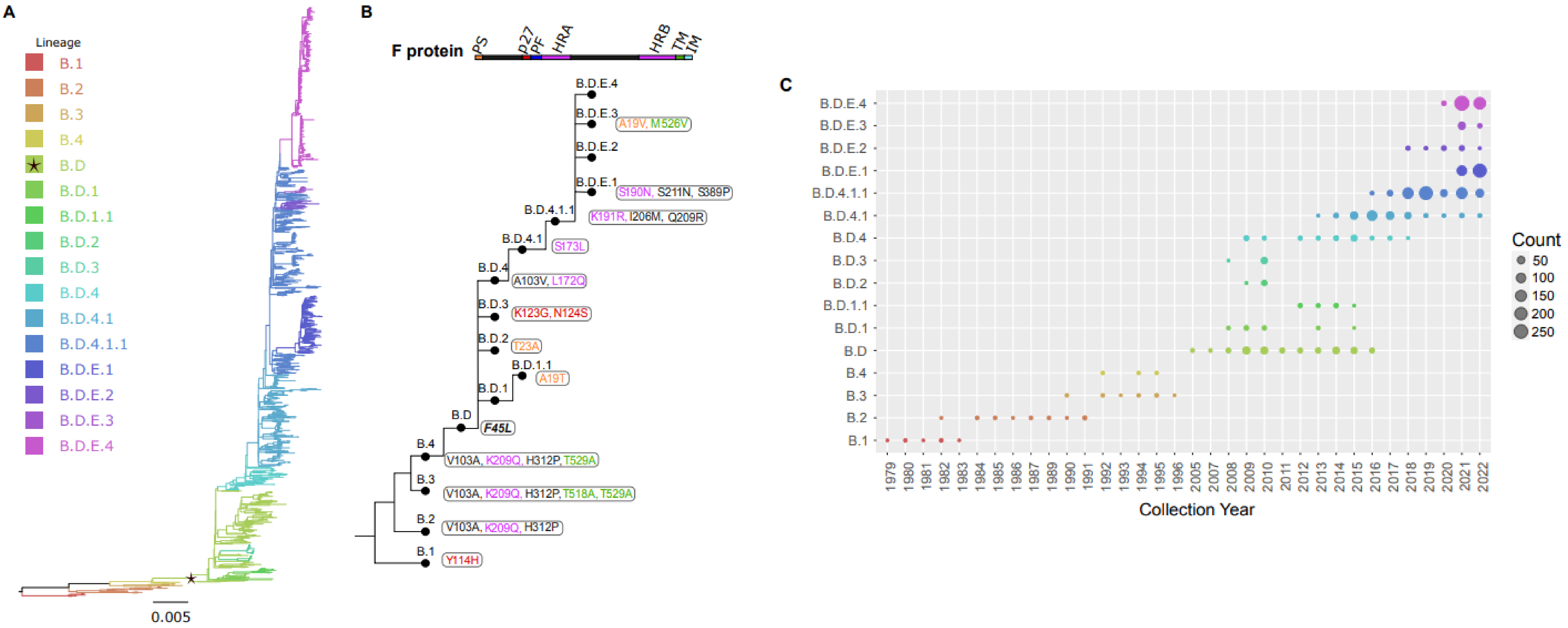
Recent definition of RSV lineages

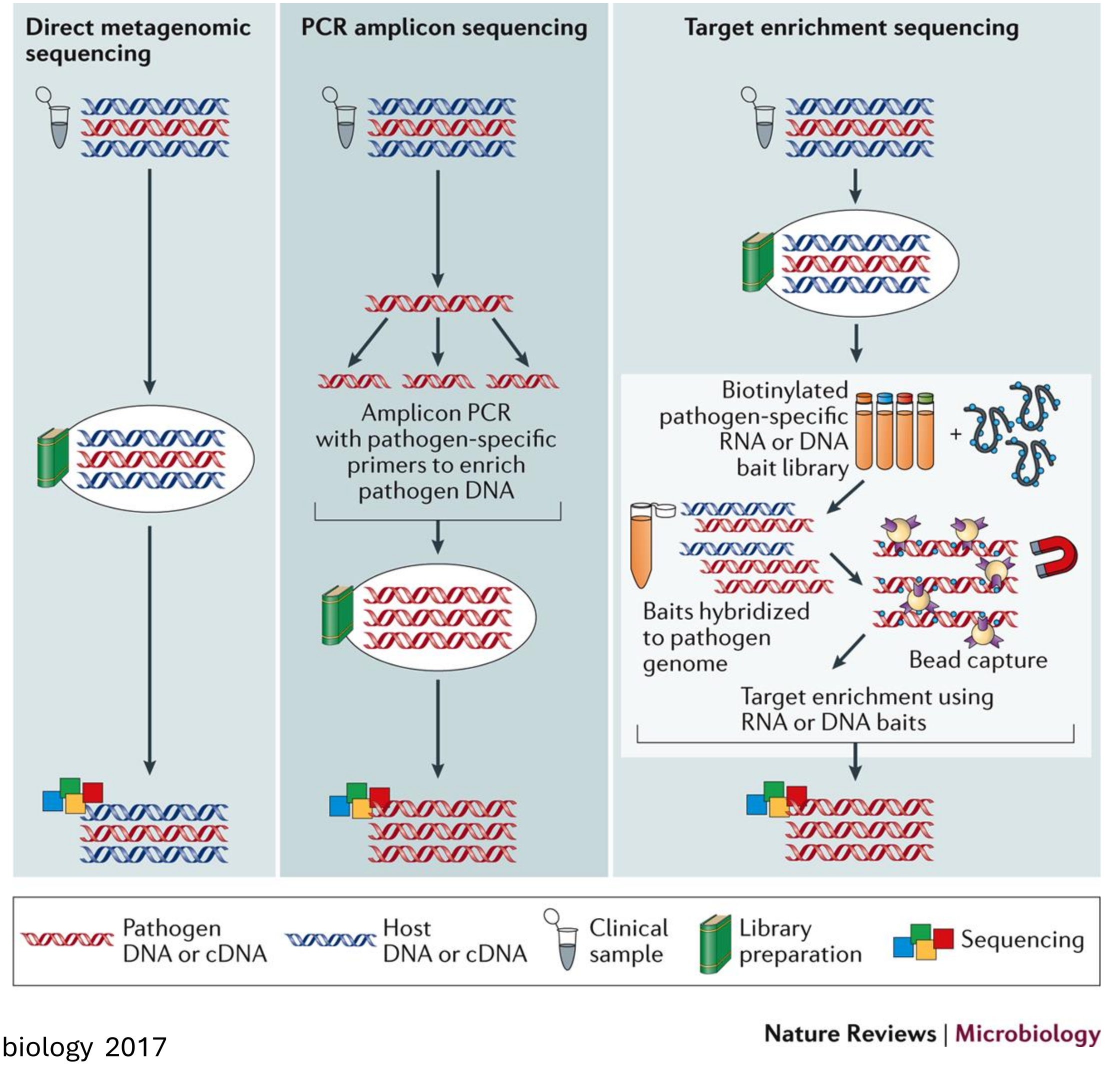


Goya 2024 medRxiv preprint doi: https://doi.org/10.1101/2024.02.13.24302237

Genotypic analysis of RSV

Sanger sequencing of G as a proxy for lineage assignment and of F to look for escape mutations

Reference method: whole genome sequencing



Charlotte J. Houldcroft, et al; Nature Reviews Microbiology 2017

Genotypic analysis of RSV

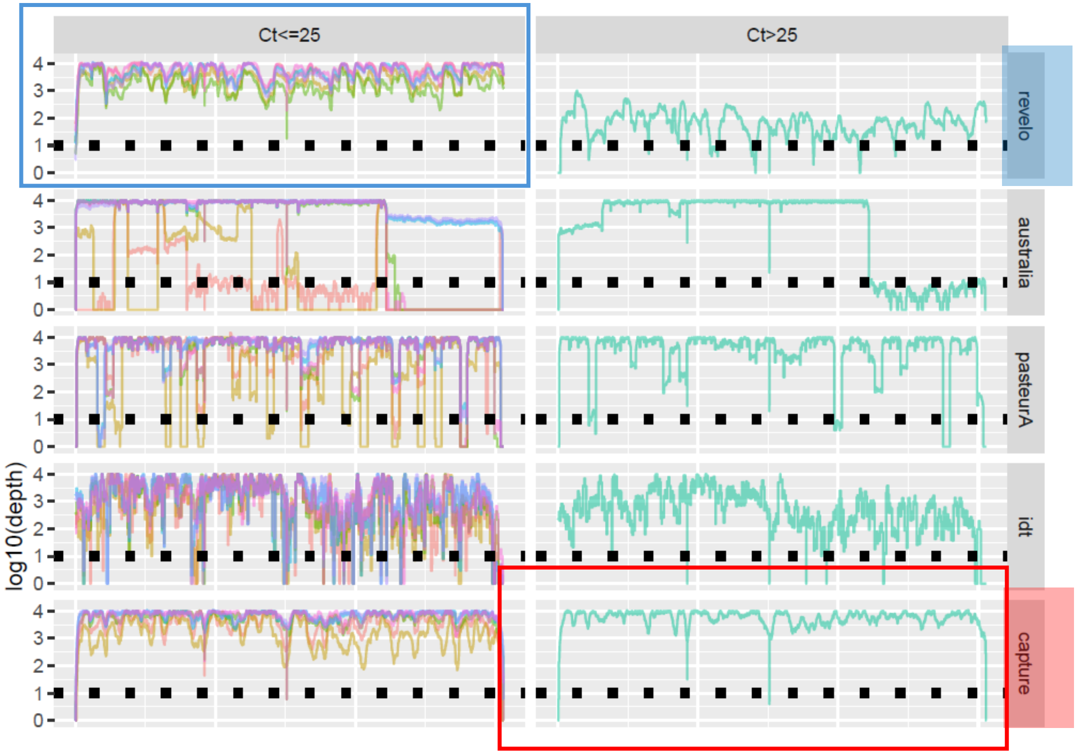
Bruno Simon *et al*; Preliminary results of a comparison of methods

GENomique
EPIdémiologique
des maladies
Infectieuses

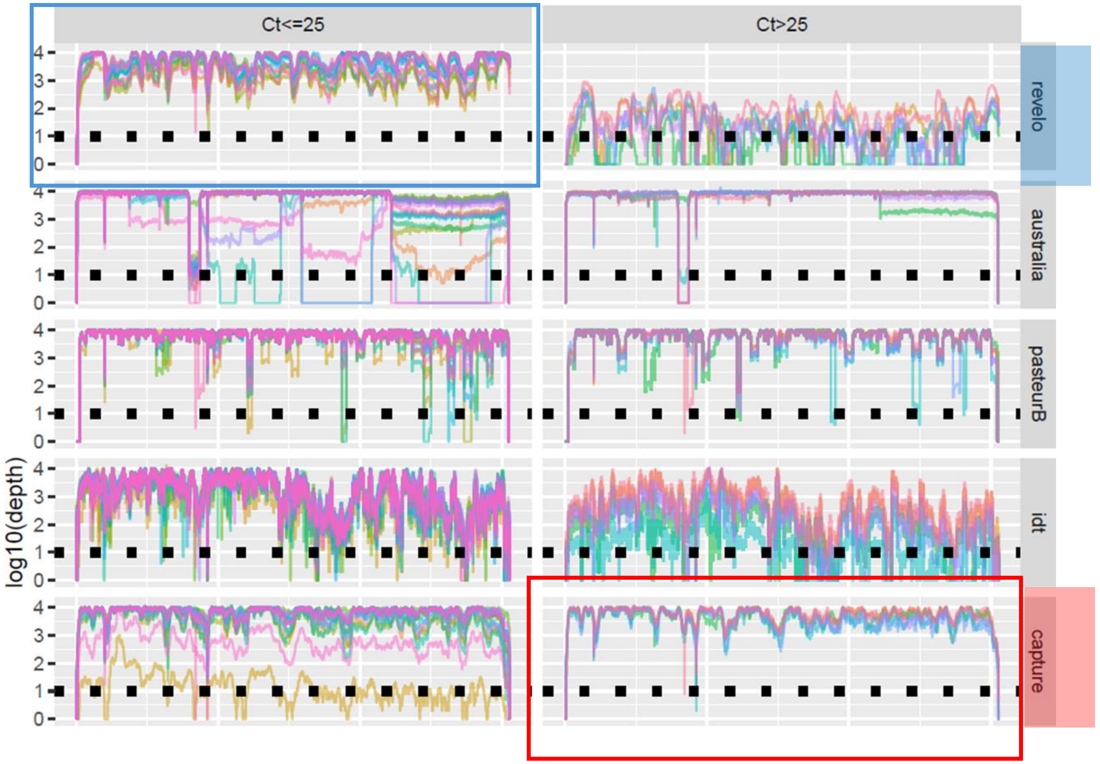
HCL
HOSPICES CIVILS
DE LYON

- ***Metatranscriptomic:* Revelo** RNA-Seq High Sensitivity *(Tecan Genomics)*
- ***Amplicon: Australia*** *(Dong et al, JCV, 2023)*
- ***Amplicon:*** G5 GEVA, **Pasteur** *Etienne Simon Lorière*
- ***Amplicon:*** xGen Repiratory Virus Amplicon Panel ***(IDT)***
- ***Capture :*** Illumina RNA Prep with Enrichment Tagmentation – RVOP2 Panel

RSV-A n=24



RSV-B n=8

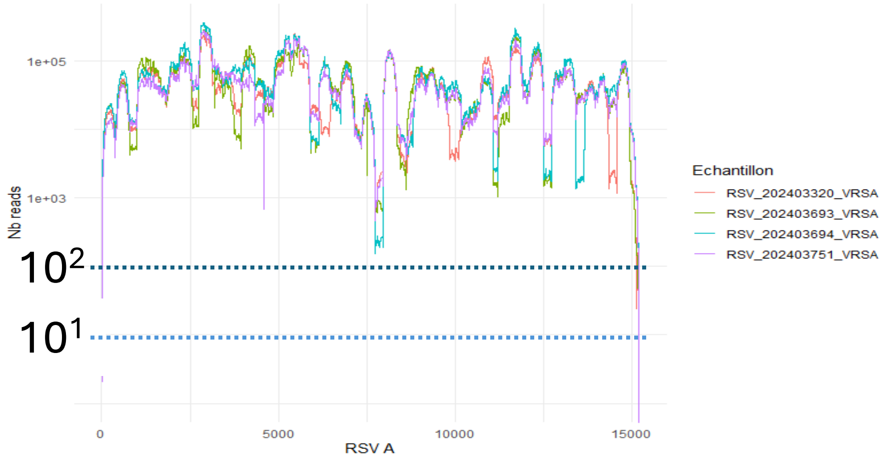


Amplicon methods

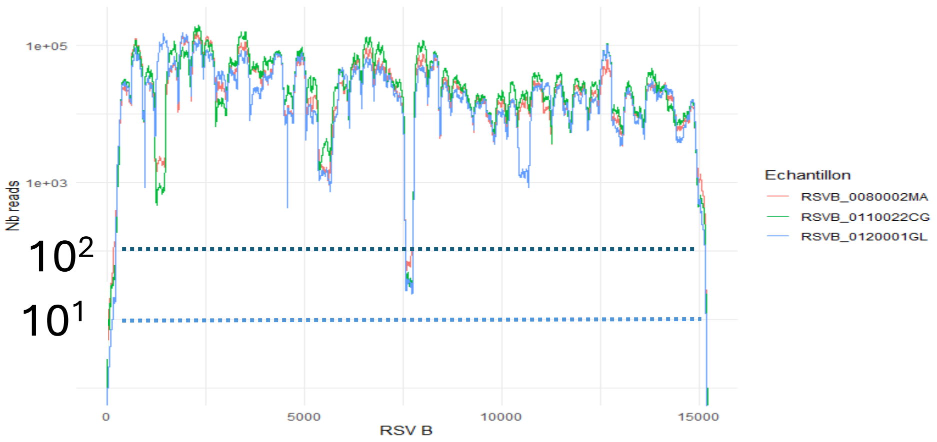
Update of Pasteur Protocol

Amplicons: 400 pb

RSV-A



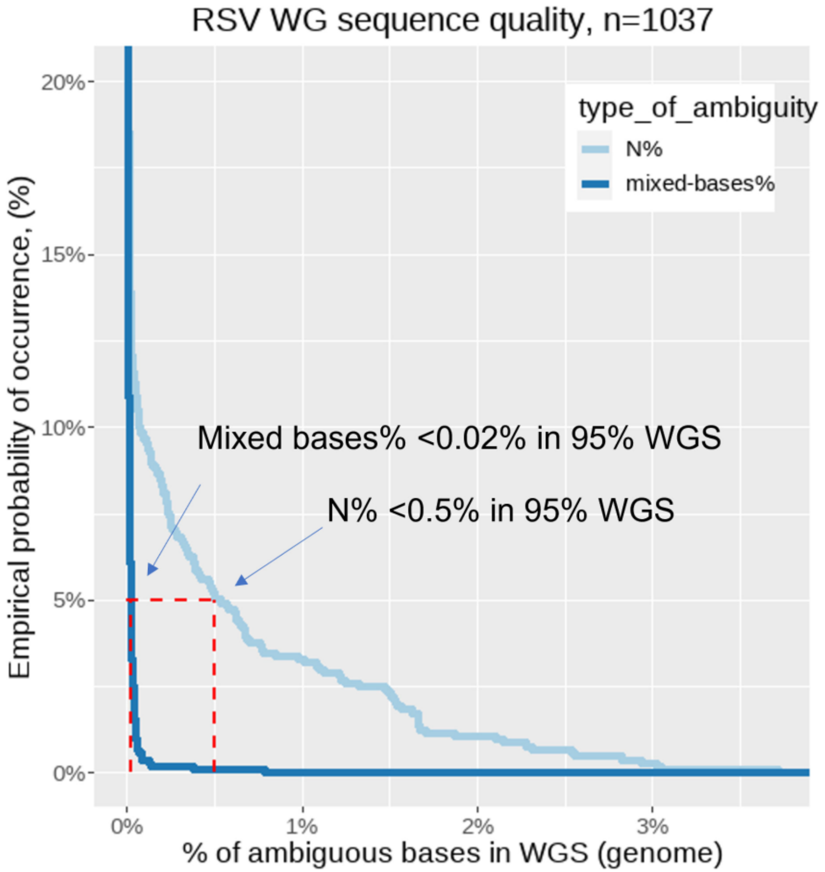
RSV-B



Charlotte J. Houldcroft, et al; Nature Reviews Microbiology 2017

Talts et al, 2024 (UK Health Security Agency, London)

Amplicons: 750-1000 pb

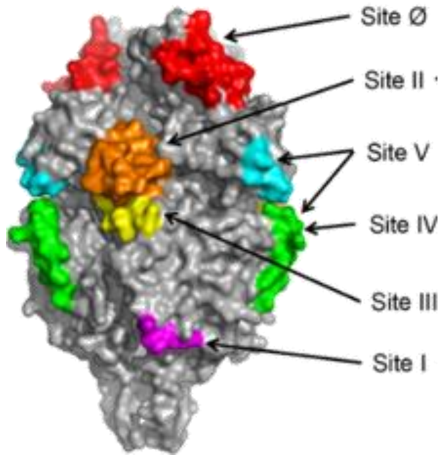


Mixed bases% <0.02% in 95% WGS

N% <0.5% in 95% WGS

Talts et al, 2024 Microbiol Spectrum 10.1128/spectrum.03067-23

Escape mutations to nirsevimab



Description of mutations in the literature

Coming soon (this summer)

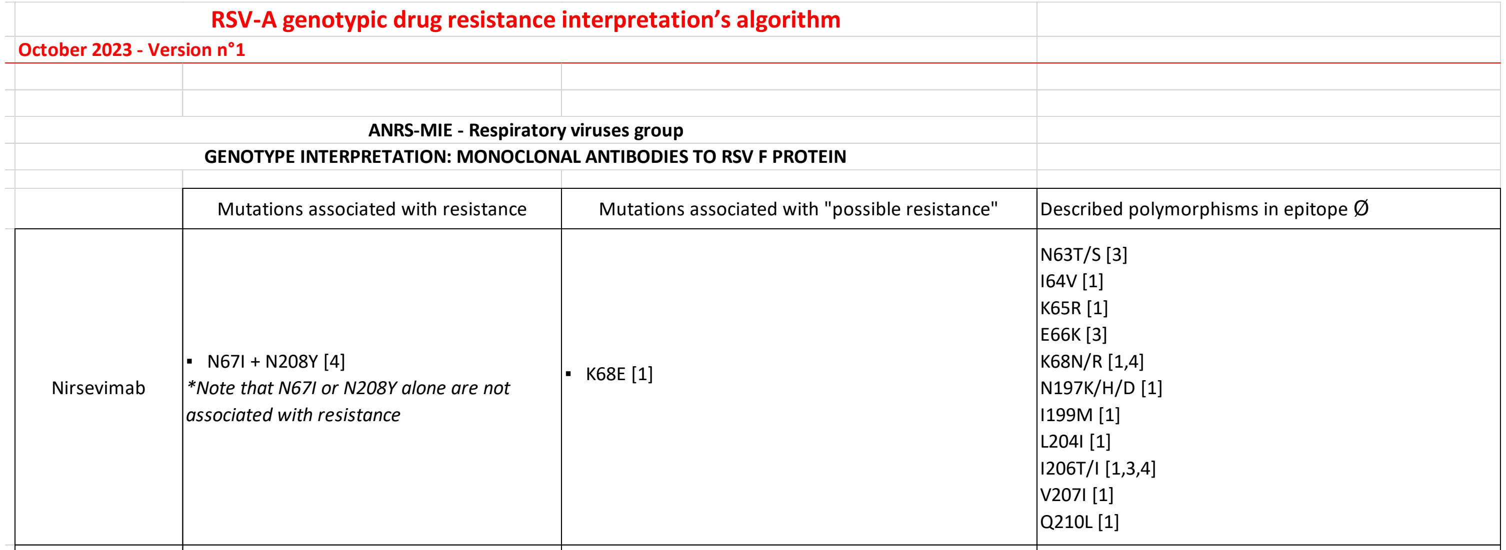
ANRS (French National Agency for Health Research)

Website with list of mutations

RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

anrs MALADIES INFECTIEUSES ÉMERGENTES Inserm

HIV-1 GENOTYPIC DRUG RESISTANCE INTERPRETATION'S ALGORITHMS



RSV-A genotypic drug resistance interpretation's algorithm

October 2023 - Version n°1

ANRS-MIE - Respiratory viruses group

GENOTYPE INTERPRETATION: MONOCLONAL ANTIBODIES TO RSV F PROTEIN

	Mutations associated with resistance	Mutations associated with "possible resistance"	Described polymorphisms in epitope 0
Nirsevimab	▪ N67I + N208Y [4] **Note that N67I or N208Y alone are not associated with resistance*	▪ K68E [1]	N63T/S [3] I64V [1] K65R [1] E66K [3] K68N/R [1,4] N197K/H/D [1] I199M [1] L204I [1] I206T/I [1,3,4] V207I [1] Q210L [1]

Escape mutations to nirsevimab

Description of mutations in the literature

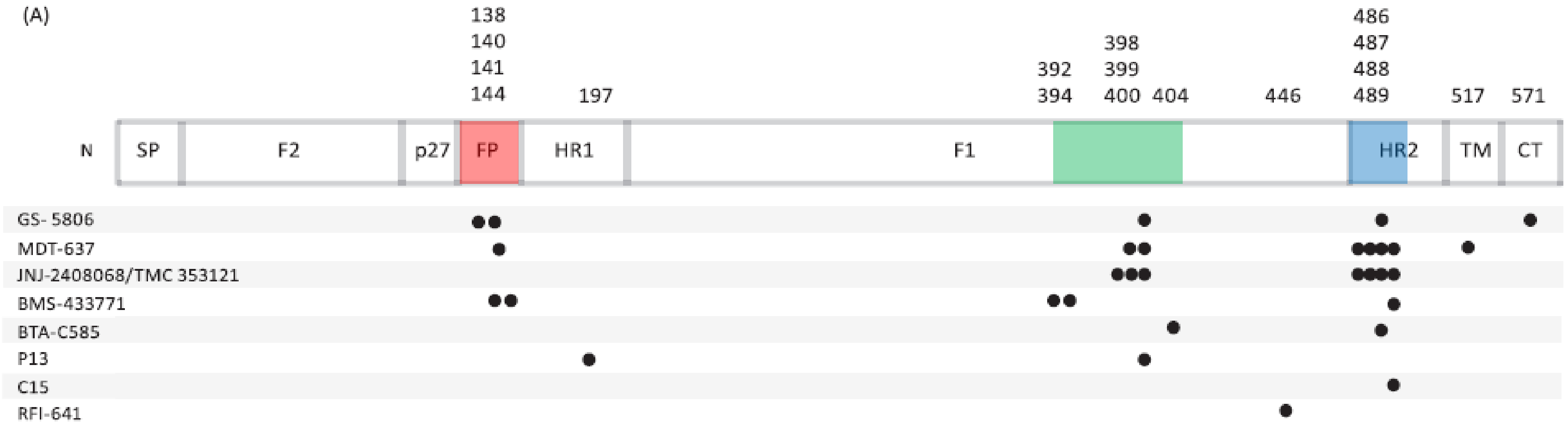
Limits of genotypic analysis

Unknown mutations ?

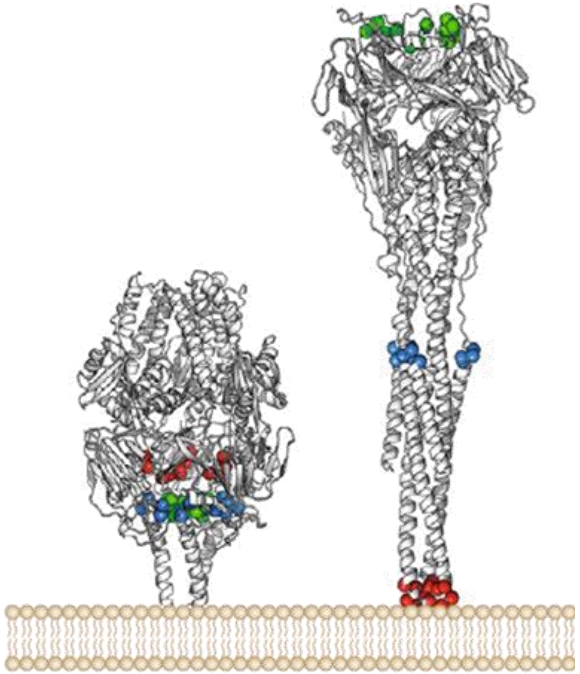
Escape mutations (might depend of context)
Ex: S201T mutation effect depends on 209 position

Escape mutations to antivirals

Known mutations on F for fusion inhibitors



Heylens et al, 2017



Battles et al, 2019

Other inhibitors

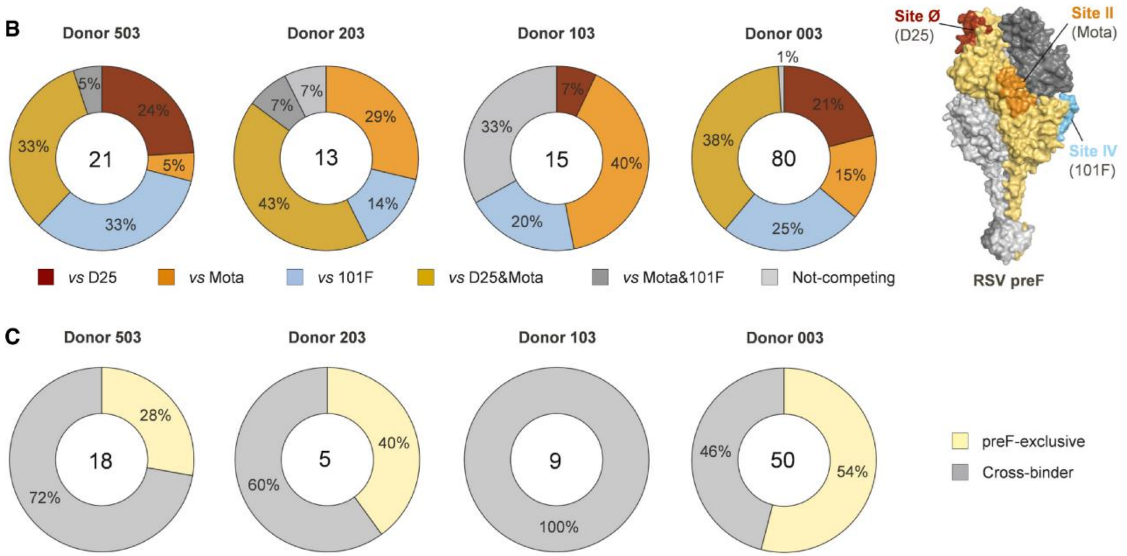
Vaccine escape ?

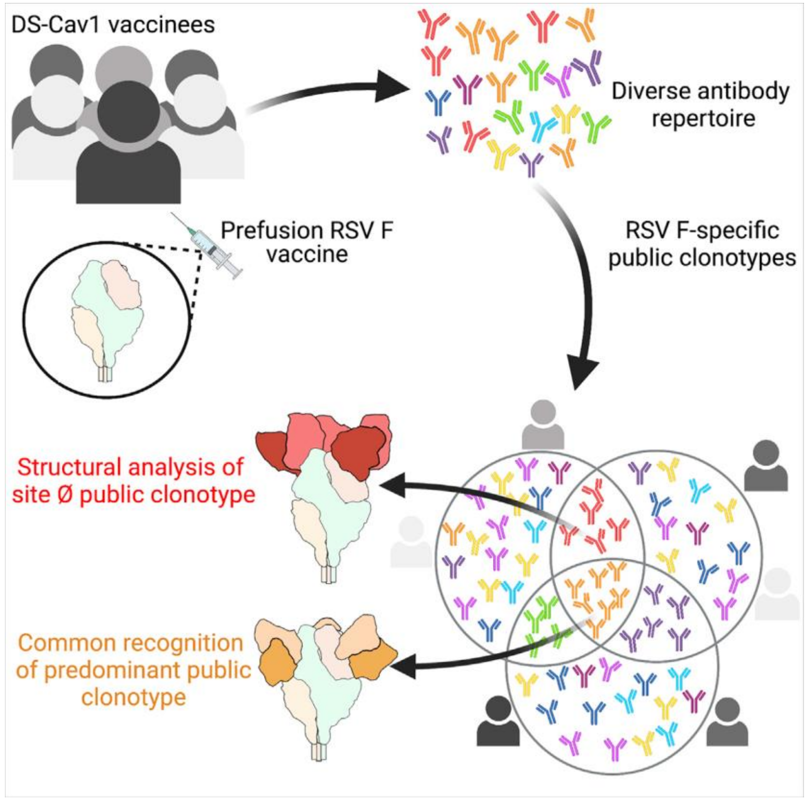
Broad efficacy: GSK A vaccine only (A2 strain)

Response to F antigen (vaccine or natural infection):

Polyclonal

Efficient neutralisation of multiple RSV A and B variants, including those with escape mutations for a monoclonal, by sera from vaccinated individuals





Sacconnay et al., Sci. Transl. Med. 15, eadg6050 (2023) 23 August 202

Andreano et al, 2021 doi.org/10.15252/emmm.202114035

Maryam Mukhamedova at al, 2021;Immunity https://doi.org/10.1016/j.immuni.2021.03.004

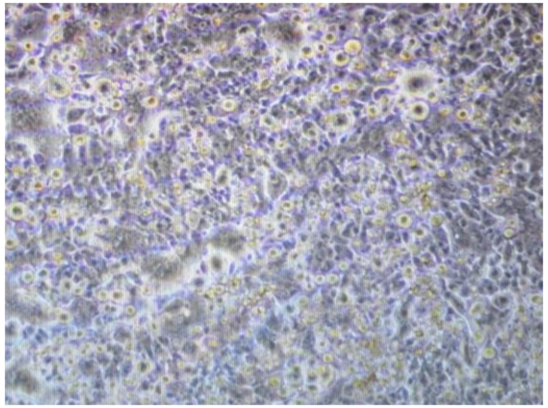
Establish consistent and simple phenotypic protocols for characterisation of RSV

Method of isolation: not fully agreed at this stage

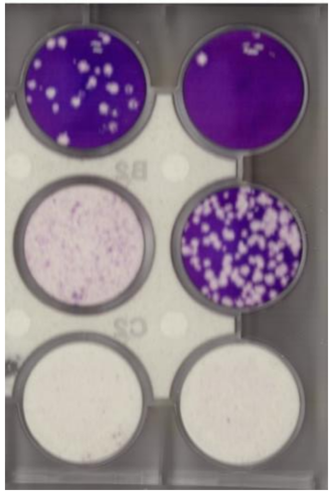
2 major issues:
- Difficult
- Mutation during cell culture

Preservation conditions ?

Cells ?
HEp-2
A549
Vero
MRC5
....



Detection of amplification
Cytopathic effect
Fluorescence, ELISA…

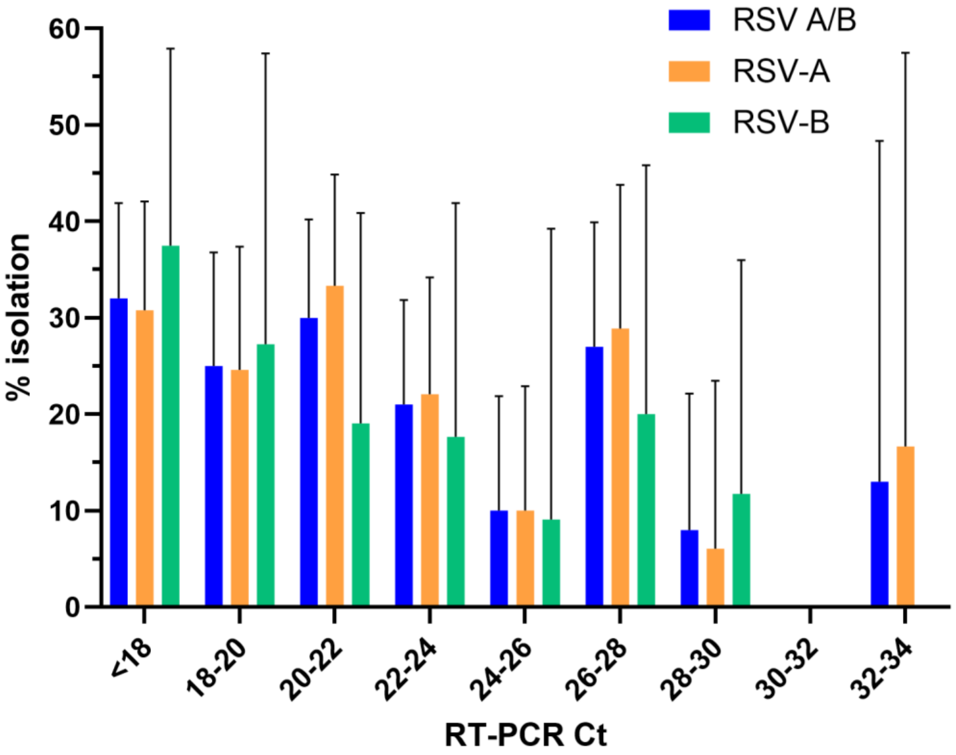


Quantification
Plaque assay
Other (fluo, ELISA, RT-PCR..)

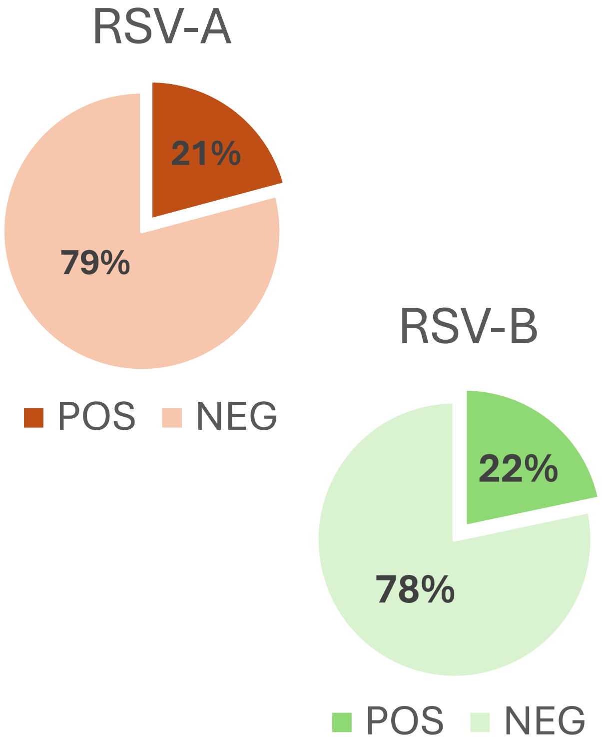
Factors affecting isolation efficiency

Isolation on HEp-2 cells monitoring cytopathic effect

Isolation effectiveness decreases with Ct

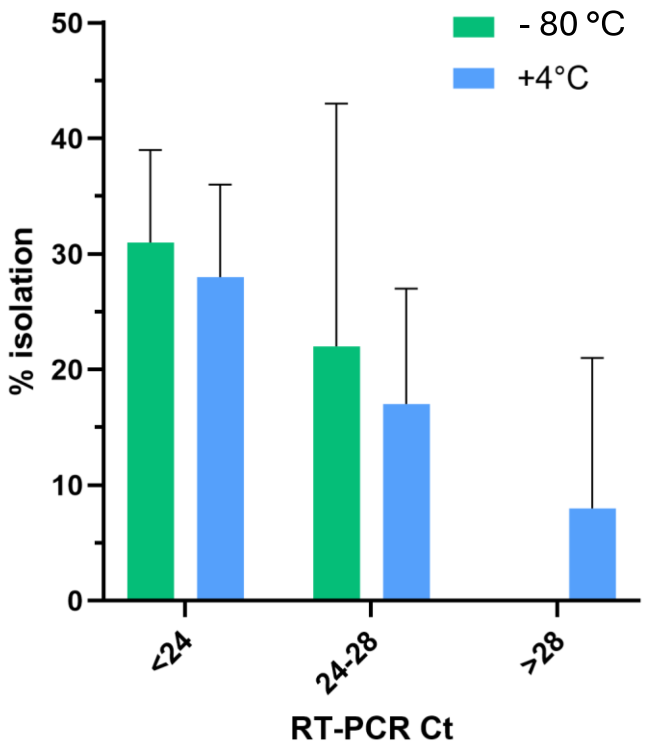


No difference between A/B



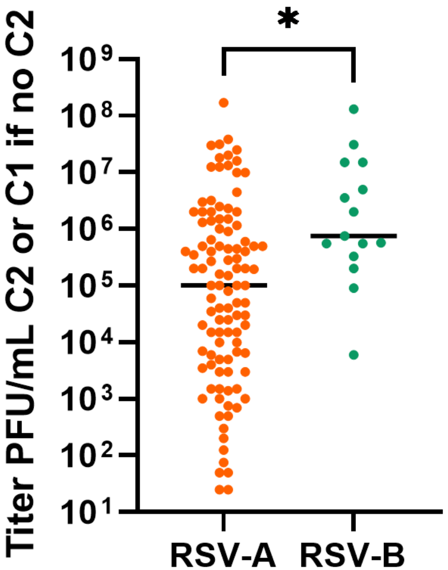
Limited effect of freezing ?

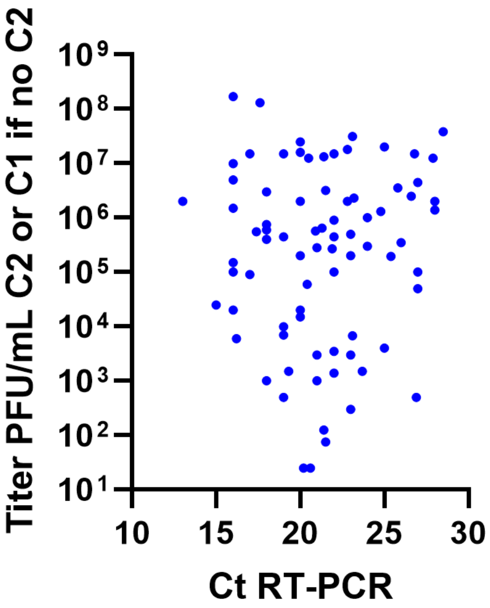
Bias for +4°C preservation



Titers and stability

No link between Titers and Ct



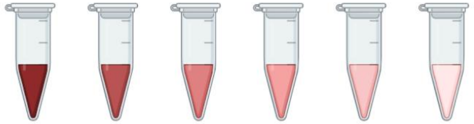


Stability assessment:
work still in progress....

Neutralisation Assay

Principles

Serial dilutions of nirsevimab (or other)



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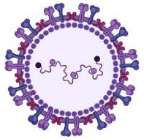
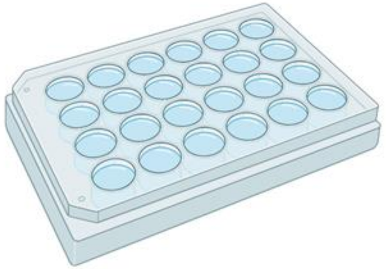
RSV



Incubation:
30-120min
RT or 37°C

Infection
Hep-2 or A549, Vero…



Incubation:
CO2 37°C
2-6 days

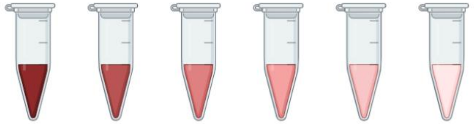
Revelation

TCID50 by ELISA or Fluo
FFU
PFU

Neutralisation Assay

Principles

Serial dilutions of nirsevimab (or other)



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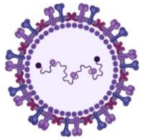
RSV



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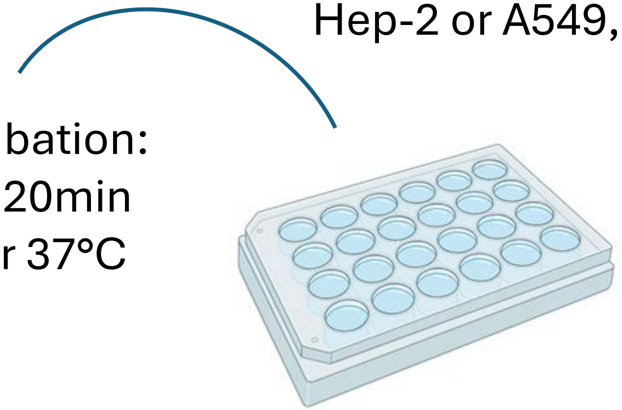


Infection
Hep-2 or A549, Vero…

Incubation:
CO2 37°C
2-6 days

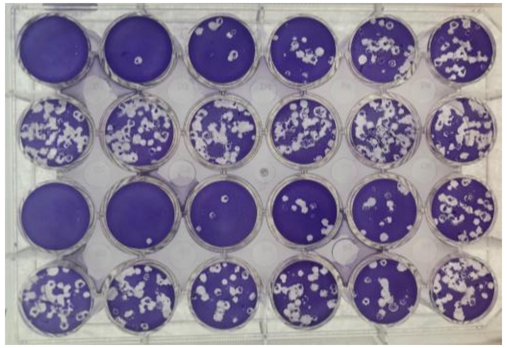
Revelation

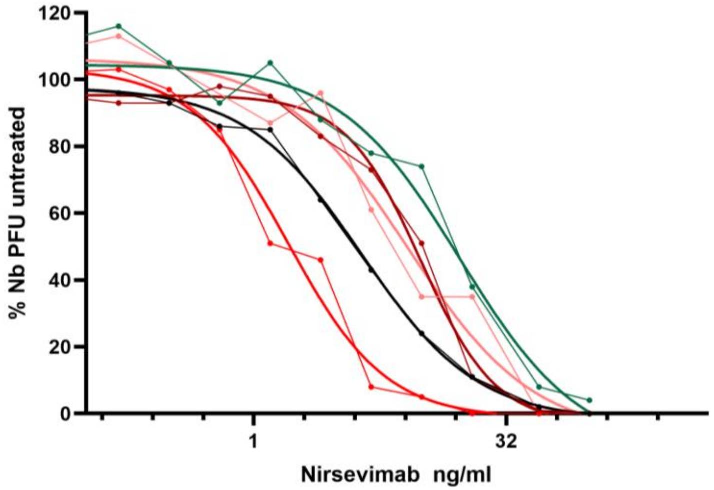
TCID50 by ELISA or Fluo
FFU
PFU

In our hands

Neutralisation assay on HEp-2 cells
Revelation of PFU after 6 days

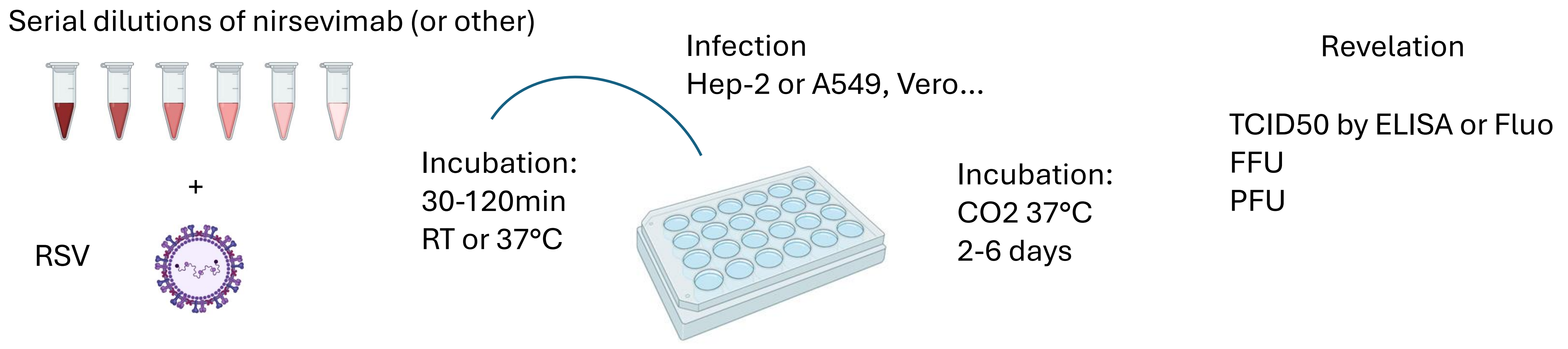
Read out: PFU cumbersome
To set up: ELISA ? Fluorescence ?





Neutralisation Assay

Principles



Serial dilutions of nirsevimab (or other)

+

RSV

Incubation:
30-120min
RT or 37°C

Infection
Hep-2 or A549, Vero…

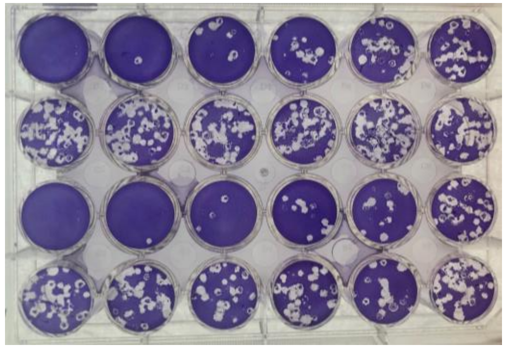
Incubation:
CO2 37°C
2-6 days

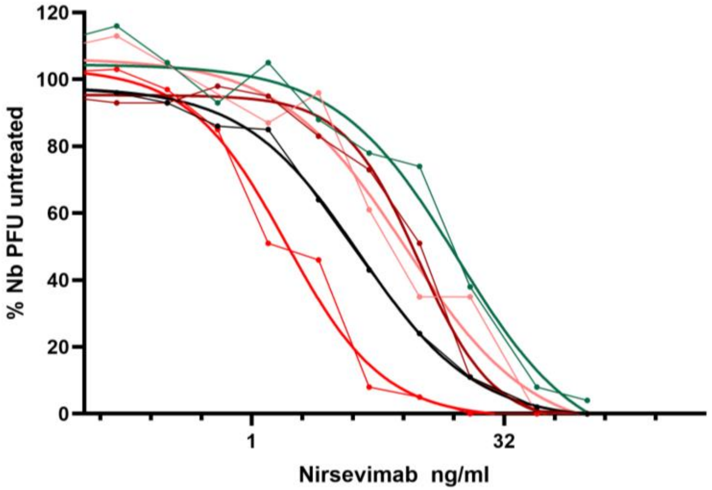
Revelation

TCID50 by ELISA or Fluo
FFU
PFU

In our hands

Neutralisation assay on HEp-2 cells
Revelation after 6 days of PFU





Read out: PFU cumbersome
To set up: ELISA ? Fluorescence ?

Antigenic characterization : **relevance for RSV ? Reagents ?**