



Surveillance of SARS-CoV-2 variants in wastewater of aircrafts

Jan - Mar 2023

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Ad-hoc guidance

Wastewater sampling of aircrafts for SARS-CoV-2 surveillance

*A guidance document for
Member States*

Status 09 Jan 2023

Deere DA, Jones DL, Ahmed W, Medema G,
Kreuzinger N, Remmonay I, Lacroix S, Hewitt J,
Tavazzi S, Gawlik BM

2023



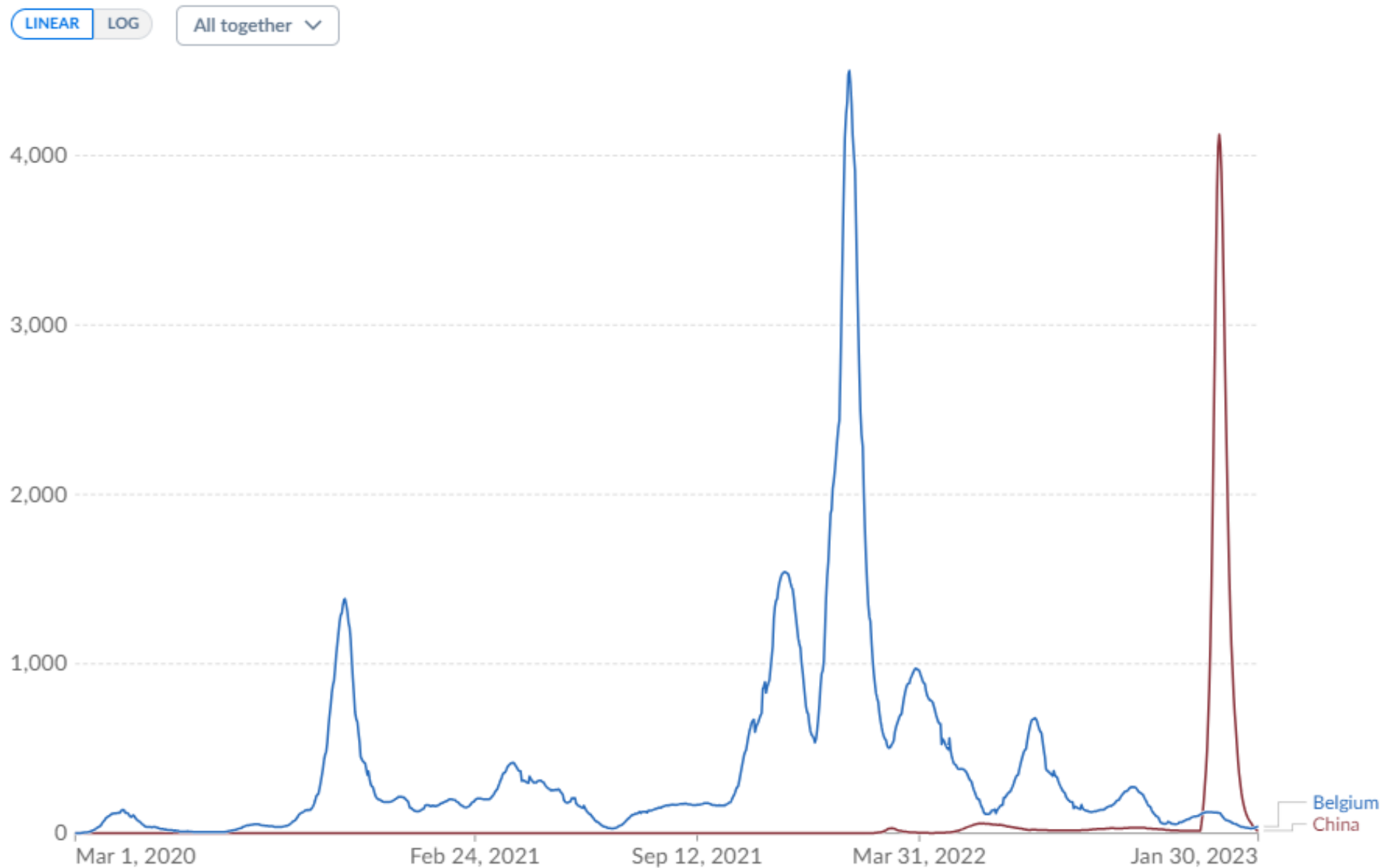
Why flights from China ?



Daily new confirmed COVID-19 cases per million people

7-day rolling average. Due to limited testing, the number of confirmed cases is lower than the true number of infections.

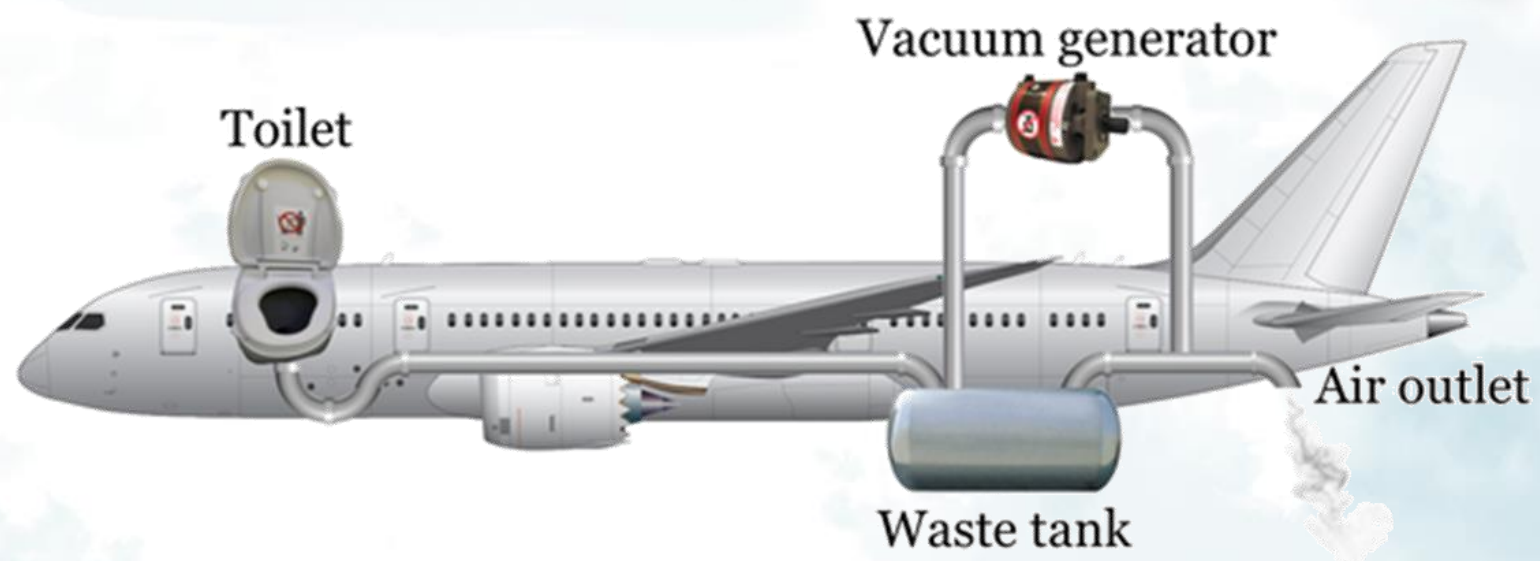
Our World
in Data

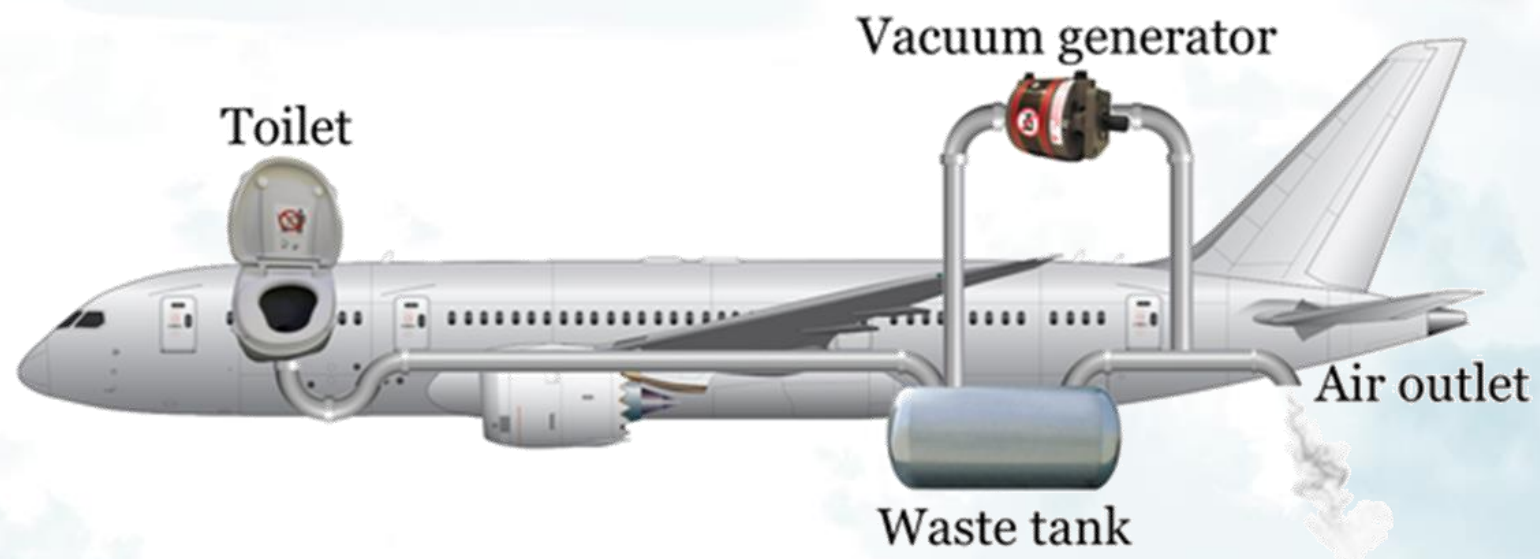


Source: WHO COVID-19 Dashboard

CC BY





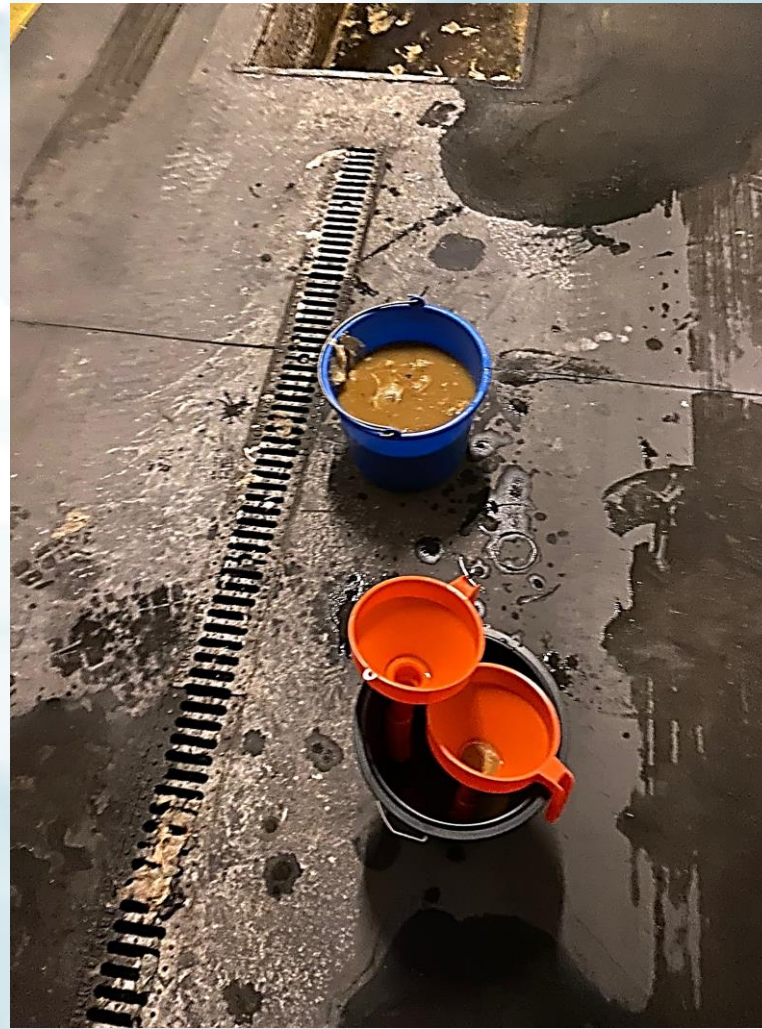


Lavatory service truck

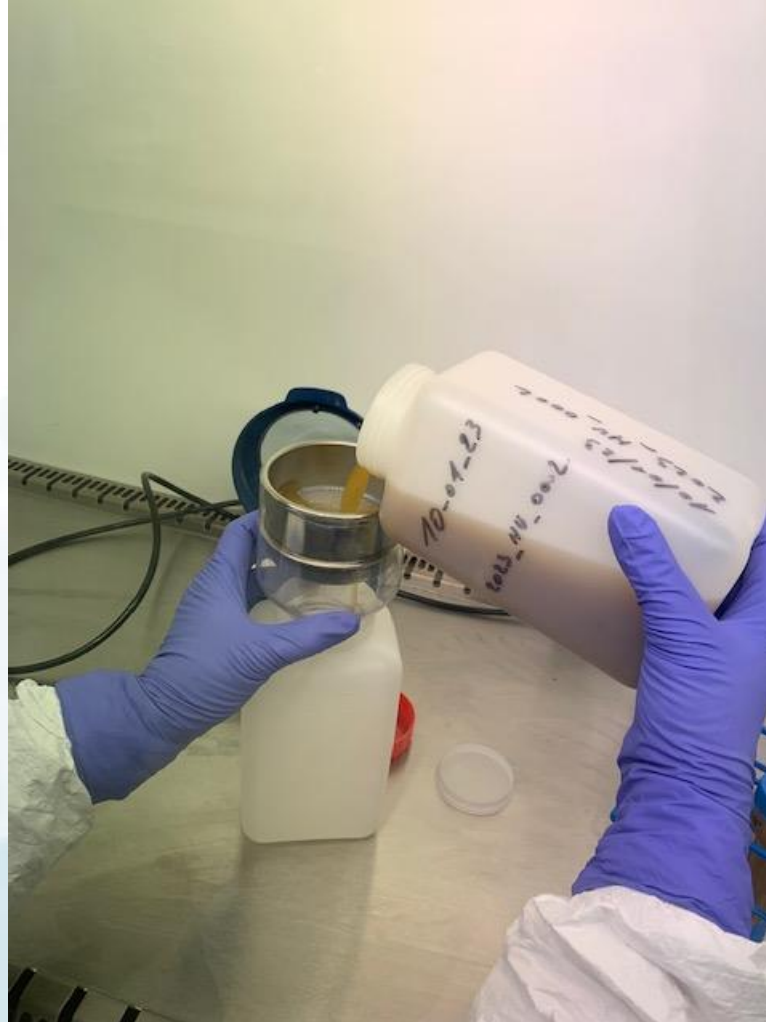


AVIAPARTNER 

Sampling of wastewater

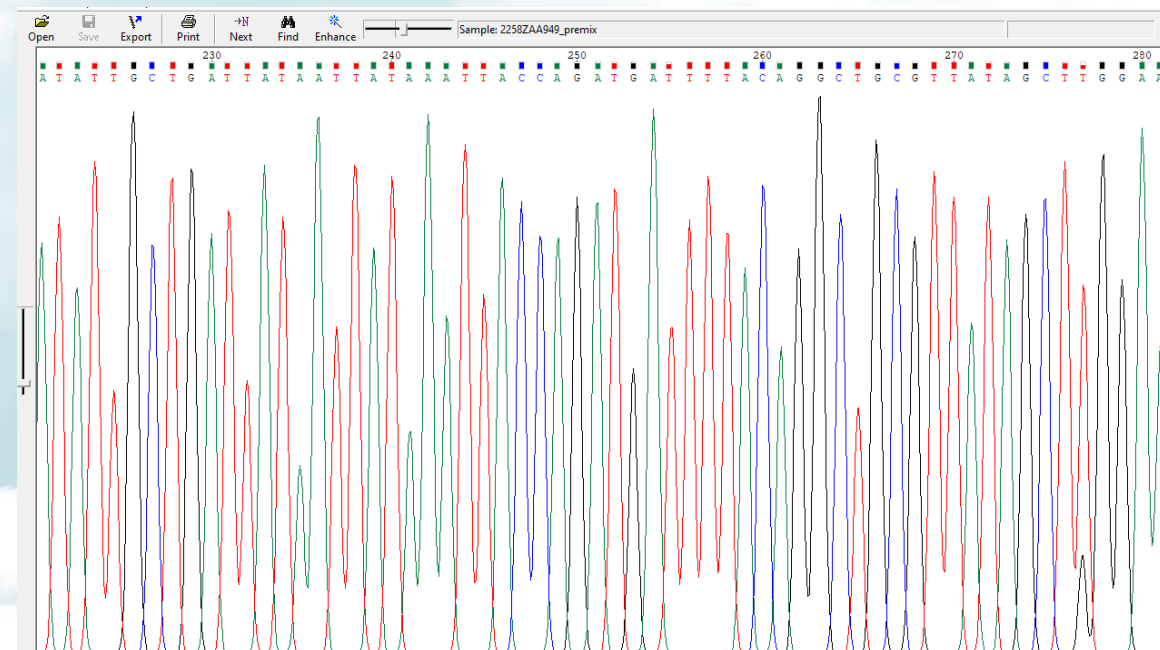


Sample preparation



Methods

1. Extraction: RNeasy kit Qiagen
2. qPCR N1 gene SARS-CoV2 : 2019-nCoV CDC EUA kit
3. Sequencing
 - RBD (Sgene) : Sanger & Illumina sequencing



Article

Monitoring SARS-CoV-2 Populations in Wastewater by Amplicon Sequencing and Using the Novel Program SAM Refiner

Devon A. Gregory ¹, Chris G. Wieberg ², Jeff Wenzel ³, Chung-Ho Lin ⁴ and Marc C. Johnson ^{1,*}

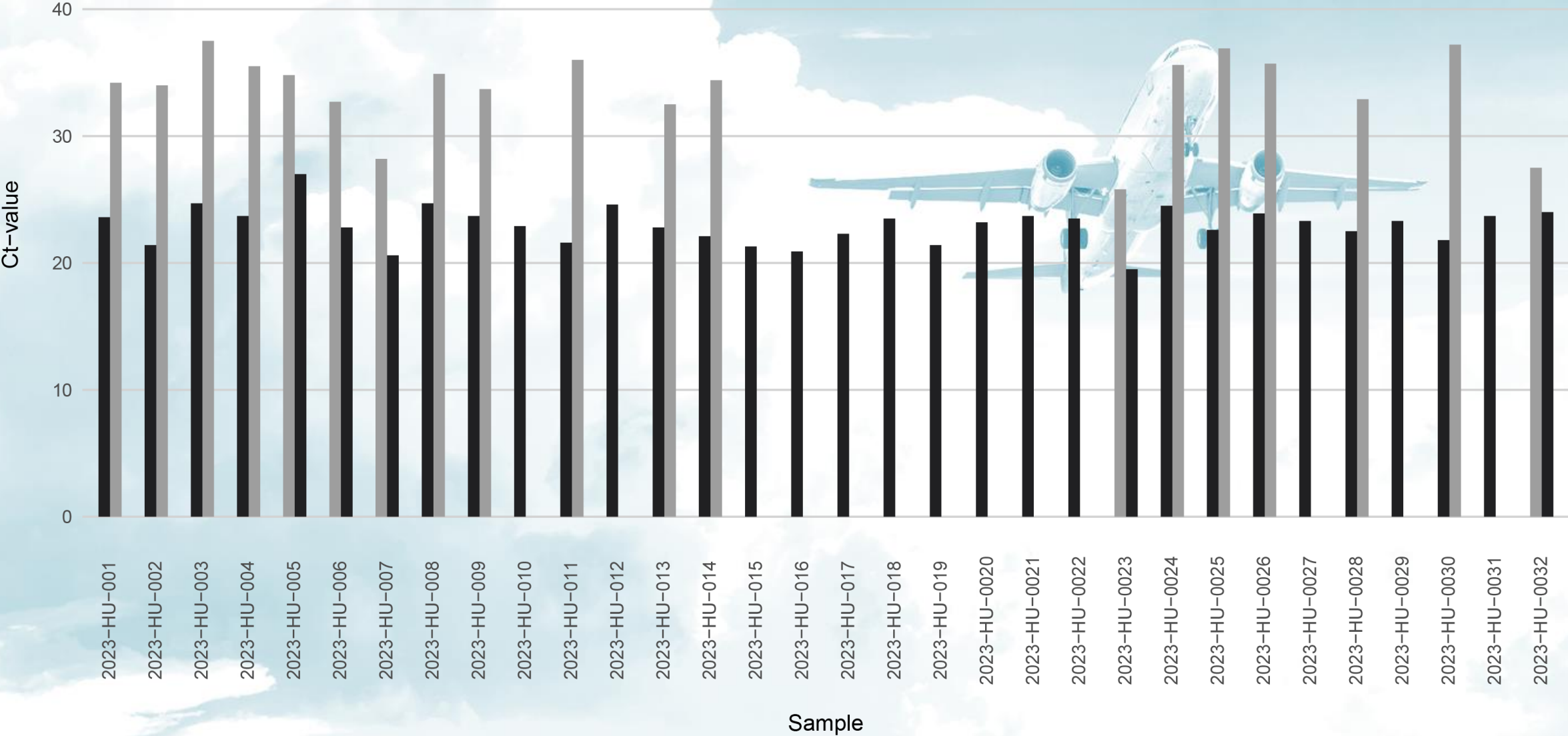
Methods

1. Extraction: RNeasy kit Qiagen
2. qPCR N1 gene SARS-CoV2 : 2019-nCoV CDC EUA
3. Sequencing
 - RBD (Sgene) : Sanger & Illumina sequencing
 - WGS : Amplicon based Illumina sequencing (ARTIC)
 - WGS : Viral Surveillance Panel Illumina (hybrid capture)
4. Other pathogens: qPCR

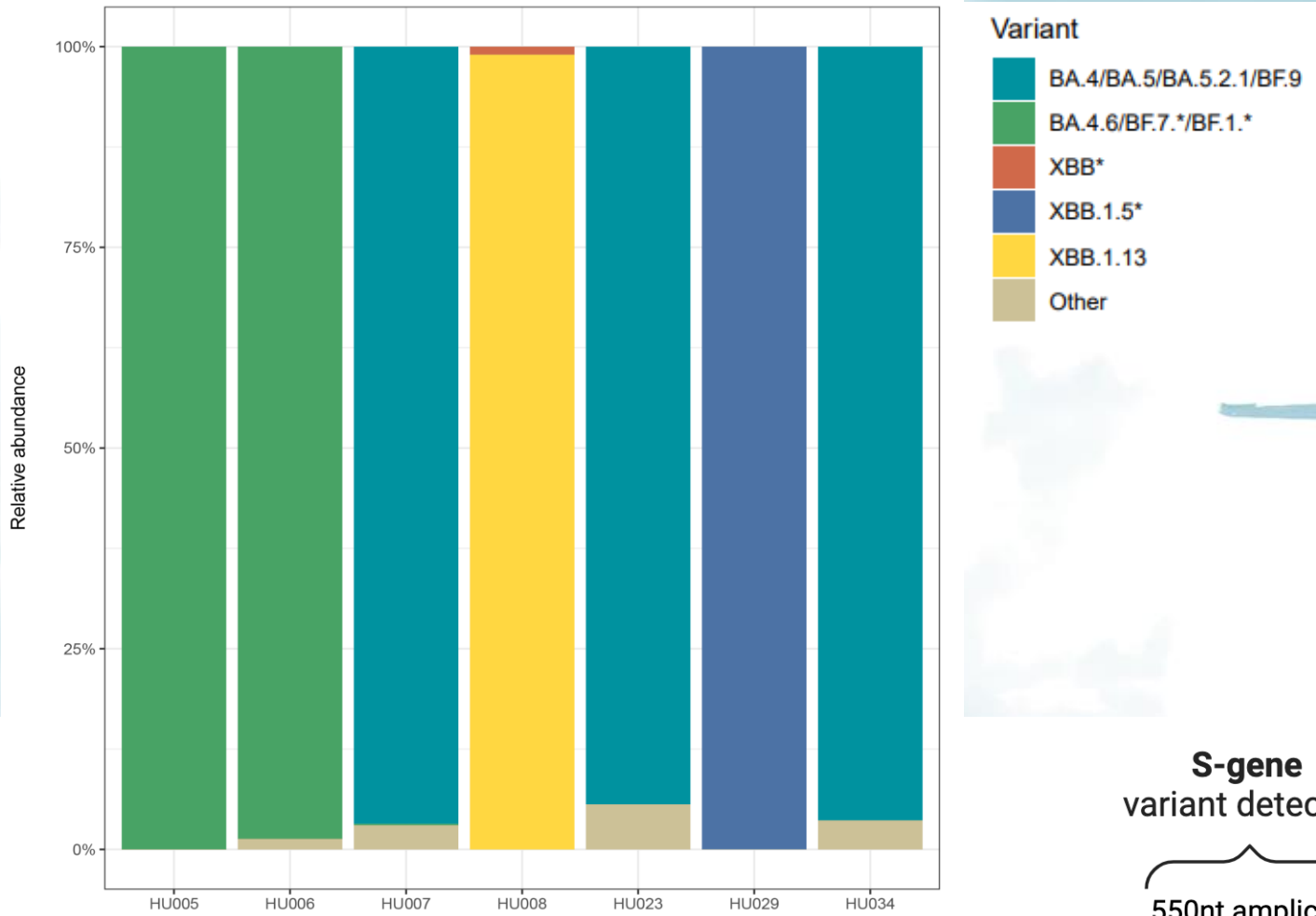


Results

SARS-CoV-2 PMMoV



RBD Illumina sequencing & SAM refiner

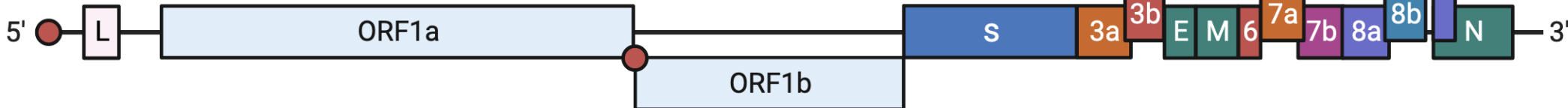


S-gene
variant detection

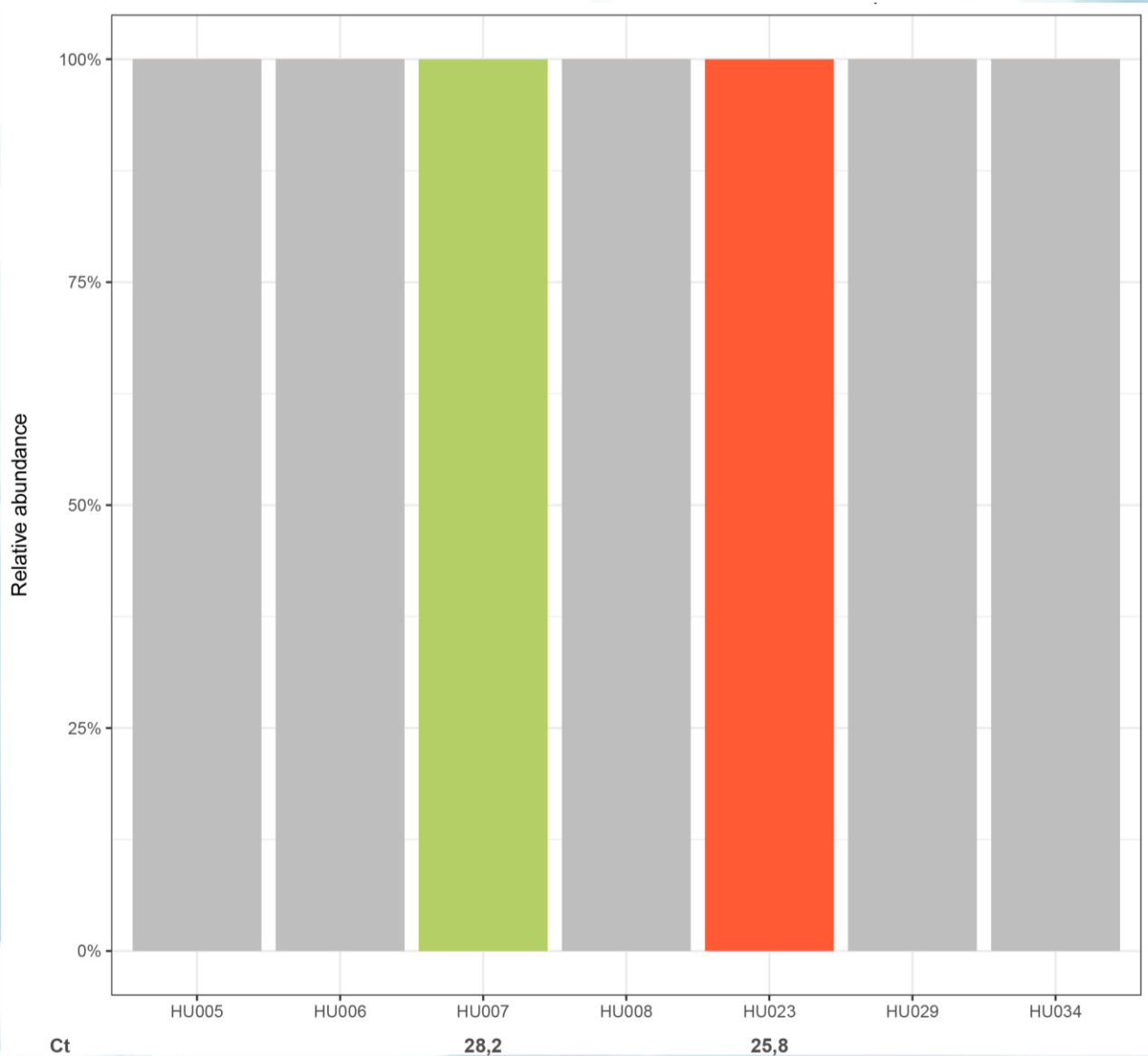
550nt amplicon

RBD

9b



WGS: Amplicon based Illumina sequencing & Freyja



Both BA.5 derivatives

HU 007

DY4=BA.5.2.48.4

718,829 mapped reads

> 90% coverage

HU 023

DY2 =BA.5.2.48.2

560,008 mapped reads

> 90% coverage

WGS: Viral Surveillance Panel by Illumina: hybrid capture



Polyomavirus JC	99,7%
SARS-CoV2: BA5 22B	99,9%
PMMoV	97,8%
Polyomavirus BK	89,1%
HPV 33	85,7%
HPV 39	84,4%
Norovirus GII.P16	86,8%
Norovirus GI.P13	54,9%

Sequencing results

Sanger RBD

HU005 : BA.4/ BA.5
HU006 : BA.4.6/ BF.7
HU007 : BA.4/ BA.5
HU008 : XBB.1.13
HU023 : BA.4/ BA.5
HU029 : XBB.1.5
HU034: BA.4/ BA.5

Illumina RBD

SAM refiner

HU005 : BA.4/ BA.5
HU006 : BA.4.6/ BF.7
HU007 : BA.4/ BA.5
HU008 : XBB.1.13
HU023 : BA.4/ BA.5
HU029 : XBB.1.5
HU034: BA.4/ BA.5

WGS ARTIC

Freyja

HU005 : /
HU006 : /
HU007 : BA.5.2.48.4
HU008 : /
HU023 : BA.5.2.48.2
HU029 : /

Hybrid Capture



HU007 : BA.5 (22B)
HU023 : BA.5 (22B)

Other pathogens

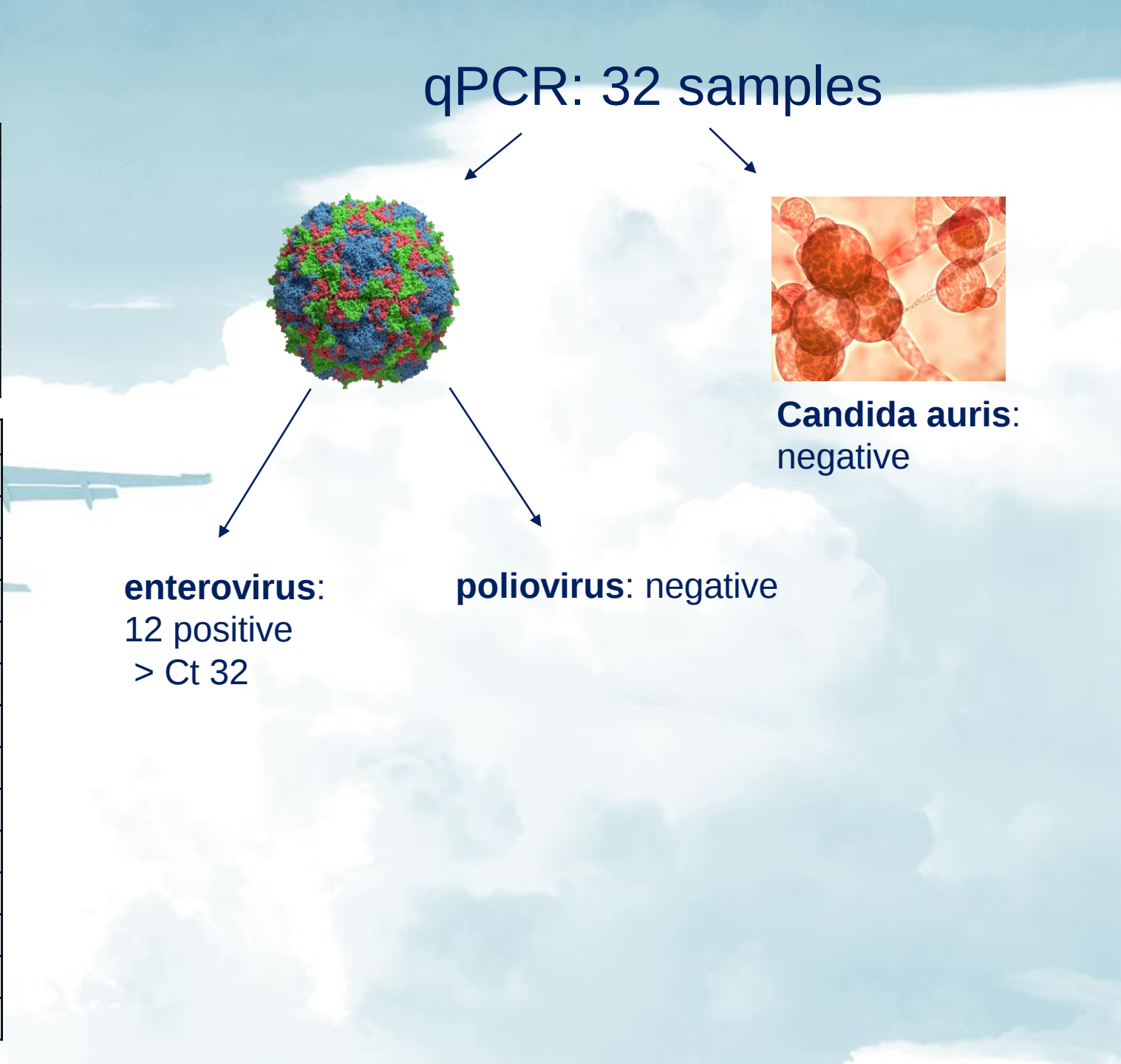
SAMPLE	DATE	RESPI PATHOGEN
2023-HU-0002	10/01/2023	Enterovirus/Rhinovirus
		Streptococcus pneumoniae
2023-HU-0004	13/01/2023	Adenovirus
2023-HU-0007	18/01/2023	Adenovirus
		Streptococcus pneumoniae

SAMPLE	DATE	GASTRO PATHOGEN
2023-HU-0002	10/01/2023	Norovirus GII
		Campylobacter
		Clostridium difficile toxin A/B
		Enterogaggative E coli
		Enteropathogenic E coli
		parasites
2023-HU-0004	13/01/2023	Adenovirus 40/41
		Enterogaggative E coli
		Enterotoxigenic E coli
		Plesiomonas shigelloides
		parasites
2023-HU-0007	18/01/2023	Enteropathogenic E coli
		Enterogaggative E coli
		Adenovirus F40/F41

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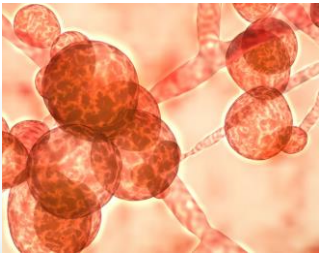
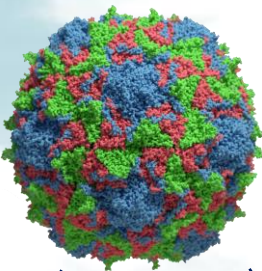


Other pathogens

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		Plesiomonas shigelloides
		parasites
2023-HU-0007	18/01/2023	Enteropathogenic E coli
		Enteroaggregative E coli
		Adenovirus F40/F41

qPCR: 32 samples



Candida auris:
negative

enterovirus:
12 positive
> Ct 32

poliovirus: negative



Bacterial Culture:
Enterobacterales:

Escherichia coli,
Klebsiella pneumoniae,
Morganella morganii;
Providencia spp,
Enterococci

Future perspectives





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USA**



**Prof. Jelle Matthijnssens
Lander De Coninck
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Belgium**

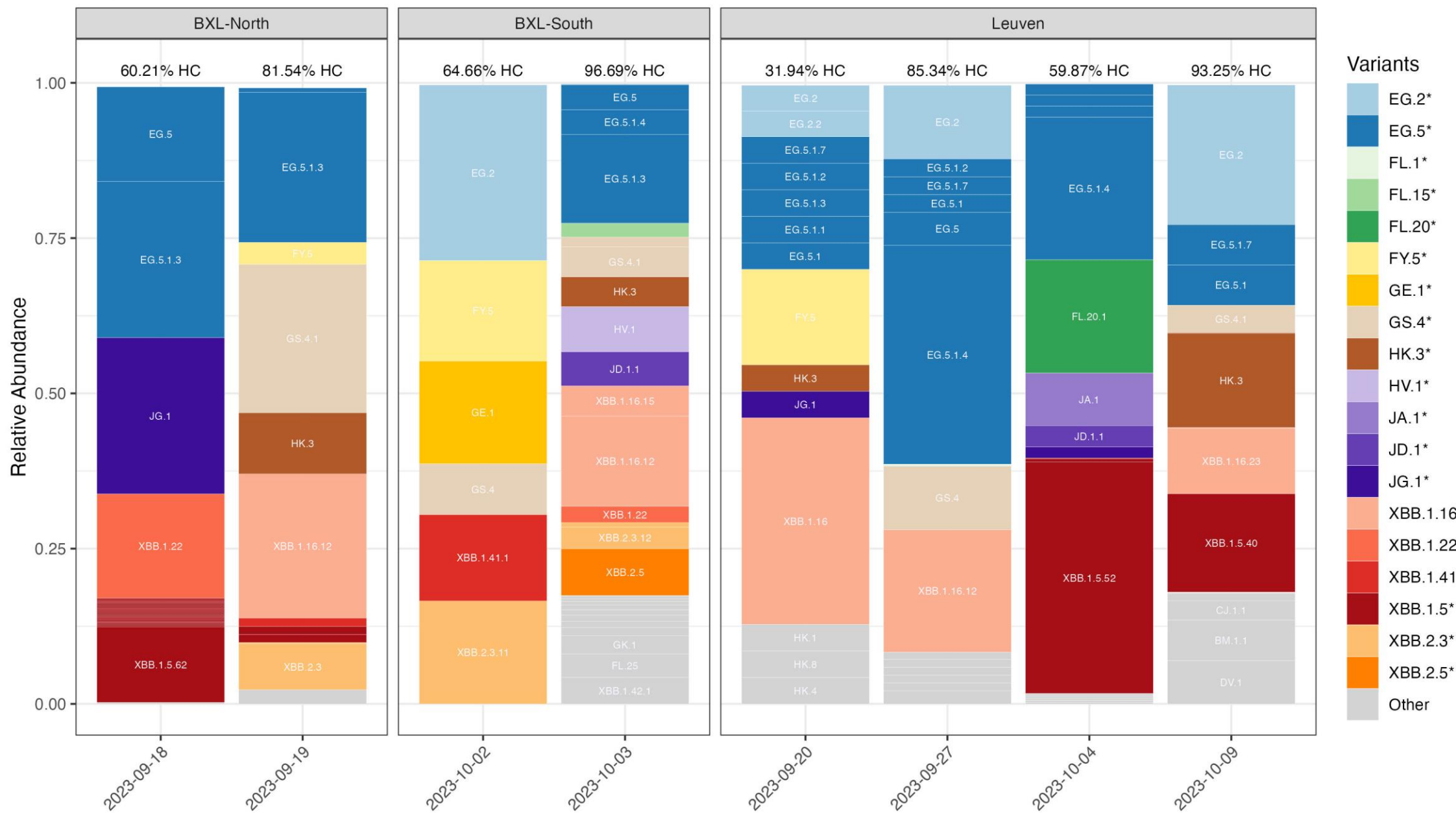


**"To measure is
to know."**

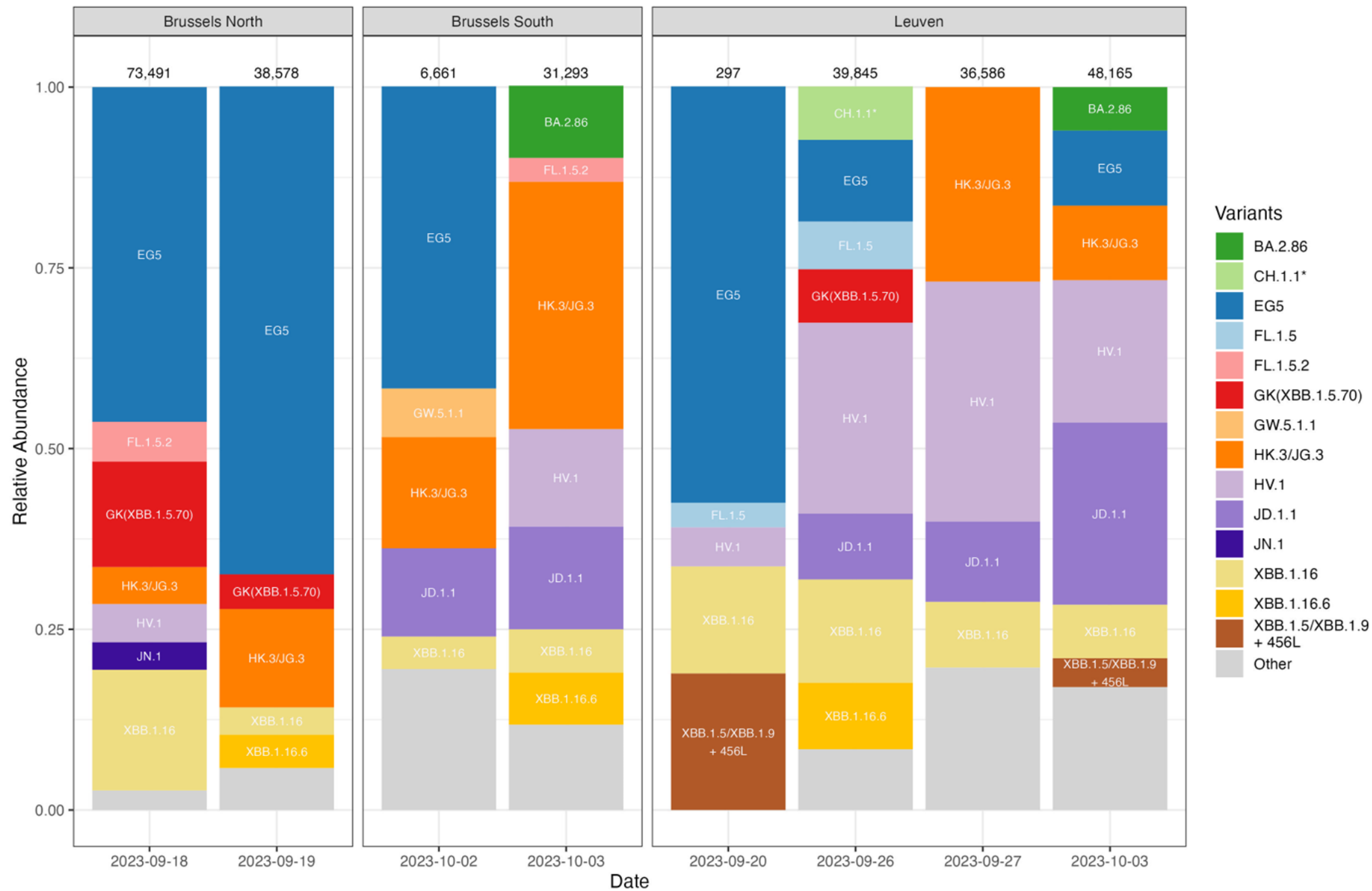
**"If you can not
measure it,
you can not
improve it."**

Lord William Thompson Kelvin

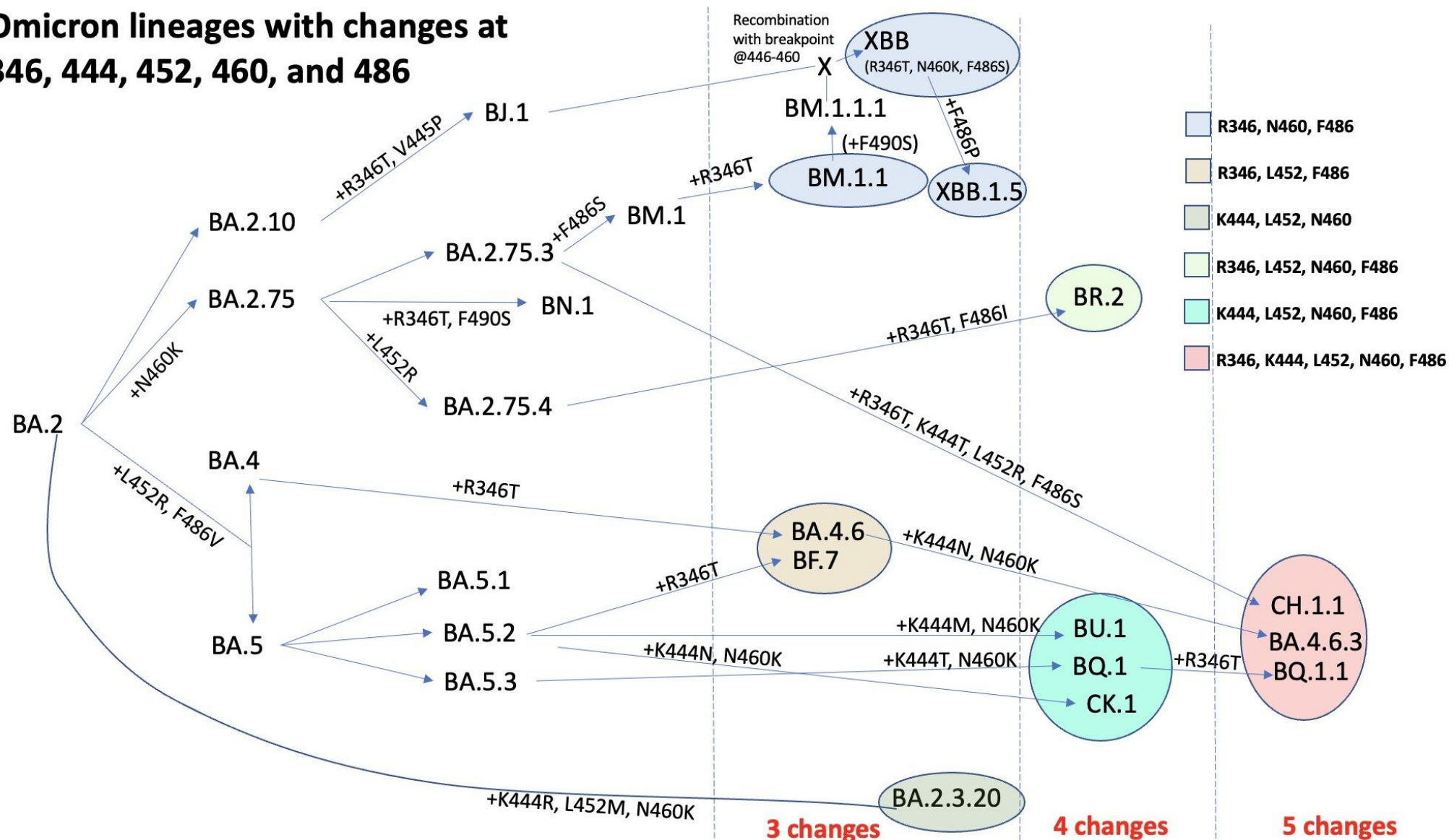
Freyja variant detection



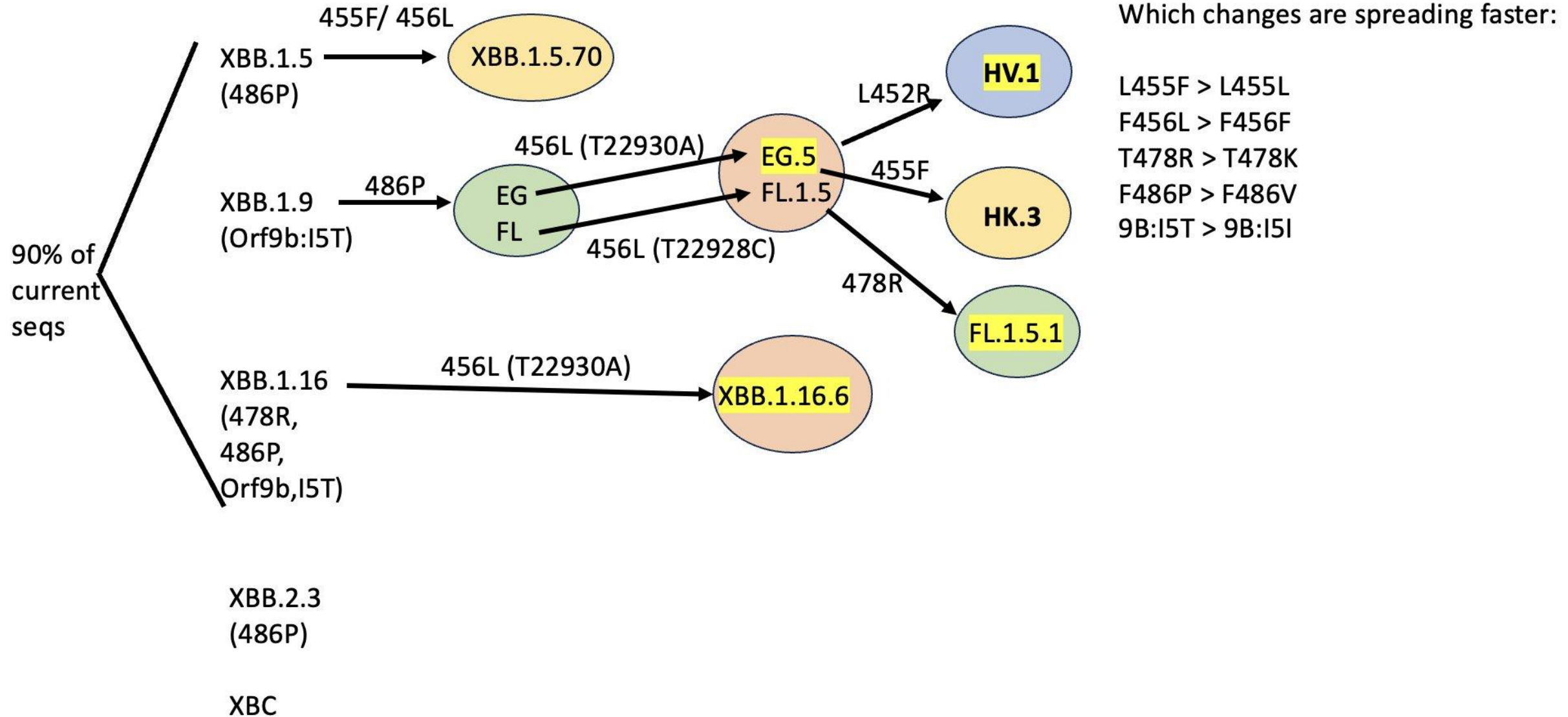
S gene variant detection



Omicron lineages with changes at 346, 444, 452, 460, and 486



Convergence: S:455F/456L/478R/486P & 9b:I5T



Adenovirus	Hepatitis B virus	Parechovirus
Aichivirus	Hepatitis C virus	Parvovirus
Astrovirus	Hepatitis E virus	Poliovirus
Chapare virus	Human Immunodeficiency Virus 1	Polymavirus
Chikungunya virus	Human Immunodeficiency Virus 2	Respiratory syncytial virus
Coronavirus-229E	Influenza A Virus	Rhinovirus
Coronavirus-HKU1	Influenza B Virus	Rift Valley fever virus
Coronavirus-OC43	Japanese encephalitis virus	Rotavirus
Coronavirus-NL63	Junin virus	Rubella virus
Coxsackievirus	Kyasanur Forest disease virus	Sabia virus
Crimean-congo haemorrhagic fever virus	Lassa fever virus	Salivirus
Dengue virus 1	Lujo hemorrhagic fever virus	Sapovirus
Dengue virus 2	Machupo virus	SARS-COV
Dengue virus 3	Marburg virus	SARS-COV-2
Dengue virus 4	MERS-CoV	Tick-borne encephalitis virus
Eastern equine encephalitis virus	Metapneumovirus	Torque Teno virus
Ebola virus	Monkeypox virus	Variola virus
Enterovirus	Nipah virus	Venezuelan equine encephalitis virus
Guanarito virus	Norovirus	West Nile virus
Hantavirus	Omsk hemorrhagic fever virus	Western equine encephalitis virus
Hendra henipavirus	Oncolytic human papillomavirus	Yellow fever virus
Hepatitis A virus	Parainfluenza virus	Zika virus

SAMPLE	DATE	SARS-CoV-2 Ct	SARS-CoV-2 copies/μl	PMMoV Ct	PMMoV copies/μl	Variants
2023-HU-001	7/01/2023	34,2	15	23,6	76300	
2023-HU-002	10/01/2023	34,0	17	21,4	330750	
2023-HU-003	12/01/2023	37,5	2	24,7	35505	
2023-HU-004	13/01/2023	35,5	7	23,7	71139	
2023-HU-005	14/01/2023	34,8	10	27,0	7449	BA4 / BA5
2023-HU-006	17/01/2023	32,7	36	22,8	129188	BA.4.6/ BF7*/B.F1*
2023-HU-007	18/01/2023	28,2	537	20,6	568357	BA4 / BA5
2023-HU-008	20/01/2023	34,9	10	24,7	596000	XBB + F490P
2023-HU-009	25/01/2023	33,7	20	23,7	184000	
2023-HU-010	27/01/2023	Negatief		22,9	119320	
2023-HU-011	31/01/2023	36,0	5	21,6	279495	
2023-HU-012	1/02/2023	Negatief		24,6	37119	
2023-HU-013	2/02/2023	32,5	42	22,8	128321	
2023-HU-014	3/02/2023	34,4	13	22,1	208244	
2023-HU-015	7/02/2023	Negatief		21,3	362217	
2023-HU-016	10/02/2023	Negatief		20,9	451442	
2023-HU-017	11/02/2023	Negatief		22,3	182864	
2023-HU-018	14/02/2023	Negatief		23,5	81505	
2023-HU-019	15/02/2023	Negatief		21,4	327638	
2023-HU-0020	17/02/2023	Negatief		23,2	100088	
2023-HU-0021	21/02/2023	Negatief		23,7	69576	
2023-HU-0022	22/02/2023	Negatief		23,5	80252	
2023-HU-0023	24/02/2023	25,8	2251	19,5	1145687	BA4 / BA5
2023-HU-0024	1/03/2023	35,6	6	24,5	41959	
2023-HU-0025	3/03/2023	36,9	3	22,6	142727	
2023-HU-0026	4/03/2023	35,7	6	23,9	61716	
2023-HU-0027	7/03/2023	Negatief		23,3	91822	
2023-HU-0028	10/03/2023	32,9	32	22,5	160578	XBB.1.5*
2023-HU-0029	14/03/2023	Negatief		23,3	91699	
2023-HU-0030	21/03/2023	37,2	3	21,8	251454	
2023-HU-0031	24/03/2023	Negatief		23,7	69529	
2023-HU-0032	28/03/2023	27,5	821	24,0	55937	BA.4.6/ BF7*/B.F1*