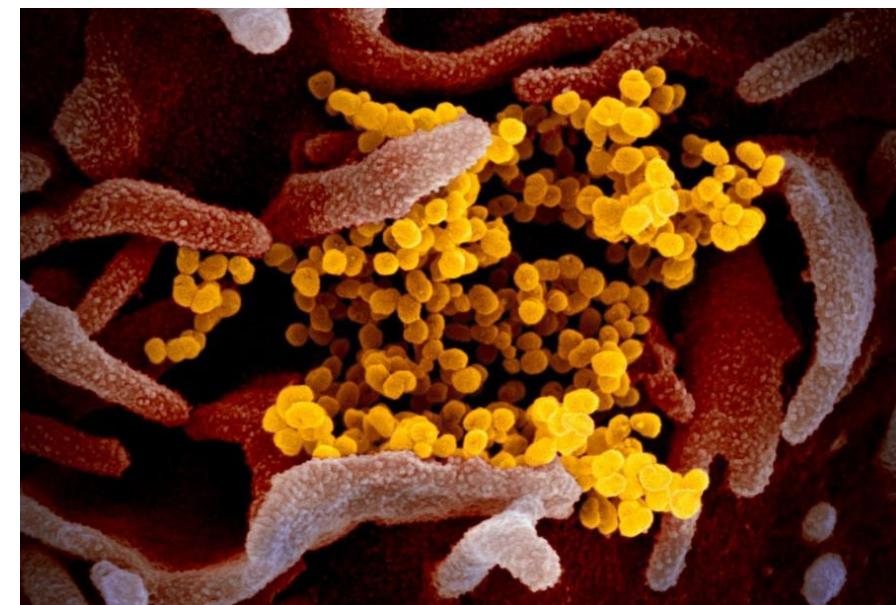


WGS – DISCRIMINATIVE PCR in the Czech Republic

Helena Jiřincová

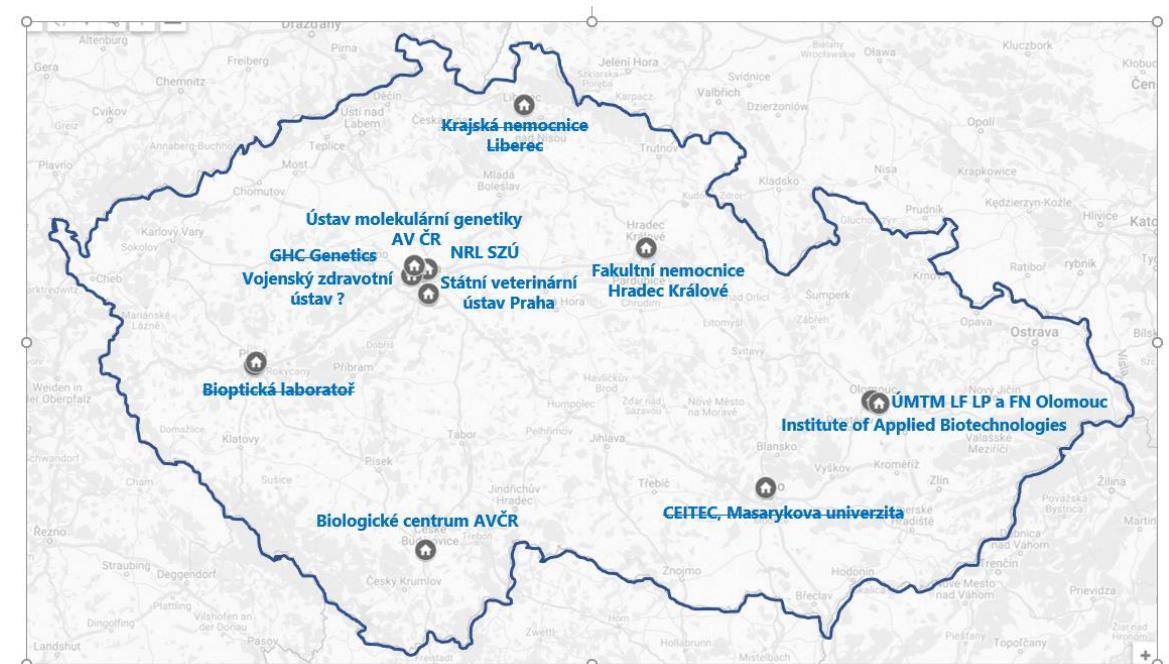
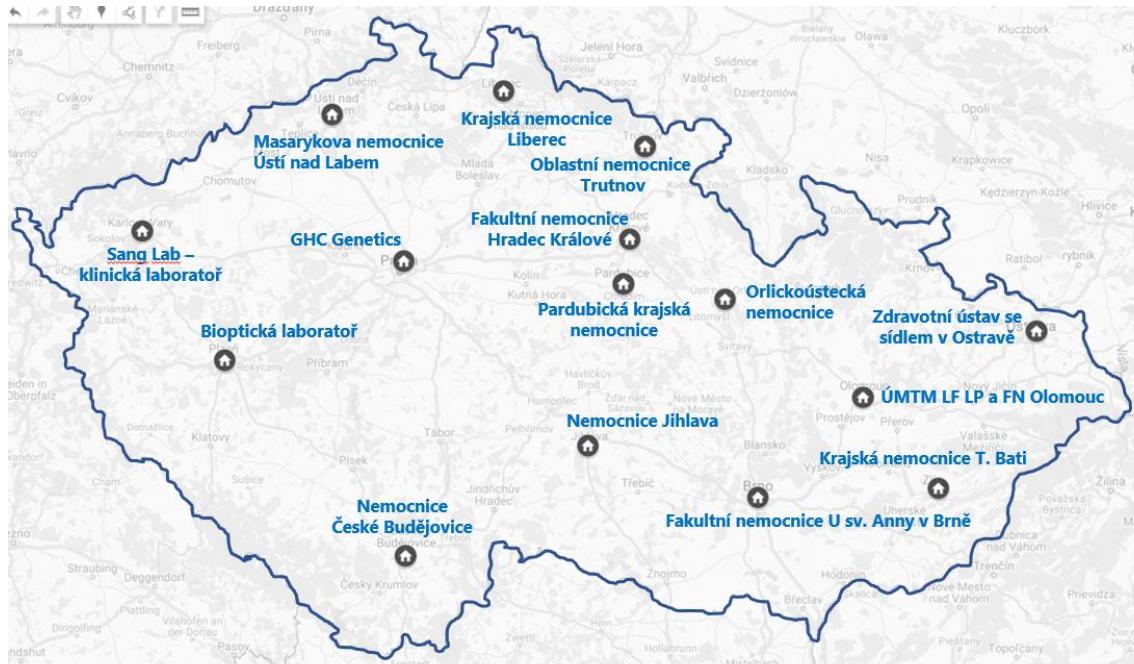
NRL FOR INFLUENZA NAD RESPIRATORY VIRUSES

CEM - NIPH



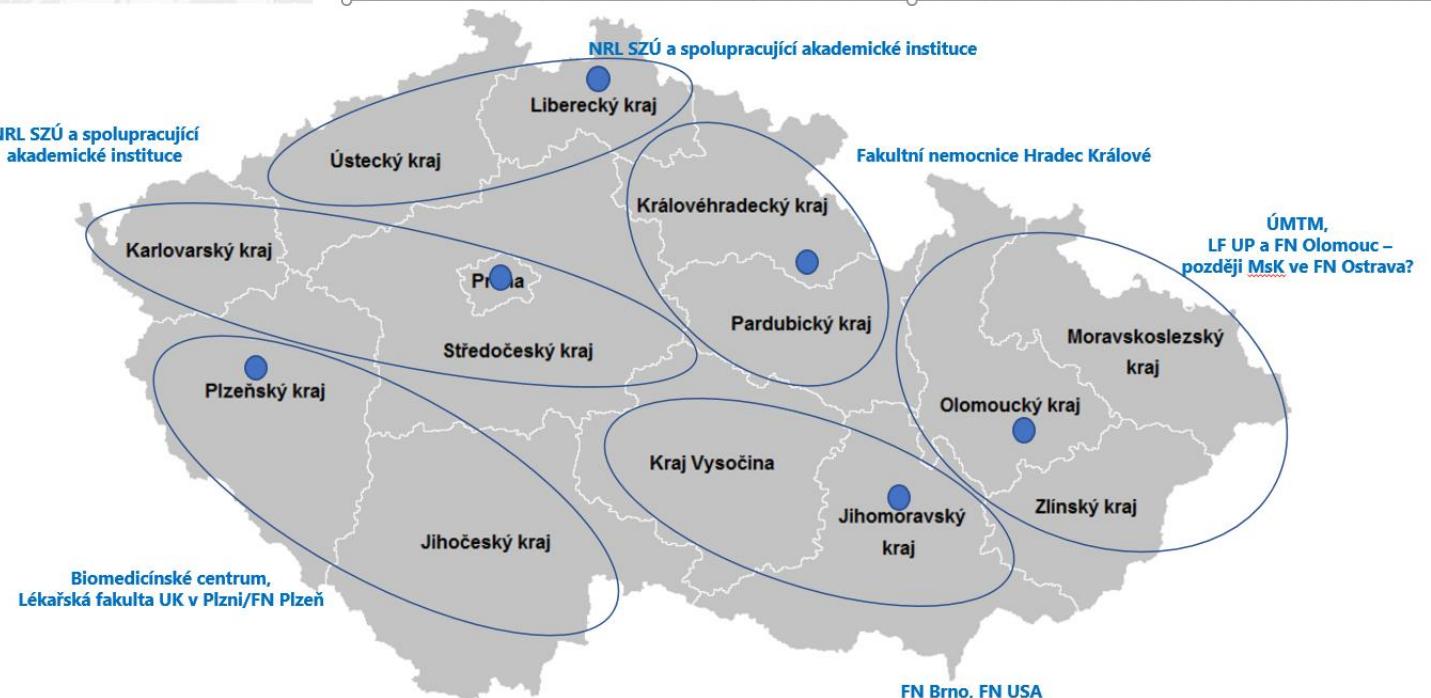
WGS strategy - target 4 500 samples per month

- Representative – 10% of positive from the sentinel labs
- Targeted
 - Import
 - PCR discriminative
 - Outbreak
 - Atypical PCR
 - Vaccination failure
 - Hospitalisation <50 YOA
 - Atypical clinical signs



Sentinel and SARS WGS net

Coverage of the whole region



Summary of the data from WGS

- Sum of WGS in GISAID 4 378
- Sum of WGS in GISAID 2021 4 096
- **Sum of WGS ECDC support 1 980**

- Alpha B.1.1.7 3 137
- Beta B.1.351 60
- Gamma P.1 11
- Delta B.1.617.2 96
- Zeta P.2 5
- B.1.620 3
- B.1.1.318 4
- B.1.617.1. 5
- B.525 2
- C.36 17
- C.36.3 1
- B.1.258 (+ x.10, 11, 12, 14, 15) 369

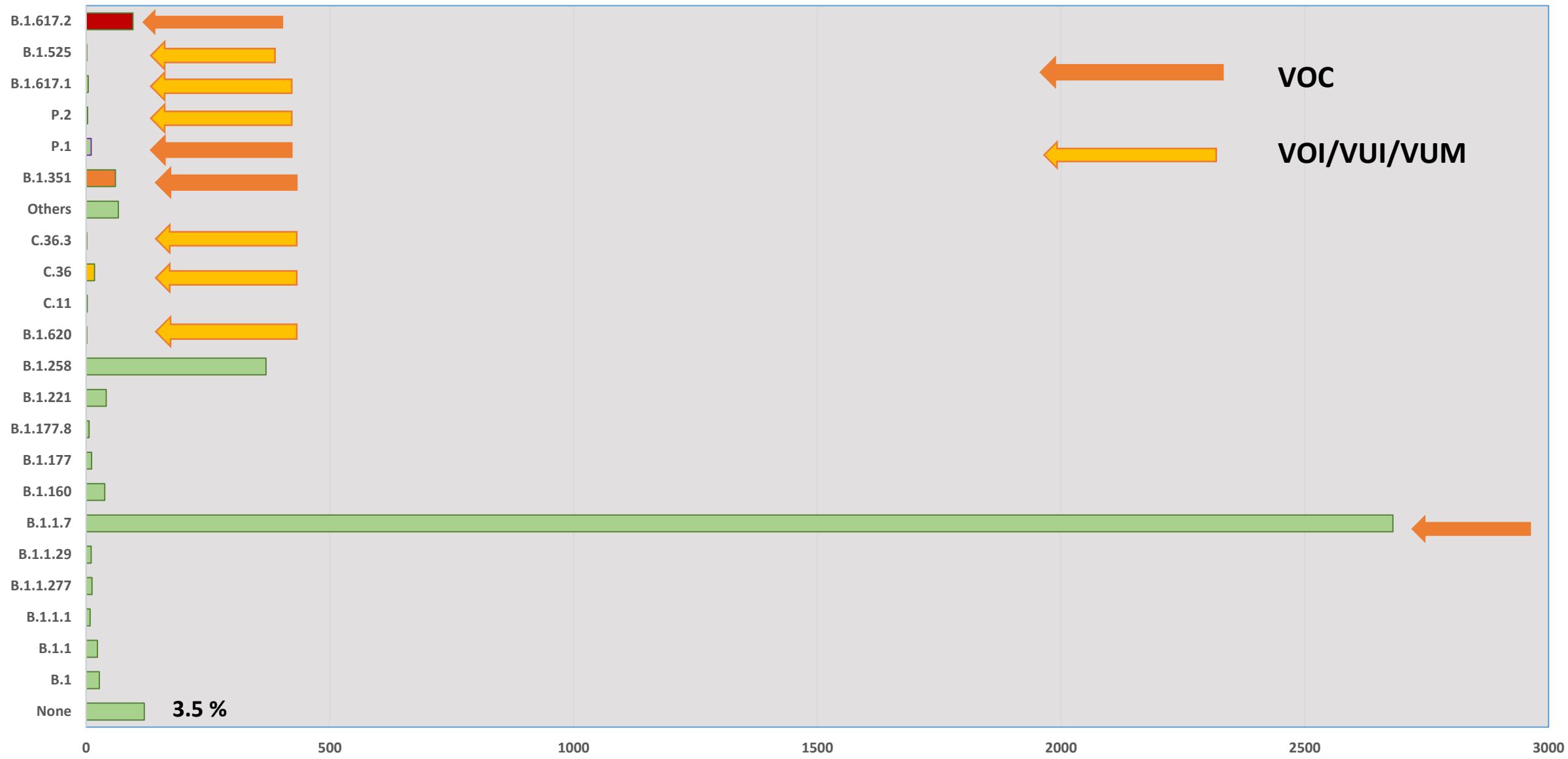
VOC – variant of concern

VUI – variant under investigation

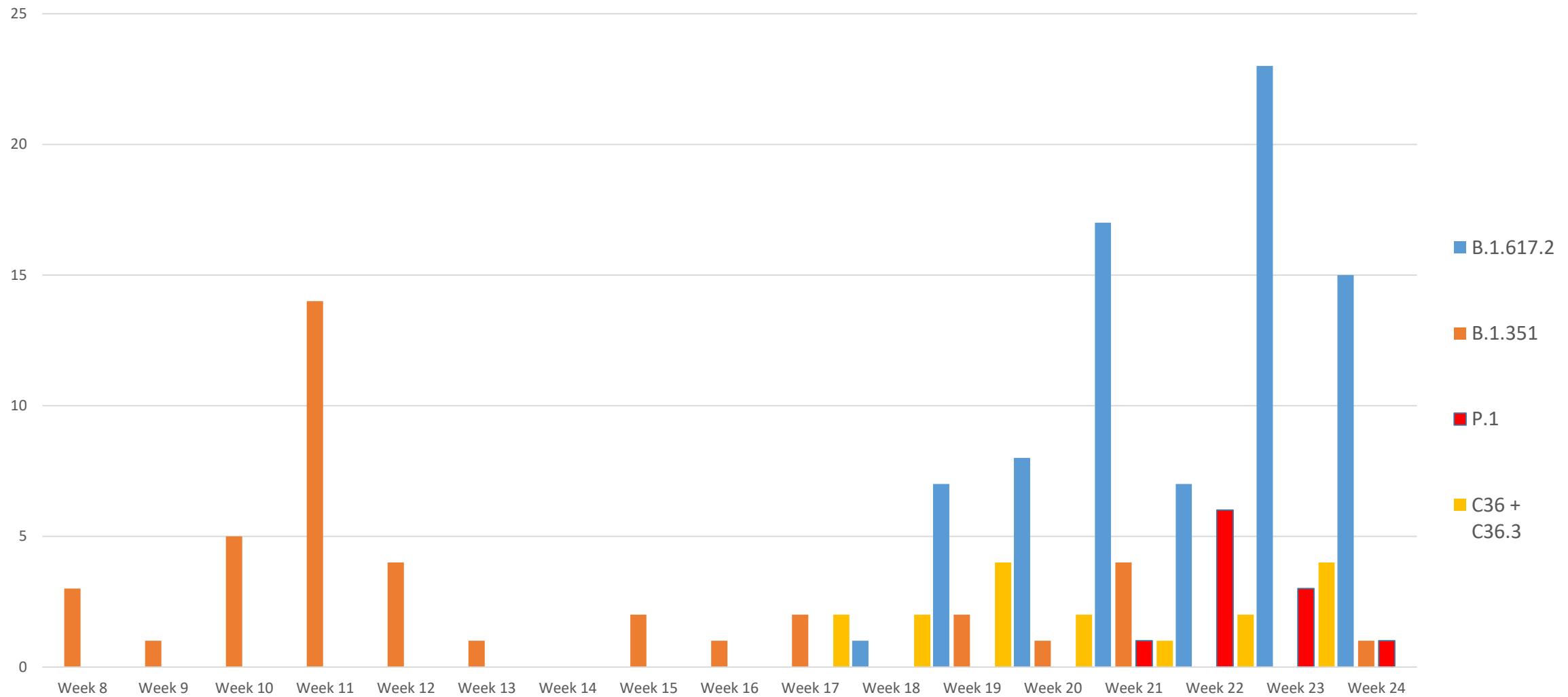
VOI – varint of interest (CDC/WHO terminologie)

VUM – variant under monitoring (ECDC terminologie)

Overview of variants detected in the Czech republic in 2021

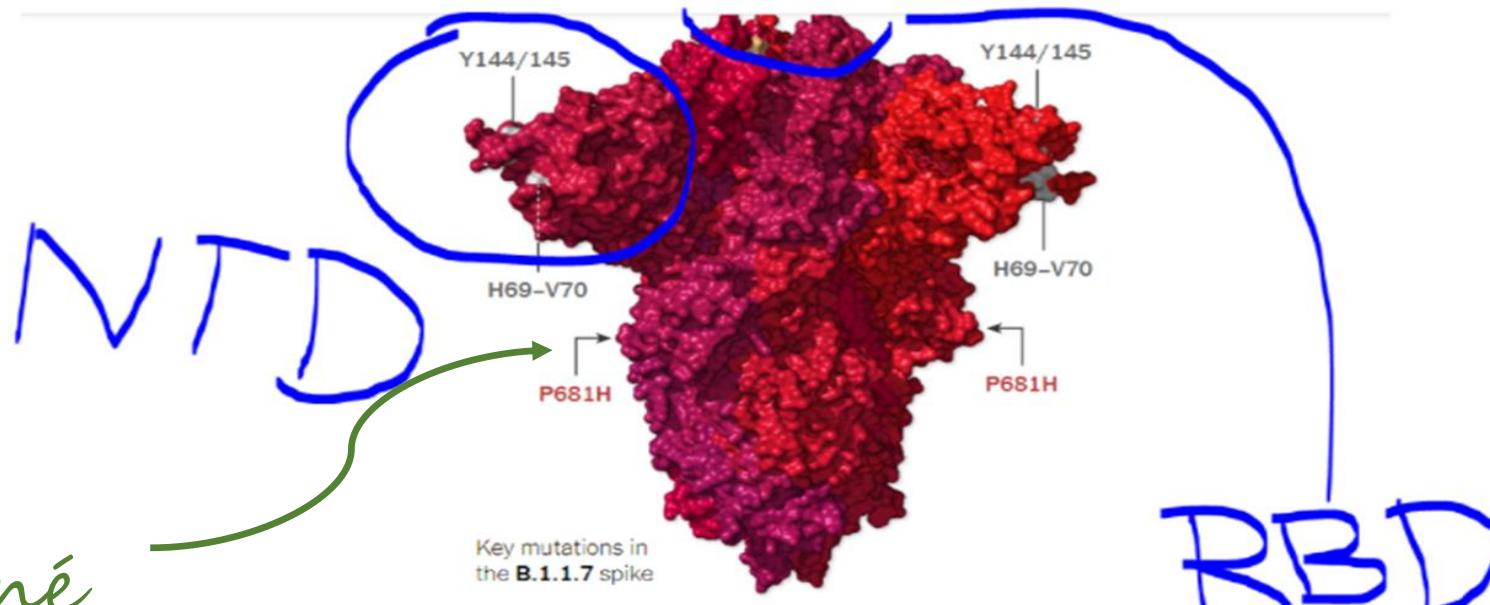


VOC detection in CW 8 to CW 24 2021 (data from NRL)

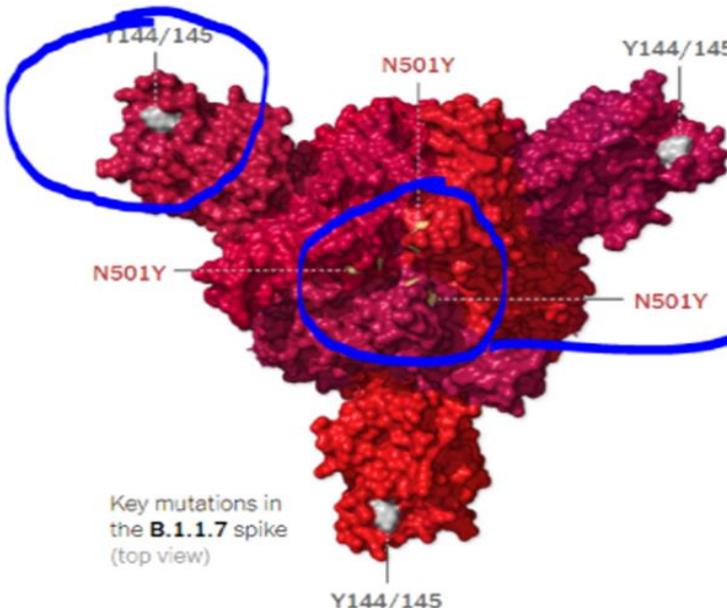


PCR

Furinové štěpné
místo



It takes three spike proteins to form one spike, so each mutation appears in three places:



For more on the B.1.1.7 mutations, see: [Inside the B.1.1.7](#)

NRL recommendation concerning PCR

The goal is not to identify a variant, but to find a hot spot mutation

1st priority: E484K L452R

Escape, resistant against bamlanivimab (BAMLAN - Eli Lilly)

EG C.36 / B.617.2 B.1.1.7, P.1, P.2, B.1.351/B.1.1.318

2nd priority: N501Y, K417N, P681 H

Or: K417N/T, P681H/R, E484K/Q, del69-70.

? **T478K** B.1.617/2, B.1.1.519 **T478R** (Tanzanie, Uganda A.VO.1)

- Import
- Outbreak
- Atypical PCR
- Vaccination failure
- Hospitalisation <50 YOA
- Atypical clinical signs

Přehled detekčních souprav pro diskriminační PCR

NRL prosí výrobce a distributory o aktualizaci a případně doplnění. Zvýrazněné jsou ty soupravy, které nabízejí vhodnou kombinaci z hlediska současné situace. Přehledná tabulka je přílohou zprávy.

Název kitu	Výrobce	Distributor	Detekované mutace*																Spike L2/L2A	OffLab	S3673_3677	deletion	vrt (S gene)	WT (E gene)	
			de69-70	D6DA	K417N	A570D	E69K	N501Y	P681H	1176F	R48P WT	Ngene D3L	N501E 106/107/108 d	W135C	L413R	K417T	P681R	E69K	S477N	D614G	A222V	T251	C677H	I478K	*V144 del
Biosphore N501Y Detection Kit v1 *	Anatolia Geneworks	GALI spol. s r.o.			x			x	x																
Biosphore SARS-CoV-2 Variant Detection Kit v1 *	Anatolia Geneworks	GALI spol. s r.o.			x			x	x																
Biosphore SARS-CoV-2 Variant Detection Kit v2 *	Anatolia Geneworks	GALI spol. s r.o.			x	x	x	x	x																
Realizing SARS-CoV-2 Variant	Bioron	GALI spol. s r.o.	x		x	x	x	x	x																
KIT-C68/SARS-CoV-2 VARIANT SET 1 (9424407190)	Roche	Roche	x		x	x	x	x	x																
VirSNIP SARS de89,70 + 484K + 501Y (9449824001)	TibMol Biol	Roche	x		x	x	x	x	x																
VirSNIP SARS B117 (Spike del-501) (9429502001)	TibMol Biol	Roche	x		x	x	x	x	x																
VirSNIP SARS B131 (484K+501Y) (9429522001)	TibMol Biol	Roche			x	x	x	x	x															x	
VirSNIP SARS-CoV-2 Spike A230531 N501Y (9405577001)	TibMol Biol	Roche	x		x	x	x	x	x																
VirSNIP SARS-CoV-2 Spike del H89_V7 (9408874001)	TibMol Biol	Roche	x		x	x	x	x	x																
VirSNIP SARS-CoV-2 Spike D614G (9405686001)	TibMol Biol	Roche																							
VirSNIP SARS-CoV-2 Spike P881H (9409572001)	TibMol Biol	Roche							x																
VirSNIP SARS-CoV-2 Spike E484K (9429549001)	TibMol Biol	Roche			x	x	x	x	x																
VirSNIP SARS-CoV-2 Spike A570D (9429557001)	TibMol Biol	Roche			x	x	x	x	x																
VirSNIP SARS-CoV-2 Spike K417N (9429565001)	TibMol Biol	Roche	x		x	x	x	x	x																
VirSNIP SARS-CoV-2 Spike V1176F (9431837001)	TibMol Biol	Roche							x																
VirSNIP SARS-CoV-2 Spike L452R (9459022001)	TibMol Biol	Roche							x															x	
LightMix® Kit SARS-CoV-2 E+N UBC (09482407001)	TibMol Biol	Roche																							
Další kity, které detekují mutace neuváděné v tabulce																									
VirSNIP SARS-CoV-2 Spike Y453F (mink) (9405658001)	TibMol Biol	Roche																							
PowerChek SARS-CoV-2 Mutation detection kit ver.1	Kogene	Svebn Biolabs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
PowerChek SARS-CoV-2 Mutation detection kit ver.2	Kogene	Svebn Biolabs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
V435-96FR/RT/B - SARS-CoV-2 British Variant Real-TM	Sacace	Dynex	1																						
V435-96FR/RT/V - SARS-CoV-2 Variant Typing Real-TM	Sacace	Dynex	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
I424-0010 PKamp™ VariantDetector™ SARS-CoV-2 RT-PCR	PerkinElmer	Dynex	1																						
Covidetect™ COVID-19 4-plex Mutation RT-PCR Assay	PentaBase	Dynex			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Covidetect™ COVID-19 Mutation N501Y Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation P681H Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation E184K Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation Del69-70 Simplex	PentaBase	Dynex	1																						
Covidetect™ COVID-19 Mutation Del69-70/N43K Multiplex	PentaBase	Dynex	1																						
Covidetect™ COVID-19 Mutation N501Y/P681H Multiplex	PentaBase	Dynex	1																						
Covidetect™ COVID-19 Mutation N43K Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation A222V Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation K417N Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation L452R Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation S477N Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation Q677H Simplex	PentaBase	Dynex																							
mutace, které nejsou v tabulce:																									
Covidetect™ COVID-19 Mutation A701V Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation L18F Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation DelY144 Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation Del242-244 Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation Y1453F Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation H655Y Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation F476L Simplex	PentaBase	Dynex																							
Covidetect™ COVID-19 Mutation V553L Simplex	PentaBase	Dynex																							
NovaPrime Plus SARS-CoV-2	Novatec	Covpoint			x	x																			
NovaType ID	Novatec	Covpoint			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
NovaType II SARS-CoV-2	Novatec	Covpoint			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
NovaType III SARS-CoV-2 (launch in June)	Novatec	Covpoint			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
NovaType Select L452R SARS-CoV-2	Novatec	Covpoint																							
NovaType Select (launch in July): basic kit with free selectable Primer Probes for the mutations. Mutations here are indicated are the first ideas to start the development. Not fixed yet.	Novatec	Covpoint			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
gb SARS-CoV-2 Variant SAV BR, UK	GeneBiotech																								
Allplex™ SARS-CoV-2 Variants II Assay	Seegene	X																							
Allplex™ TM SARS-CoV-2 Variants II Assay	Kogene Biotech	Medista																x	x	x	x	x	x	x	
DbDirect™ COVID-19 Multiplex RT-PCR Kit	Diana Biotechnologies																								
DbDirect™ COVID-19 Multiplex RT-PCR Kit + primer additive S6484K	Diana Biotechnologies																								
ElisGen™ COVID19 UKV/SAV RT	Elizabeth Pharmacogen		x																						
GemProof SARS-CoV-2 Variants Escape PCR Kit	GemProof		x?	x?																					
GemProof SARS-CoV-2 South African/UK Typing PCR Kit	GemProof		x?	x?																					
COVID-19 Variant Catcher	Cliniti		x																						
PhoenixDx™ SARS-CoV-2 Mutant Screen (N501Y)	PhoenixDx																								
PhoenixDx™ SARS-CoV-2 Mutant Screen (K417N)	PhoenixDx																								
PhoenixDx™ SARS-CoV-2 Mutant Screen (E484K)	PhoenixDx																								
DIOS-RT-qPCR SCR	Institute of Applied Biotechnologies																x?	x?							
SARS-CoV-2/UK/SA Variant Triplex	id solutions		x																						
SARS-CoV-2 / N501Y / E484K Quadruplex kit	id solutions																x	x							
ViroQ SC2 Variant	BAG Diagnostics																x	x	x	x	x	x	x	x	
coban™ SARS-CoV-2 Variant Set 1 Test	Roche		x														x	x	x	x	x	x	x	x	
RIDA GENE SARS-CoV-2 Lineage	r-biopharm																		x	x					
COVID-19 Mutation N501Y Simplex	PentaBase																x	x	x	x	x	x	x	x	
COVID-19 Mutation E484K Simplex	PentaBase																								

Detekovaná mutace varianty (červeně pozitivní detekce, černě neprokázána přítomnost mutace)	Suspektní varianta* červeně—VOC (alertní varianta) černě—VOI (varianta zájmu) černě—VUM (sledovaná varianta)
N501Y , K417N, L452R, E484K, E484Q, P681R	Suspektní alfa (B.1.1.7)
N501Y , E484K, K417N, L452R, E484Q, P681R	Suspektní alfa (B.1.1.7) + E484K Suspektní gama P.1 Suspektní theta P.3 Suspektní B.1.621
N501Y , E484K, K417N, L452R, E484Q, P681R	Suspektní beta (B.1.351)
N501Y , L452R, K417N, E484K, E484Q, P681R	Suspektní alfa (B.1.1.7) + L452R Suspektní A.27
E484K, K417N, L452R, E484Q, N501Y, P681R	Suspektní zeta P.2 Suspektní iota B.1.526 Suspektní B.1.525 Suspektní B.1.620 Suspektní A.28 Suspektní AT.1 Suspektní B.1.1.318 Suspektní AV.1
L452R, K417N, E484K, E484Q, N501Y, P681R	C.16 C.36 + L452R C.36.3 C.37 B.1.526.1
L452R, P681R, K417N, E484K, E484Q, N501Y, P681R	Suspektní delta B.617.2
L452R, E484Q, P681R, K417N, E484K, N501Y, P681R	Suspektní kapa B.1.617.1 Suspektní B.1.617.3 Suspektní B.1.617

Pozn. Není-li uvedeno řecké písmeno, nebylo ještě přiřazeno WHO

* VOC—variant of concern, VOI—variant of interest, VUM—variant under monitoring (dle ECDC)

Tabulka 1: Aktuální přehled variant a mutací zájmu

NLČP klíče a kódy pravidelně AKTUALIZOVÁNY uveřejněny např. zde: <https://dastacr.cz/SARS-CoV-2.html>

VOC – variant of concern

1

VII = variant under investigation

1

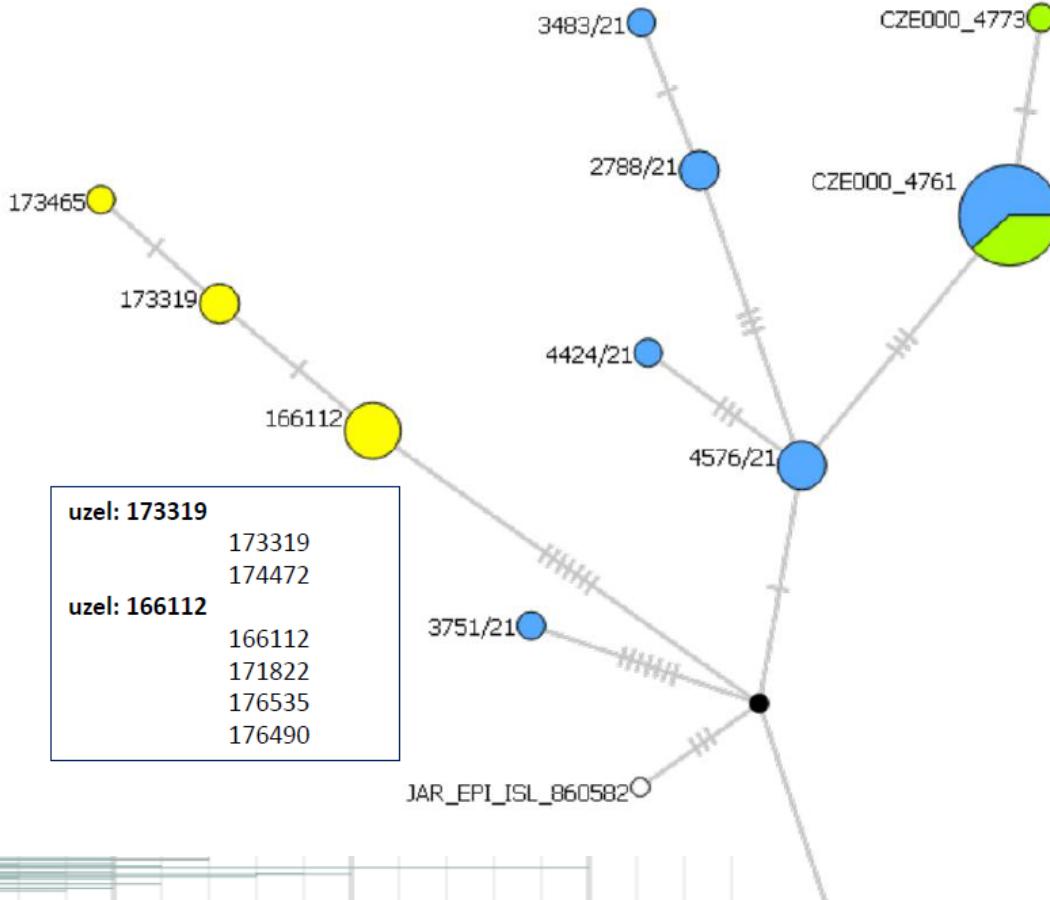
VOI variant of interest (CDC/WHO terminology)

1

VUM = variant under monitoring (ECDC terminologie)

B.1.351 - ČR Example of the tracing

Vzorky 3751, 2788, 3483 představují návaznost na import ze Zanzibaru. Vzorky detekované v Olomouci (žlutě označené) naznačují jedno ohnisko ze společného zdroje, který se liší od ostatních detekovaných variant, kde lze rovněž usuzovat do „společný“ zdroj infekce.



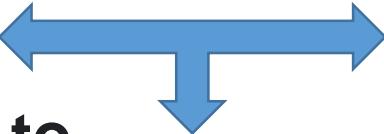
uzel: CZE000_4761		
CZE000_4761	Ústí n L	
CZE000_4762	Ústí n L	
CZE000_4764	Liberec	
CZE000_4770	Liberec	
CZE000_4774	Liberec	
4421/21	Liberec	
4765-21	Liberec	
4767-21	Liberec	
4771/21	Liberec	
4772/21	Liberec	
4775/21	Liberec	
4776/21	Liberec	
4777-21	Liberec	
2788/21	Hradec Králové	
3244/21	Brno	
3483/21	Hradec Králové	
4224/21	Brno	
3246/21	nezařazeno	Praha
(ECDC)		Mělník
3751/21		
(pitva)		
4589/21	nezařazeno	Praha
Uzel: 4576/21	4576/21	Praha
	4692/21	Praha
	4744/21	Liberec
	4773/21	Liberec

Other NRL activities:

- Local EHK (SARS-CoV-2, respiratory panel : influenza A/B, RSV, ADV)
- Webinars for the labs and epidemiologist
- Variant virus strain isolation – distribution of:
 - B.1.258
 - B.1.1.318
 - P.1.1.248
 - B.1.351
 - B.1.1.7
 - B.1.617.1
 - B.1.617.2
 - P.1
- Standards for PCR – to labs nad local manufacturers
- Standards for VNT
- Standards for the research

Summary - PCR or WGS?

- PCR
not to identify a variant, but to
find a hot spot mutation



- WGS – PANGO LIneages
- Variants (VOC- VOI - VUI – VUM)

- Import
- Outbreaks
- Atypical PCR
- Vaccination failure
- Hospitalisation <50 YOA
- Atypical clinical signs

10% / 4 500 per months
Representative - pool



Namasté

