Sampling framework for SARS-CoV-2 sequencing in Ireland – version 2.0

Version	Date	Description of changes
2.0	18/01/2024	Version circulated to National SARS-CoV-2 WGS Programme Steering Committee. Signed off with agreed minor edits

Next Review by 18/01/2025

Key Summary

- As the SARS-CoV-2 testing policy in Ireland has changed from one of mass SARS-CoV-2 PCR population-based testing to more targeted testing, the National SARS-CoV-2 Whole Genome Sequencing Surveillance Programme (NSWGSSP) has consequently revised the national SARS-CoV-2 sequencing sampling framework
- The revised SARS-CoV-2 sequencing sampling framework will therefore focus on cases with severe disease (hospitalisation, ICU admission) and deaths, outbreaks in health and care settings, sentinel surveillance programmes in the community and acute hospitals and targeted sequencing based on public health risk assessment/clinical requests and virological changes e.g. new variant of concern
- As per ECDC and WHO recommendations, the NSWGSSP will focus on quality sequencing, rather than quantity and will also work towards improving the representativeness of samples selected
- The NSWGSSP is also collaborating with the national wastewater surveillance programme – which will also provide additional sequencing information on SARS-CoV-2 variants in the community

Background of NSWGSSP

In the wake of the emergence of the Alpha variant in December 2020 and its significant impact on the Irish healthcare system, it became clear that a national strategy was needed to scale up sequencing efforts, to ensure timely monitoring of new and emerging variants and to facilitate "genomic epidemiology", which combines genomic "fingerprinting" of the virus with track and trace epidemiology, to understand how and where transmission is occurring. In response to this, the National Public Health Emergency Team (NPHET) requested that a group of experts develop a proposal to establish a National SARS-CoV-2 Whole Genome Sequencing Surveillance Programme. The proposal, which was subsequently agreed by NPHET, recommended that the Programme be led by HPSC, with NVRL as the lead laboratory partner, with logistic support from, and under the oversight of HSE laboratory operations.

The Programme framework that was developed and implemented is a "hub-and-spoke" model, with NVRL as the "hub (lead) laboratory" and seven acute hospital laboratories as "spoke laboratories" (one from each of the HSE Health Regions[1], plus a paediatric hospital, Appendix). The spoke laboratories are in Beaumont Hospital (HSE Dublin and North East), St James's Hospital (SJH) (HSE Dublin and Midlands), St Vincent's University Hospital (SVUH) (HSE Dublin and South East), Cork University Hospital (CUH) (HSE South West), University Hospital Limerick (UHL) (HSE Mid West), Galway University Hospital (GUH) (HSE West and North West) and Children's Health Ireland, Crumlin (CHI). HPSC, as the national health protection surveillance centre, is the lead organisation and surveillance partner for the Programme.

The primary aim of the Programme is to track the molecular epidemiology of SARS-CoV-2 in Ireland to inform and enhance the public health response to COVID-19. Associated programme goals include contributing to the global knowledge base on SARS-CoV-2 WGS to inform the global public health response. For more details on the Programme please visit the webpage here.

[1] https://healthservice.hse.ie/staff/news/staff-news-listing-page/hse-health-regions-will-commence-on-1-march-2024/

Changes to SARS-CoV-2 Testing Policy in 2023 and impact on NSWGSSP

The Public Health advice for COVID-19 was updated on 30th March 2023 to state that SARS-CoV-2 testing is no longer recommended for the vast majority of people. In line with this advice, community testing centres in Ireland closed as of 30th March 2023.[2]

SARS-CoV-2 PCR testing of symptomatic cases will continue to be used in hospital settings and in General Practice for patients at risk of severe disease, for the purpose of diagnosing and deciding on the provision of treatment. Respiratory virus PCR testing will also be used for sentinel surveillance programmes – sentinel GP Acute Respiratory Infection (ARI) and Severe Acute Respiratory Infection (SARI) surveillance. Respiratory virus PCR testing is also recommended for the management of ARI outbreaks in healthcare and other settings.[3]

The impact of the change from mass population-based SARS-CoV-2 PCR testing on the NSWGSSP is as follows:

1. There has been a marked decline in the number of positive SARS-CoV-2 samples nationally and therefore also a decline in the samples available to the NSWGSP for sequencing.
2. The majority of PCR testing for SARS-CoV-2 is now occurring in hospitals and is therefore not representative of the entire population.
3. Flash surveys[4] which were a key component of previous versions of the framework are no longer being undertaken.

The reduction in SARS-CoV-2 PCR testing and available samples for sequencing is a challenge which is arising across the EU and globally. Countries are sequencing clinical samples at much lower levels due to re-prioritisation of resources and the huge reduction in the number of specimens available for sequencing due to limited PCR testing.[5,6]

Subsequently, the focus of the NSWGSSP has moved to sequencing cases from sentinel surveillance systems, and to the identification of variants causing severe disease and death, outbreaks in health and care settings, and changes in SARS-CoV-2 characteristics such as transmissibility and outbreak potential.

Routine PCR testing and sequencing of wastewater samples will provide additional information on SARS-CoV-2 variants circulation in the community. The National Wastewater Surveillance Programme (NWSP) collect and analyse samples from 30 wastewater catchment areas on a weekly basis for SARS-CoV-2 viral loads. In October 2023, sequencing of SARS-CoV-2 genomic material in wastewater was added to the NWSP. This currently is undertaken on samples from the Ringsend wastewater catchment area, which is the largest wastewater catchment area in Ireland, servicing a residential population of approximately 1.2 million people. This initiative will facilitate unbiased evaluation of viral lineages circulating in the community and may help to reduce the time to detection of a novel variant or lineage. Although a separate programme, given the cross over of technical expertise across both methods, and the complimentary nature of the intelligence provided by both types of surveillance, the need for close collaboration is recognised.

[2] COVID-19 Community Testing Centres and PCR Self-Referral Portal will close this week - HSE.ie
[3] Public Health Advice for the management of cases and contacts of COVID-19.pdf (hpsc.ie)
[4] Flash surveys are cross-sectional type surveys which consist of testing all positive SARS-CoV-2 PCR samples or a certain proportion of samples from a particular day each week
[5] https://www.who.int/publications/m/item/covid-19-epidemiological-update---24-november-2023
[6] Country overview report: week 44 2023 (europa.eu)

ECDC recommendations for SARS-CoV-2 WGS sampling

As the pandemic has progressed and with the shift away from mass population testing to more targeted testing, SARS-CoV-2 genomic surveillance strategies have also become more focussed. Both the ECDC and WHO[7,8] recommend that genomic sequencing for SARS-CoV-2 surveillance should target risk groups and individuals with moderate or severe symptoms as well as representative sentinel surveillance systems such as surveillance for acute respiratory infections (ARI and SARI). They also stress the timely sharing of sequence data on public platforms such as Global Initiative on Sharing All Influenza Data (GISAID)[9] for global monitoring of variant trends and early detection of emerging variants.

The ECDC have guidance on sequencing volume thresholds needed to detect circulating variants at various levels of prevalence (Table 1, page 5). These thresholds apply under the assumption that reported sequences are from representative surveillance systems and are as follows[7,10]: 1% prevalence (610 sequences); >1% to 2.5% (241–609 sequences); >2.5% to 5% (118–240); >5% to 10% (57–117); >10% to 15% (37–56); >15% to 20% (26–36); >20% to 30% (16–25); >30% (>16 sequences).

Programme Sampling Framework

The NSWGSSP sampling framework will focus on sampling from nationally representative surveillance systems including all laboratory confirmed SARS-CoV-2 hospitalised and ICU cases and deaths processed in spoke laboratories and SARS-CoV-2 positive samples from COVID-19 outbreaks in health and care settings. The sampling framework will also include samples from sentinel surveillance programmes: sentinel GP ARI and SARI[11] surveillance systems.

It is also recommended that the NSWGSSP sampling framework be expanded to include SARS-CoV-2 positive hospital healthcare worker samples identified via the European Vaccine Effectiveness, Burden and Impact Studies (VEBIS) hospital HCW surveillance programme; currently at two hospital sites in Ireland.[12] Samples from the VEBIS programme are currently being PCR tested by the commercial laboratory Enfer and WGS is not currently part of this programme.

In addition, there will be targeted sequencing for Public Health/clinical information. It will include Public Health requests for sequencing of cases as part of outbreak investigations following public health risk assessment, HCAI cases/outbreaks, investigation of epidemiological changes/viral pathology shifts, chronic infection cases, investigation of antiviral resistance and sequencing from risk groups. It may also include sequencing of imported cases and cases who are close contacts of imported cases, although this is currently not taking place.

Note: the suitability of a specimen for sequencing depends on the amount of starting material i.e. viral RNA in the specimen. A proxy for viral RNA quantity is the Real-Time Reverse Transcription PCR (RT-PCR) critical threshold (Ct) value and the general recommendation for suitability of a specimen for WGS is a Ct value ≤25 for the SARS-CoV-2 diagnostic specimen. As a general guide, a technical maximum of 60% of RT-PCR positive SARS-CoV-2 specimens are generally suitable i.e. have a Ct value ≤25 for genomic sequencing.[13]

[7] Operational considerations for respiratory virus surveillance in Europe - July 2022 (europa.eu)
[8] https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance-Guidance-Addendum-2023-1
[9] https://gisaid.org/
[10] https://erviss.org/ SARS-CoV-2, Variant characterisation, Methods section
[11] The SARI surveillance programme is expanding to improve the representativeness of the programme
[12] Vaccine Effectiveness, Burden and Impact Studies (VEBIS) of COVID-19 and Influenza - Epiconcept
[13] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8661879/

Distribution and volume of sequencing at spoke sites versus NVRL

The seven spoke hospital sites cover 40% of the population, based on hospital catchment area[14]. (Appendix). In addition, other non-spoke hospital group sites can refer samples to the Programme spoke laboratories. NVRL has a current maximum capacity to sequence 1,600 SARS-CoV-2 positive clinical samples suitable for WGS per week. The seven spoke sites – St James's Hospital, University Hospital Limerick, St Vincent's University Hospital, University Hospital Galway, Cork University Hospital, Beaumont Hospital and CHI Crumlin Hospital have a maximum capacity to sequence up to 48 SARS-CoV-2 positive clinical samples suitable for WGS per week at each site. Therefore, the **surge sequencing capacity is up to 1900 sequences per week**.

There is flexibility in the division of sequencing. Should there be more cases than are possible to sequence at a spoke site, NVRL can assist with sequencing of overflow cases. Based on the reduction in available samples for sequencing, estimated routine sequencing volume per week and surge sequencing capacity are outlined in Table 1 below. The majority of current sequencing is taking place in spoke sites. Each spoke site is requested to sequence at minimum 24 cases per week.

Table 1. NSWGSSP sequencing volume and capacity, 2023/2024, Ireland and variant detection thresholds as per ECDC 'Operational considerations for respiratory virus surveillance in Europe'

Lab	Min per week	Max per week	Average per week (May 2023-Feb 2024)
NVRL	24	1600	17
UHG	24	48	28
SVUH	24	48	26
Beaumont	24	48	18
SJH	24	48	11
UHL	24	48	11
CUH	24	48	10
CHI Crumlin	24	48	4
Total	**192**	**1936**	**125**
Detection threshold[15]	*2.5% to 5%*	*1%*	*2.5% to 5%*

Repeated sequencing of samples from same case

Within the Programme, in general, each COVID-19 case should only have one specimen sequenced per episode of infection, with the exception of suspected prolonged SARS-CoV-2 RNA detection in immunocompromised or other individuals.

[14] Population coverage based on hospital catchment area sourced from https://finder.healthatlasireland.ie/

[15] Detection threshold indicates the prevalence of a circulating variant that could be detected given the volume of sequencing or genotyping undertaken in the sequencing week period. Higher volumes enable variants circulating at a lower prevalence to be detected. These thresholds are based on the calculations published in 'Operational considerations for respiratory virus surveillance in Europe' and apply under the assumption that reported sequences are from representative surveillance systems. The categories correspond to the following underlying sequencing volumes: =1% prevalence (=610 sequences); >1% to 2.5% (241–609 sequences); >2.5% to 5% (118–240); >5% to 10% (57–117); >10% to 15% (37–56); >15% to 20% (26–36); >20% to 30% (16–25); >30% (>16 sequences).

Sampling Framework

Group to be sequenced	Lab reporting template/CIDR field	Considerations	Number of cases / Proportion to be sequenced per week[16]	Population/Sites under surveillance[12]	Sequencing Laboratory
Sentinel GP Surveillance	'Sentinel GP case'	GP sentinel surveillance network expanding during 2023/2024 to 100 practices	Each practice tests up to 5 ARI patients per week, when ARI cases are presenting. The upper threshold of specimens PCR tested in one week will be 500. Based on 2023/2024 no. of specimens tested, it peaked at 214 in a single week with 24 positive for SARS-CoV-2[17]	Approximately 18% of Irish population	Hub
Sentinel SARI surveillance	'Severe acute respiratory infection surveillance'	Currently operating in one hospital site (SVUH). Plans to expand to 4 hospital sites in total during 2023/2024 – impacted by current HSE recruitment embargo	Likely small numbers. During 2023/2024 (up to week 13 2024) from one hospital site: 369 specimens tested and 44 positive for SARS-CoV-2	With one site – SVUH, 6% of Irish population under surveillance. Coverage will expand with additional sites	Spoke
Hospitalised Cases	'Hospitalised case'[18]	Sample all cases admitted to hospital WITH or DUE to COVID-19 and sequence suitable specimens. Community/healthcare/unknown site of acquisition	All suitable hospital case specimens to be sequenced. Average no. per week April 2023 – March 2024 n=278	40% coverage – all 7 spoke hospitals	Spoke (where capacity is available), overflow to Hub
ICU cases	'ICU admissions due to COVID'	Sample all lab confirmed SARS-CoV-2 ICU cases and sequence suitable specimens. ICU surveillance is limited to those where admission to ICU is DUE to COVID-19	All suitable ICU case specimens to be sequenced. Average no. per week April 2023 – March 2024 n= 2	40% coverage – all 7 spoke hospitals	Spoke, overflow to Hub
Deaths	'Death'	Suitable specimens from all COVID-19 deaths	Currently small numbers nationally. Average per week April 2023 – March 2024 n=15	40% coverage – all 7 spoke hospitals	Hub/Spoke
Hospital outbreaks	'Hospital or HCAI outbreak'	Sequence up to 5 suitable SARS-CoV-2 positive symptomatic case specimens from each hospital outbreak to be sequenced, unless exceptional circumstances	April 2023 – March 2024, 601 hospital outbreaks with an average of 6 cases[19] per outbreak. 13 outbreaks per week average. 76 outbreak cases per week	40% coverage – all 7 spoke hospitals	Spoke
Nursing home outbreaks	'Nursing home outbreak'	Up to 5 ARI samples per ARI outbreak PCR tested, unless exceptional circumstances. All suitable SARS-CoV-2 positive case specimens to be sequenced[20]	April 2023 – March 2024, 717 Nursing Home outbreaks with an average of 10 cases per outbreak. 15 outbreaks per week average. 154 outbreak cases per week	All sites included	Hub/Spoke

[16] Number sequenced based on theoretical maximum number of positive specimens found as suitable for sequencing based on maximum threshold Ct value of 25

[17] Exact numbers dependent on sample availability, capacity of the GP Sentinel Surveillance network and SARS-CoV-2 incidence in the community. Higher numbers are only likely during peak respiratory season. Ideally, all eligible sentinel samples should be referred for WGS where widespread community testing is unavailable

[18] Previous option 'Hospitalised case w/ community acquired infection' to be removed

[19] As per interim measure for cases applied in Weekly COVID-19 Outbreak Report i.e. higher number among number ill, aggregate and outbreak linked confirmed case numbers

[20] Algorithm for testing for ARI in RCF.pdf (hpsc.ie)

Group to be sequenced	Lab reporting template/CIDR field	Considerations	Number of cases / Proportion to be sequenced per week[16]	Population/Sites under surveillance[12]	Sequencing Laboratory
Other outbreak settings	'Outbreak in residential / other healthcare setting' 'Other outbreak'	Following Public Health request	Likely small numbers	Coverage to be determined	Hub/Spoke
Public Health request following public health risk assessment e.g. Severe illness, high mortality, unexpectedly high attack rate	e.g. 'Outbreak with severe illness, high mortality or high attack rate'	Consider prioritising samples requested following a public health risk assessment	Likely small numbers	As requested	Hub/Spoke
Investigation of epidemiological changes/viral pathology shifts	'Investigation of epidemiological or virus changes'	Points to consider: • Changes in epidemiological patterns of disease • Changes in behaviour of SARS-CoV-2 e.g. high attack rates in certain clinical contexts, changes in diagnostic performance etc.	Likely small numbers	As requested	Hub/Spoke[21]
Healthcare worker	'Healthcare worker'	Recommended source: HCW VEBIS study at two hospital sites Spoke sites if HCW identified	Likely small numbers	TBD	To be determined for VEBIS Spoke
Chronic infection	'Risk group - Immunocompromised patient with prolonged viraemia'	Cases with prolonged detection of SARS-CoV-2	Very small numbers	As requested	Spoke
Antiviral Resistance	'Antiviral resistance'	Likely to be a hub responsibility	N/A currently	As requested	Hub
New Variant of Concern	'Other'	Surge capacity/escalation when new VOC arises	TBD	Surge capacity	Hub/Spoke
A proportion of imported cases (Hx of overseas travel) + cases who acquire their infection from an imported case	'Travel related outbreak or case'	Currently paused – can be stood up as required	No more than 50 suitable case specimens per week (however capacity up to 150 with alignment of resources)	Paused	Hub. Surge WGS capacity in private laboratories
Wastewater (WW) surveillance programme[22]	n/a	As part of WW surveillance programme	Currently amplicon sequencing underway from samples in Ringsend site	1.2 million population coverage at Ringsend site	UCD/NVRL

[21] Should a pattern of concern be observed, all sites will be alerted as soon as possible, to investigate if the pattern is observed elsewhere and if coordination across sites is required

[22] SARS-CoV-2 wastewater sequencing is based on amplicon sequencing of an internal fragment of the SARS-CoV-2 spike protein gene rather than the whole SARS-CoV-2 genome

Appendix

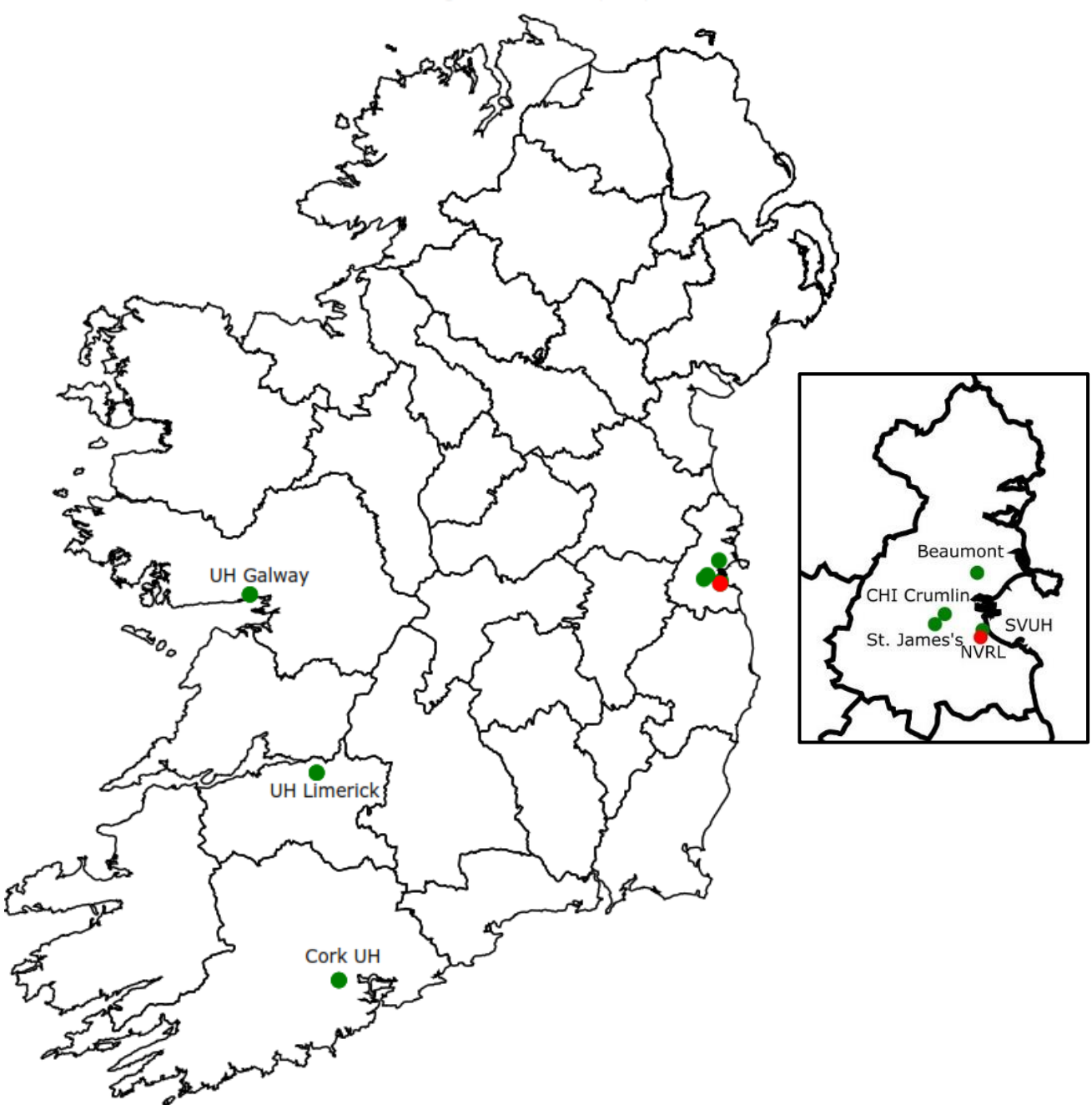

Figure A1: Map of SARS-CoV-2 WGS Programme laboratories (n=8)