

HERA – ECDC visit 09-02-2022

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Objective 1

Delayed, but resolved – fully automatisation of most of the procedures (except of quantification, no integration possible)

Specific Objective ID	[2.2.1] Specific Objective Title and Description	
1	Automation of preparation of sequencing libraries	
Process Indicator(s)		Target value
Publish call for tender – 2 pipetting NGS robotic machines (conditional tender)	1	😊
Publish call for tender - 1 archiving system (conditional tender)	1	
Acquisitions of the 2 pipetting NGS robotic machines	2	
Acquisitions and installation of the archiving system	1	
Connection of Omics Genomic laboratory to the NGS SARS-CoV-2 reporting system (ISIN).	1	
Set up and verification of the standard operation procedure (SOP) of NGS library preparation and archiving system.	2	
Operator admission and staff training	2	
Output Indicator(s)		Target value
D1.1: Two installed pipetting automats, validated protocols and standard operation procedure (SOP) for NGS library preparation (Delivery in month 5)	1	
D1.2: Archiving system (AS) is verified and connected to LIS data, the records/samples are accessible and AS enables easy storage and retrieval of archived samples. (Delivery in month 8)	1	
Outcome/Impact Indicator(s)		Target value
Reaching the weekly sequencing capacity		376
SARS-CoV-2 sequences stored in GISAID (expected 5% failure)		2 800
Archives of SARS-CoV-2 samples, viral strains, standards, primers		15 000

D.1.1

1. FoS CU – manual /automated sequencing capacity -100 samples per 2 weeks
 - [Biomek 4000 Automated Liquid Handler with integrated PCR cycler Applied Biosystem](#)
 - installed, validation of the process finished
 - NEB protocol v 3, without quantification and normalisation
2. NIPH NRL – manual sequencing capacity 50 samples per week
 - [Biomek i5 4000 Automated Liquid Handler with integrated PCR cycler Analytics Jena](#)
 - [10-03-2022 instalation](#)
 - [PCR cycler will by delivered in the end of Match](#)
 - [Integration in manufacturer site - succesfull](#)
 - [NGS protocol setup preparedaccording to pipetting scheme](#)
 - [CleanPlex® SARS-CoV-2 Panel - Paragon Genomics](#)
 - [Mass Array protocol - setup prepared \(obj 4\)](#)

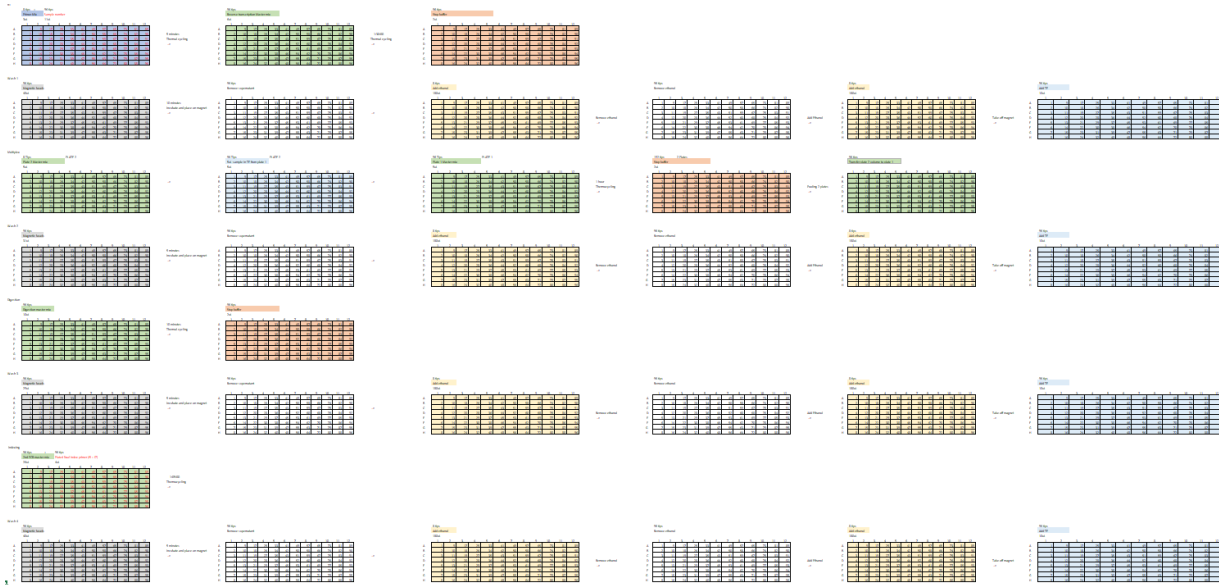
D.1.2

3. Archive:

- [Structure prepared based on: Open Access](#)
- [Hardware on site](#)
- [Architecture prepared](#)



Pipetting scheme – NGS: Clean Plex protocol (Paragon Genomics)



Primary primer mix
Thermocycler steps
Washing – reuse tips
MGB – reuse tips
Stop of reaction
Add TE – reuse tips

Manual procedure NGS: 2 x 8 to 10 hours + Illumina (20 hours) – 3 days, last day in week - Wednesday

Another rented NGS machine (non HERA): Genexus: 32 samples/25 hours – in validation phase - fully automated procedure

Archive – structure of DB D.1.2

- Primary db – clinical material - connected to LIS
- Secondary db – RNA/DNA
- Secondary db - Isolated viral strains
 - dependent on primary db recent strains
 - Independent on primary db and LIS: old archive of strains (from 1957 - 2021) – independent on primary db
- Secondary db - Virtual db of strains/clinical material/RNA/DNA transferred to external institution
- Terciary db - optionally conected to LIS
 - Standards- viral strains: inhouse/outsorced
 - Standards – cell lines
 - Standards – RNA/DNA/oligo
- Terciary db – db in house primers/probes

Objective 2

Specific Objective ID	[2.2.2] Specific Objective Title and Description
2	Capacities for sequence data storage and management
Process Indicator(s)	Target value
Publish call for a tender	1 ☺
Purchase of the server (50TB NAS with a raid-6 array)	1 ☺
Data and services transferred from the transitional storage at ELIXIR-CZ/CESNET	5 000 genomes
Output Indicator(s)	Target value
D2.1: Installed and operating server (Delivery in month 6)	1
Outcome/Impact Indicator(s)	Target value
Stored individual data	7 000 genomes

Biosecurity ☺

IT ☺

Agreement with users – ongoing - acceptance form (under preparation with layer)

Objective 3

Specific Objective ID	[2.2.3] Specific Objective Title and Description	
3	Establishing of bioinformatics pipelines for semi-automatic analysis of WGS data	
Process Indicator(s)		Target value
Preparation and verification of pipeline for NGS data retrieval		1 ☺
Development of algorithms for automatic NGS data analysis		4
Development of algorithms for merging of NGS data and metadata		1
Output Indicator(s)		Target value
D3.1: Scripts for metadata retrieving and data analysis (Delivery in month 8)		8
D3.2: Scripts for NGS data and metadata retrieving and analyses, followed by the merge assembled into workflow (Delivery in month 9)		9
D3.3: Public health data reports summarizing spread and frequency of SARS-CoV-V2 variants and mutations in the Czech Republic for policy making authorities (Delivery in month 12)		25
Outcome/Impact Indicator(s)		Target value
Infrastructure set up for NGS data assembled into the automatic workflow with a clear outcome for the public health authority		1

Objective 3 – Python script

covid-xls2dasta

```
<?xml version="1.0" encoding="UTF-8" ?>
<dasta xmlns:xsd="http://www.w3.org/2001/XMLSchema" xmlns:xi="http://www.w3.org/2001/XMLSchema-instance" xsi:schemaLocation="urn:cz-mzcr:ns:dasta:d4:ids_d4"
  <?ref:is_kod_firmy="STAPRO" is_kod_program="OpenDPO" verze_program="5.35.56" ?>
  <xml:lang="cs" />
  <as type="I">
    <vnitri:ISIM/vnitri>
    </as>
  <as type="P">
    <jmeno=J2IS/vjmeno>
    <adr=Palackého náměstí 24/adr>
    < mesto=Praha 2/mesto>
    </as>
  </as>
  <is icz="" icp="" icl="" icom="">
    <as type="I">
    </as>
  <as type="P">
    <jmeno=Jjmeno>
    <adr=4/adr>
    <dup=4/dup>
    <dup=4/dup>
    <psc=4/psc>
    < mesto=4/mesto>
    </as>
  <?ref:is_kod_firmy="STAPRO" is_kod_program="OpenDPO" verze_program="5.35.56" ?>
  <xml:lang="cs" />
  <is id_base="{ record.personal_number }" xmlns:urn:cz-mzcr:ns:dasta:d4:ids_d4"
    <rodcis={ record.personal_number }/rodcis>
    <jmeno={ record.first_name }/jmeno>
    <prjmeno={ record.last_name }/prjmeno>
    <dat_uzn={ record.birth_date.strftime('%Y-%m-%d') }/dat_uzn>
    <stat_pria={ record.sex }/sex>
    <sex={ record.sex }/sex>
    <dia type="I" xmlns:ds="urn:cz-mzcr:ns:dasta:d4:ids_dasta">
      <ds:jmeno=ds:jmeno>
      <ds:adr=ds:adr>
      <ds:psc=ds:psc>
      <ds:mesto=ds:mesto>
    </dia>
  </is>
</dasta>
```



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Interní ID	Jméno	Příjmení	Datum narození	Rodné číslo	Pohlaví	Číslo žádanky	Datum odběru	Datum vyšetření	Datum výsledku	Pango linie	Accession number (GISND)	Varianta dle číselníku	
2	hCoV-19/CzechRepublic/BC_test_1/2021	John	Doe	3.23.1954	123456/7890	M	123122313	6.16.2021	6.16.2021	7.2.2021	B.1.1.7	EPI_ISL_2989594	VOC2020	
3	hCoV-19/CzechRepublic/BC_test_2/2021	Jane	Doe	4.20.1993	123456/7890	Ž	123122314	6.17.2021	6.17.2021	7.2.2021	B.1.1.7	EPI_ISL_2989595	VOC2021	
4	hCoV-19/CzechRepublic/BC_test_3/2021	Jan	Nezamy	8.20.1986	123456/7890	M	123122315	6.18.2021	6.18.2021	7.2.2021	B.1.1.7	EPI_ISL_2989596	VOC2022	
5	hCoV-19/CzechRepublic/BC_test_4/2021	Jana	Nezama	3.15.1988	123456/7890	M	123122316	6.19.2021	6.19.2021	7.2.2021	B.1.1.7	EPI_ISL_2989597	VOC2023	
6	hCoV-19/CzechRepublic/BC_test_5/2021	Josef	Novak	7.12.2012	123456/7890	Ž	123122317	6.20.2021	6.20.2021	7.2.2021	B.1.1.7	EPI_ISL_2989598	VOC2024	
7	hCoV-19/CzechRepublic/BC_test_6/2021	Josefa	Novakova	2.24.1984	123456/7890	Ž	123122318	6.21.2021	6.21.2021	7.2.2021	B.1.1.7	EPI_ISL_2989599	VOC2025	
8														

<https://github.com/Seraff/xls2dasta>

```
</text>
<ku_z_lab klic_nclp="50805" syst_klic="RNA" komp_klic="SCV2RNVP" typ_cispol="N" typpol_fh="X" stav_vys="A" urg_zprac="R"
  <dat_du typ="I">2021-03-23T15:00:00</dat_du>
  <!--Datum odběru-->
  <nazev_lclp> RNA viru SARS-CoV-2 - varianta „pango“ (RNA, SEQVAR [-] SEQ)</nazev_lclp>
  <!--Název lab položky-->
  <vrx>
    <hodnota_nt>B.1.1.7</hodnota_nt>
    <!-- Hodnota pangolin linie -->
    <pozn>ABCDEF123</pozn>
    <!--Volitelný text odkaz do mezinarodni databaze-->
  </vrx>
```



Objective 4

Specific Objective ID	[2.2.4] Specific Objective Title and Description
4	Enhancing the capacity of a rapid variant detection system by monitoring a large set of mutations by a method involving a combination of RT-PCR and MassARRAY analysis
Process Indicator(s)	Target value
Publish a call for tender: 3 pieces of PCR-MassARRAY system (MALDI TOF technology based) - open system adopted to the detection at least 36 mutations in two end point PCR amplicons	1 
Acquisitions of the 3 PCR-MassARRAY system (MALDI TOF technology based) - open system	3 
Instruments' installation and staff training	3
Adaptation and verification of standard operation procedure for sample analysis	1
Output Indicator(s)	Target value
D4.1: Verified SOP for sample analysis (Delivery in month 5)	1
D4.2: Report on analyzed samples (min 10 000) (Delivery month 11)	1
Outcome/Impact Indicator(s)	Target value
Reaching minimal weekly operational capacity for sample analyses	1 500

Installation – 2 to 5 days
 Training - 2 to 3 days
 Validation - ongoing

NIPH NRL – 1st week in March

ZU OVA - 2nd week in March

ZU UnL - 3rd week in March

Capacity: 380 samples/week
 per machine
 37 AA changes (SNIPs)

Mass Array library 8 – 10 hours

target segment PCR -SAP-extension PCR

Mass Array analysis 3 to 5 hours (depending of the amount of sample)

Run – maximum of 94 samples (C19)



Objective 5 and 6




- Obj 5 - retrospective WGS analysis
- during the drop of covid 19 cases
 - May to August
- Obj 6 – prospective WGS study
 - Ongoing
 - Connected to Czech serological study PREVAL2



Specific Objective ID	[2.2.5] Specific Objective Title and Description
5	Perform a retrospective analysis of samples from critical phases of the SARS-CoV-2 epidemic in the Czech Republic
Process Indicator(s)	Target value
Selection of archived samples for sequencing	4 000
Selection of laboratories involved in the NSSS with capacity to sequence	3
Output Indicator(s)	Target value
D5.1: Report on sequences of samples uploaded in GISAID (included 3 800 samples, expected 5% failure) (Delivery in month 11)	1
D5.2: Report characterizing diversity of SARS-CoV-2 and its development in time during the autumn-spring 2020/21 waves in the Czech Republic (Delivery in month 13)	1
Outcome/Impact Indicator(s)	Target value
Science based evidence of virus evolution under specified circumstances as a base for the future policy making decision and definition of public health strategies.	1

Specific Objective ID	[2.2.6] Specific Objective Title and Description
6	Perform a population survey in 2021/2022 in order to determine the degree of subthreshold spread of the virus in the population and critical elements enabling mitigation.
Process Indicator(s)	Target value
Selection of inclusion criteria for target population	1
Creating a questionnaire	1
Distributing the sample collection kits and questionnaires	100 000
Analysis of returned samples for SARS-CoV-2	Max 20 000
Output Indicator(s)	Target value
D6.1: Report on percentage of positive samples (expected return rate of 20 %) (Delivery in month 5)	1
D6.2: Report on sequences of samples uploaded in GISAID (included 500 samples, expected 5% failure) (Delivery in month 6)	1
D6.3: Report characterizing diversity of SARS-CoV-2 within targeted population the Czech Republic (Delivery in month 12)	1
Outcome/Impact Indicator(s)	Target value
Updated tools for the surveillance of SARS-CoV-2, influenza and respiratory viruses based on self-sampling of targeted population as a base for the future policy making decision and definition of public health strategies.	1

Obj 6 – Preval questionnaire

 **MINISTERSTVO ZDRAVOTNICTVÍ
ČESKÉ REPUBLIKY**

Dotazník ke Studii kolektivní imunity (SARS-CoV-2-CZ-Preval-II)

Jméno a příjmení: _____

Datum odběru: _____

1. Pohlaví Muž Žena 2. Státní příslušnost ČR Jiná, uveďte jaká.....

3. Výška: cm Váha: kg

4. Kouříte?
 NE ANO → kolik cigaret denně kouříte Bývalý kuřák → Uveďte, kolik let jste kouřil(a).....

5. Měl (a) jste pozitivní PCR test na COVID-19?
 ne
 jednou rok: měsíc:
 opakovaně rok: měsíc: (datum posledního pozitivního PCR testu)

6. Jak veliká je vaše domácnost?
 Žiji sám
 Žiji s 1-4 lidmi → bez dětí s dětmi do 18 let → doma školka 1. stupeň ZŠ 2. st. ZŠ a starší
 Žiji s 5 a více lidmi → bez dětí s dětmi do 18 let → doma školka 1. stupeň ZŠ 2. st. ZŠ a starší

7. Měl (a) jste v posledním roce příznaky onemocnění dýchacích cest?
 NE ANO → uveďte jaké (Je možné zaškrtnout více odpovědí.)
 teplota >37,0°C kašel bolest v krku
 obtížné dýchání ztráta chuti nebo žichu Jiné

8. Byl jste očkován proti viru SARS-CoV-2?
 NE ANO → pokud ano, pokračujte s otázkami níže

9. Objevily se u vás nežádoucí stavy po očkování? NE ANO → prosím upřesněte (Je možné zaškrtnout více odpovědí.):


	po 1. dávce	po 2. dávce
• reakce v místě vpichu (bolest, zarudnutí, otok, svědění, zduření apod.)	<input type="checkbox"/>	<input type="checkbox"/>
• celkové příznaky (horečka, zimnice, únava, slabost, pocit horka apod.)	<input type="checkbox"/>	<input type="checkbox"/>
• alergická reakce (kopřivka, ekzém, otok obličeje apod.)	<input type="checkbox"/>	<input type="checkbox"/>
• bolest hlavy, závrat, spavost, mravenčení, křeče apod.	<input type="checkbox"/>	<input type="checkbox"/>
• bolest svalů, kloubů, šlach, zad, ztuhnutí šlje apod.	<input type="checkbox"/>	<input type="checkbox"/>
• nevolnost, zvracení, průjem, plynatost apod.	<input type="checkbox"/>	<input type="checkbox"/>
• dušnost, kašel, zrychlené dýchání, bolest v krku, krvácení z nosu apod.	<input type="checkbox"/>	<input type="checkbox"/>
• Jiné	<input type="checkbox"/>	<input type="checkbox"/>

10. Prosím upřesněte rekonvalescenci po nežádoucím stavu po očkování:

- **po 1. dávce:** bez omezení klidový režim doma do 2 dnů bez lékaře klidový režim doma nad 2 dny bez lékaře návštěva lékaře hospitalizace
- **po 2. dávce:** bez omezení klidový režim doma do 2 dnů bez lékaře klidový režim doma nad 2 dny bez lékaře návštěva lékaře hospitalizace

Děkujeme za vyplnění dotazníku.

1 z 1

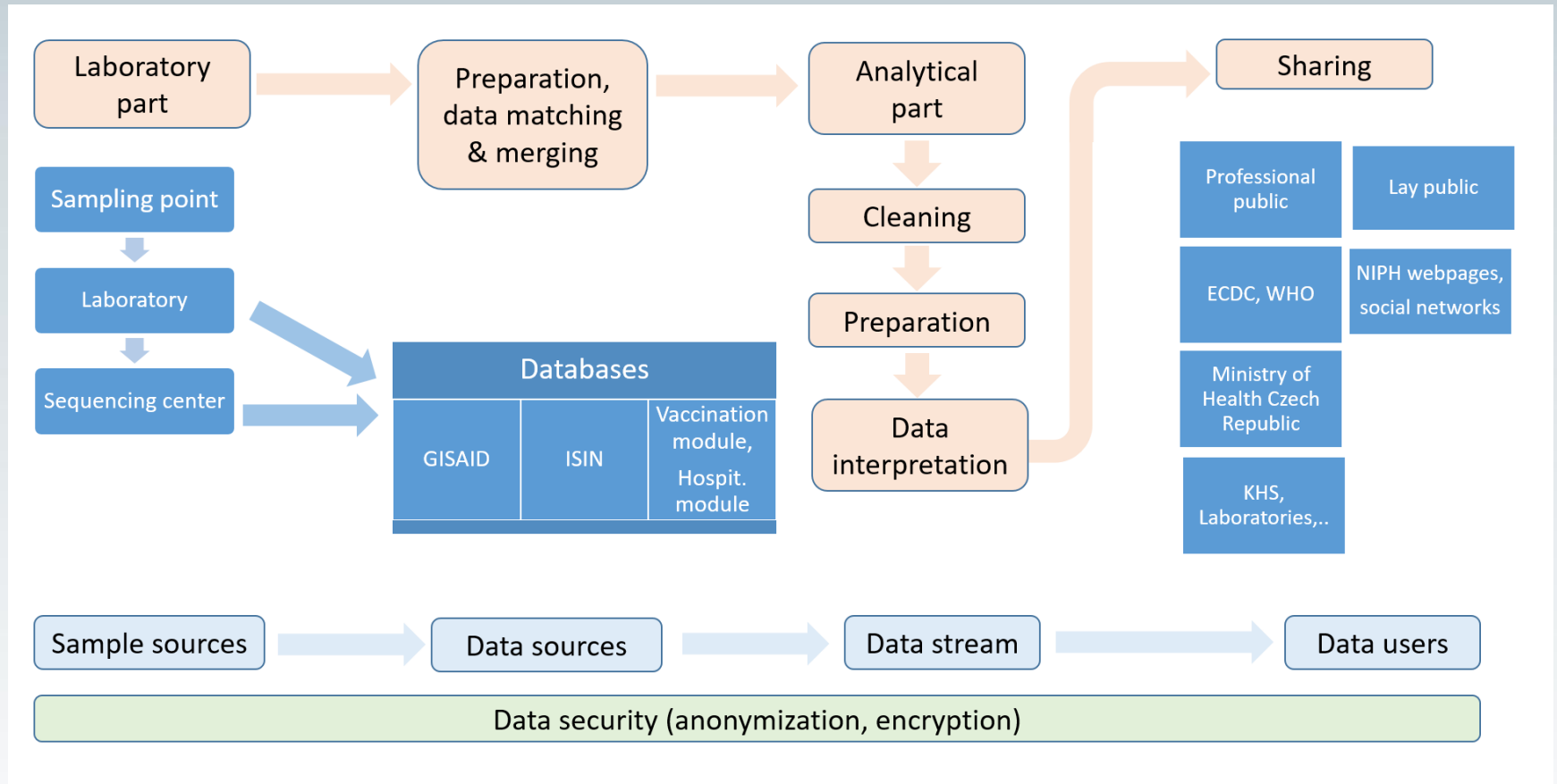


Objective 7

Specific Objective ID	[2.2.8] Specific Objective Title and Description	
7	Develop infrastructure to facilitate the timely reporting of genotype data to ECDC and other supra-national institutions, and the sharing of data in the public domain	
Process Indicator(s)		Target value
Standardizing of procedure for data mining from central public health databases (ISIN) and diagnostic laboratories		1
Setup the workflow to analyze population wide distribution of genotypes in the Czech Republic		1
Implementation of reporting workflow to incorporate virus genotype data from Czech Republic into ECDC and GISAID EpiCoV		1 per week
Output Indicator(s)		Target value
D7.1: SOP for the data mining from public health sector databases (Delivery in month 9)		1
D7.2: Reports published in public domain of ECDC and GISAID EpiCoV including genotype data (Delivery in month 12)		5
Outcome/Impact Indicator(s)		Target value
Enhanced surveillance capability and capacity to contribute to the international database with timely and actual data for future policy making and definition of public health strategies at the EU level.		1

- Data pipeline scheme 😊
- Script for the definition of representative sample – WGS 😊
- Data access to National register of covid data and infectious diseases data – still under negotiation
- Access via export of several metadata sets – very slow

Obj 7 – data pipeline



Objective 8

Specific Objective ID	[2.2.9] Specific Objective Title and Description
8	Increasing the professional capacity of laboratory experts and epidemiologists, deepening interdisciplinary cooperation between institutions with different specializations
Process Indicator(s)	Target value
Organization of the individual consultations and participation at the laboratory meetings	100
Organization of the interdisciplinary webinars for epidemiologist and diagnostic laboratories on good practices in data reporting of SARS-CoV-2, and methodology of their interpretation (target number of participants – 200)	10
Publication of training materials online, via project website	10
Output Indicator(s)	Target value
D8.1: Report on individual consultations, laboratory meetings interdisciplinary webinars (Delivery in month 10)	1
Outcome/Impact Indicator(s)	Target value
Operational interdisciplinary network of laboratory and public health experts across the Czech Republic	1

1x sequencing workshop
– experiences from Czech sequencing centers

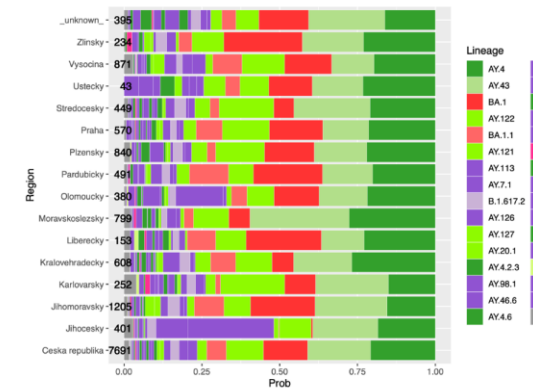
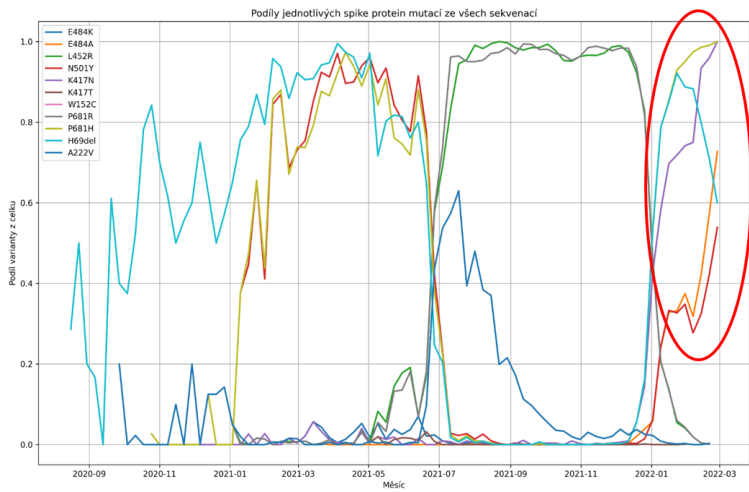
Sequencing meetings – monthly base

One day conference for epidemiologist and laboratories - interdisciplinary

3 lectures for the bioinformatics - beginners in

- NGS data interpretation
- NGS principle
- NGS raw data assembly
- AA structure
- Haplotype analyses

Thank you for your attention



Zdroj: virus.img.cas.cz



Project „Enhancing Whole Genome Sequencing (WGS) and/or Reverse Transcription Polymerase Chain Reaction (RT-PCR) national infrastructures and capacities to respond to the Covid-19 pandemic in the European Union and European Economic Area“ had received funding from the European Centre for Disease Prevention and Control under the Grant Agreement number ECDC/HERA/2021/004 ECD.12218.



More information about the project: <http://www.szu.cz/ecdc-1>

NOTE:

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