GENOMIC SURVEILLANCE OF RESPIRATORY VIRUSES

DECEMBER 2023

RECOMMENDATIONS

- The main objective of the genomic surveillance of respiratory viruses in Belgium is to monitor trends of circulating variants.
- Monitoring trends of circulating variants can be achieved through the existing sentinel surveillance networks (GPs and hospitals).
- Sequencing of environmental samples (such as wastewater) should be developed to complement genomic surveillance via the sentinel networks.
- In addition, a targeted genomic surveillance for respiratory viruses should be performed in specific situations (unusual outbreaks, severe unusual clinical presentations, immunocompromised patients, cases suggestive of zoonotic transmission) to assess the characteristics of new strains.
- The genomic surveillance should be enhanced when needed, by targeting additional relevant population groups or settings.
- The genomic surveillance of respiratory viruses should include Influenza, SARS-CoV-2 and RSV.
- All genomic data available should be promptly shared via existing international platforms such as GISAID.
- The RAG reiterates the importance of (multiplex) PCR testing in hospital settings as a first essential step of the surveillance of respiratory infections.

CONTEXT

Genomic surveillance of SARS-CoV-2 in Belgium has been carried out, since 2021, by a nationwide SARS-CoV-2 Sequencing Consortium gathering 17 laboratories. This coordinated sequencing strategy was set-up by the NRC Respiratory Pathogens UZ/KULeuven in collaboration with Sciensano. The objective of the Consortium was to (i) monitor circulating variants of SARS-CoV-2 in Belgium (baseline genomic surveillance), as well as (ii) detect emerging variants and analyze variant characteristics (targeted genomic surveillance).

The knowledge of circulating variants and variant dynamics provided understanding of the spatio-temporal spread of the virus, the impact of emerging mutations on treatment and vaccine efficacy (1,2).

The epidemiological surveillance of COVID-19 has evolved in the past year and currently mostly relies on sentinel systems in primary and secondary care, which cover acute viral respiratory infections in general, not only COVID-19. These sentinel systems include sample analyses for a subset of cases. The

wastewater-based surveillance has also become an important data source for monitoring virus circulation, especially when the number of clinical samples is low.

In this context, Sciensano developed a proposal for microbiological surveillance of Influenza, RSV and SARS-CoV-2 through the sentinel network of general practitioners and hospitals, as well as from the waste water for the period 2024-2026 *(Integrated Surveillance Note 2024-2026).*

As the SARS-CoV-2 Sequencing Consortium activities are foreseen to end by December 2023, the RAG discussed the genomic surveillance of SARS-CoV-2 and other respiratory viruses beyond 2023, in the context of an integrated analysis of the main respiratory infections:

(i) What should a genomic surveillance program for respiratory pathogens cover? (baseline and/or targeted genomic surveillance)
(ii) Is there a need to maintain a sequencing capacity for upscaling?
(iii) How can the objectives of the genomic surveillance be achieved?

Note that this advice focuses on the scientific aspects of a genomic surveillance for respiratory viruses in Belgium, but does not address questions such as financing of the surveillance, operationalization of the strategy or organization of the laboratories performing sequencing.

BACKGROUND INFORMATION

European and international recommendations for genomic surveillance

The ECDC stressed the importance to monitor changes and characteristics of circulating and emerging respiratory viruses, particularly virological changes of influenza viruses, SARS-CoV-2, and other respiratory viruses to inform treatment, drug, and vaccine development (3).

In the “Operational considerations for respiratory virus surveillance in Europe” from July 2022 the ECDC highlighted the importance of establishing sentinel surveillance systems in primary and secondary care for acute respiratory infections, as those systems provide (i) robust epidemiological data routinely collected using common syndromic case definitions with reliable denominators and (ii) integral microbiological testing that can be extended to multiple viruses (3,4).

In terms of sample analyses, the ECDC suggested that, where possible, **all specimens from primary and secondary care sentinel surveillance testing positive** for influenza viruses or SARS-CoV-2 should be sequenced. In addition, a carefully selected sample (balanced across age groups, geography, and clinical spectrum, including primary and secondary care settings) of influenza virus- and SARS-CoV-2-positive specimens from **non- sentinel** and registry-based systems could also be sequenced to achieve desired sequencing volumes. Sequencing samples from sentinel and non-sentinel samples serve the purpose of **monitoring SARS-CoV-2 variant circulation**.
However, specimens testing positive for influenza viruses and SARS-CoV-2 from **specific population groups and settings** (targeted surveillance) should also be sequenced, for the purpose of **detecting signals of emergence of novel virus variants** with potentially changed characteristics, and as a minimum, a subset should be sent to the SARS-CoV-2 reference laboratories, and/or WHO reference laboratories for further characterization.

Upscaling of testing for Influenza viruses and SARS-CoV-2 should be foreseen if needed, in response to the emergence of a new SARS-CoV-2 variant of concern or Influenza variant.

The ECDC also mentioned that wastewater surveillance may also be considered for monitoring genomic trends for SARS-CoV-2, but detailed guidelines are not yet available.

In the *Global genomic surveillance strategy for pathogens with pandemic and epidemic potential, 2022–2032,* WHO provided a unifying vision for using genomics as a powerful addition to address public health needs for pandemic and epidemic preparedness and response, independent of the pathogen (5).

Evolution of the surveillance of COVID-19 in the last year

The epidemiological and virological COVID-19 surveillance in Belgium, as in the EU, has decreased significantly in the past year, with the revised testing strategy leading to less samples available for sequencing (Figure 1 and 2) (6,7). The monitoring of COVID-19 through counts of positive cases, hospitalizations and deaths has progressively been replaced by sentinel systems in primary and secondary care covering respiratory infections in general (Figure 3 and 4), as well as by surveillance of the wastewater (Figure 5).

Figure 1: Number of COVID-19 tests performed in Belgium over time since 21/11/2022 (source (7)*)*

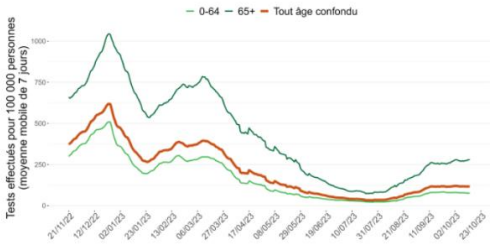


Figure 2: Number of COVID-19 cases reported in Belgium since 21/11/2022 (source (7)*)*

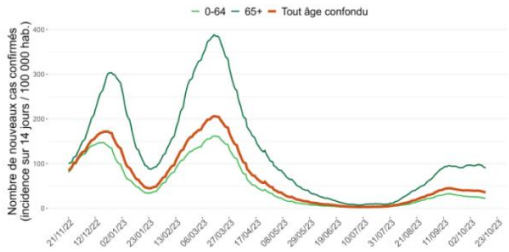


Figure 3: Sentinel surveillance of respiratory infections in primary care: number of consultations of GP practices for ILI symptoms, by respiratory season (source (7)*)*

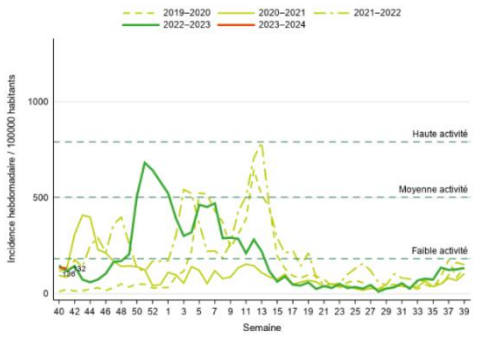


Figure 4: Sentinel surveillance of respiratory infections in secondary care: number of hospitalizations for SARI, by respiratory season (source (7)*)*

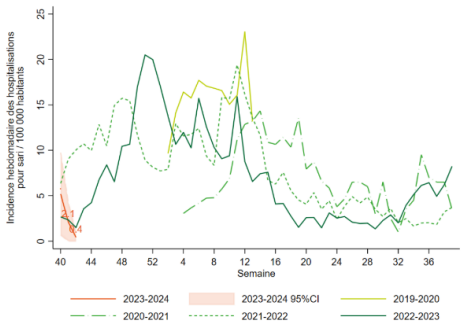
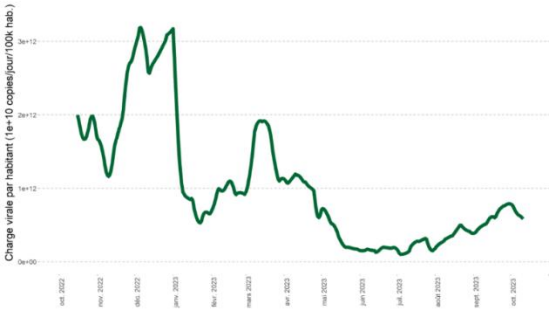


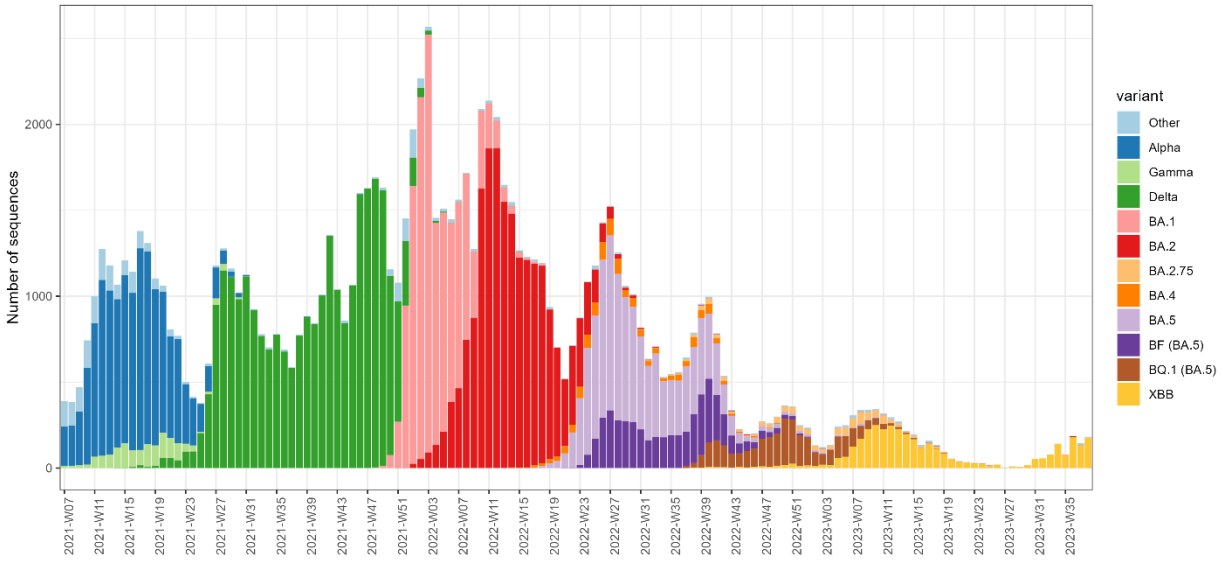
Figure 5: SARS-CoV-2 viral loads in waste water, aggregated at national level (source (7)*)*



The Belgian SARS-CoV-2 Sequencing Consortium

At the beginning of 2021, a nationwide SARS-CoV-2 Sequencing Consortium was established in Belgium to substantially increase the country's genomic sequencing efforts for SARS-CoV-2 (both in terms of intensity and representativeness), to perform quality control among participating laboratories, and to enable coordination and collaboration of research projects and publications. The aim was to sequence 5% of all COVID-19 cases diagnosed in Belgium (1). From January 2021 to October 2023, the SARS-CoV-2 Sequencing Consortium submitted 175 824 SARS-CoV-2 sequences to the GISAID platform achieving on average a coverage of 4,2 % of the COVID-19 positive cases. Figure 6 shows the number of samples sequenced by the SARS-CoV-2 Sequencing Consortium and the variants detected overtime in the baseline genomic surveillance.

Figure 6: Circulating variants and number of samples sequenced by the SARS-CoV-2 Sequencing Consortium in the baseline genomic surveillance (source: HealthData.be)



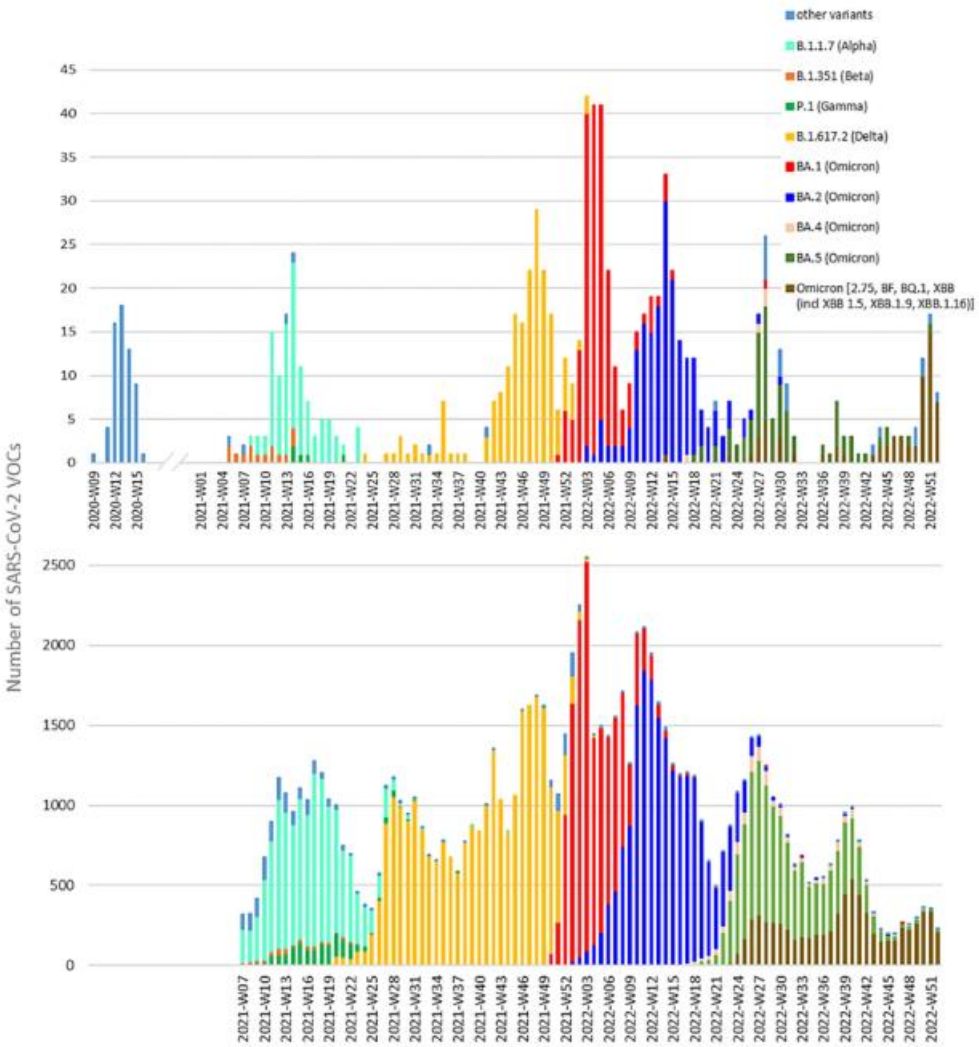
Sequencing of samples from the sentinel surveillances

The **sentinel network of general practitioners** (GPs) exists in Belgium since 1979, it covers 108 GP practices including 182 GPs. Every week, the GPs of the network record the number of consultations for Influenza-Like-Illness (ILI) and acute respiratory tract infections. They also take respiratory samples from five patients per week for analysis (8). All Influenza positive samples are subtyped using qPCR. Sequencing is performed at three specific time points (beginning, middle and end of the respiratory season). All samples positive for SARS-CoV-2 and eligible for sequencing (Ct<25) are sequenced.

The **sentinel network of hospitals** exists in Belgium since 2012. It has recently been expanded from 6 to 10 hospitals and now covers 19 % of the Belgian population. Hospitals from the network collect respiratory samples and clinical information from patients presenting severe acute respiratory infections (SARI). Influenza positive samples are sequenced at three specific time points (beginning, middle and end of the respiratory season), whereas all samples testing positive for SARS-CoV-2 and eligible for sequencing (Ct<25) are sequenced. In a recent publication, Denayer et al analyzed the performance of a SARS-CoV-2 genomic surveillance based on the Belgian sentinel hospital network during the pandemic. They evaluated the ability of the system to correctly follow major trends of variant circulation, the ability to promptly detect major introduction events, and the ability to detect minority variant populations. They

showed that the SARI surveillance network is an appropriate and possibly cost-effective tool to monitor the circulation of SARS-CoV-2 variants or variants of other respiratory pathogens (Figure 7) (2).

Figure 7: SARS-CoV-2 variant dynamics during the COVID-19 pandemic registered by the SARI surveillance (upper graph) and the national baseline genomic surveillance (lower graph) (Source (2)*)*



In addition, a **sentinel network of nursing homes** has been set up in 2021 to monitor the start, the duration and the intensity of the ILI season among nursing home residents. The network includes 44 nursing homes. During the respiratory infection season, two nasopharyngeal samples per week and per nursing home are collected. Samples are subtyped using qPCR but sequencing is currently not performed.

Sequencing of samples from the wastewater surveillance

Starting in January 2024, the wastewater-based surveillance will cover 37% of the Belgian population by analysing samples from 30 wastewater treatment plants once per week. Brussels airport will be monitored by the wastewater-based surveillance as it may be an important entry point of COVID-19 variants. The circulation of COVID-19 virus will be monitored through SARS-CoV-2 viral loads estimated according to the established protocol (9). The monitoring of RSV and Influenza will be integrated. Genomic wastewater-based surveillance will inform on the genomic viral diversity circulating in the general population, without enrichment according to health-seeking behaviour.

Sequencing for SARS-CoV-2 will be performed at specific time points, namely at surges and at peaks of COVID-19 infection outbreaks. Sequencing of other respiratory viruses is not yet in place.

DISCUSSION POINTS

Objectives and purposes of a genomic surveillance in general

- The objectives of genomic surveillance can be twofold: (i) following trends of circulating variants which can be obtained through a sentinel genomic surveillance and (ii) detecting low-abundant emerging variants, which is carried out by specific targeted genomic surveillance programs.
- These objectives can serve different purposes: (a) epidemiological surveillance at national level, (b) outbreak management, (c) clinical/patient management, (d) policy support, (e) contribution to international surveillance efforts.

Sentinel and targeted genomic surveillance in the Belgian context

- In the current Belgian context, a genomic surveillance for respiratory viruses is essential to follow trends of circulating variants.
- The knowledge on the trends of circulating variants provides significant and sufficient insights for planning vaccination campaigns or estimating the efficacy of current treatments.
- During the COVID-19 pandemic, measures depending on variant characteristics (such as infectivity and severity of the virus) were developed (see RAG advice 8/02/2022). Nevertheless, mitigation measures implemented by the authorities have never been variant-specific.
- Sequencing samples from specific populations or settings (targeted genomic surveillance) would have an added value in specific situations to assess characteristics of new strains in terms of transmissibility, severity or immune escape. Specific populations or settings include outbreaks with unusual number of secondary cases, immunocompromised patients, cases with severe or unusual clinical presentation, cases suggestive of zoonotic transmission.
- Genomic surveillance of respiratory viruses is also carried out at the European level by the ECDC through participation of Member States (3,9). The collaboration to an EU-level surveillance and to international platforms such as GISAID provides a broader perspective of the Belgian data. Conversely, Belgium can also benefit from information shared by EU Member States on these platforms. It is therefore essential that all genomic data generated is promptly shared on these platforms.
- A genomic surveillance for respiratory viruses should include Influenza, SARS-CoV-2 and RSV, as vaccination programs are now implemented for these three viruses.
- The genomic surveillance data will feed the qualitative analysis of the epidemiological situation of respiratory infections performed through the Respi-Radar.

Surveillance systems available in Belgium (see also table 1 below)

- While the sequencing strategy for SARS-CoV-2, as developed by the Sequencing Consortium, was dependent on the COVID-19 testing strategy, this is not the case for sequencing via the sentinel networks. In the current situation, where testing of symptomatic patients for COVID-19 (or other respiratory pathogens) is not recommended and where the amount of samples available for sequencing has decreased; the added value of the existing sequencing strategy can be questioned.
- The genomic surveillance via the sentinel network of hospitals (SARI patients) showed timely detection of SARS-CoV-2 variants of concern, as compared to the baseline genomic surveillance carried out by the Sequencing Consortium (2), but this analysis was done retrospectively. The current SARI sentinel surveillance system shows some delays, thus the timeliness of the genomic

surveillance through this network is uncertain. Nevertheless, it enables to meet the main objective of the genomic surveillance, i.e. following trends of circulating variants. Of note, delays in the SARI surveillance systems are expected to be reduced in the coming weeks given the implementation of an electronic reporting system.

- In a surveillance through the SARI sentinel network, emerging variants might be detected with a delay compared to community circulation, and variants causing severe disease might be overrepresented. The surveillance based on samples from SARI patients should therefore be complemented by samples from the sentinel network of GPs, which covers mild diseases. And ideally, a third component covering the asymptomatic and minimally symptomatic infections at the community level, should be added (Figure 8).
- The performance of a genomic surveillance via the sentinel network of GPs is currently unknown as the surveillance system has been heavily disrupted during the COVID-19 pandemic; a retrospective analysis of samples and comparison with the results of the Sequencing Consortium has therefore not yet been possible. Better understanding the complementarity between these two sentinel surveillance networks is necessary.
- The number of samples collected via the sentinel network of GPs is currently limited due the fact that written informed consent is needed.
- Sequencing of samples from the environment such as wastewater could provide complementary results to the SARI and ILI sentinel surveillances. Even though wastewater samples sequencing shows limitations, it would allow for monitoring community transmission, independently of the health-seeking behavior of (symptomatic) people. Another potential component of environmental surveillance could be via sampling of indoor air, which would allow a more targeted sampling (per age group or in specific sectors). This strategy will be further developed during the following months.
- Surveillance of patient and environmental samples should not be limited to SARS-CoV-2, but extended to (at least) Influenza and RSV. However, additional developments are needed in order to allow sequencing and analysis of Influenza and RSV (as performed for SARS-CoV-2) from wastewater samples.

Enhancing genomic surveillance efforts

- A capacity for upscaling testing for respiratory pathogens should be maintained if required, as recommended by the ECDC. However, merely increasing the number of samples to sequence from the sentinel surveillances will not provide additional information.
- Genomic surveillance efforts could be enhanced when needed by diversifying the groups or settings in the targeted genomic surveillance approach, in order to allow for early detection of emerging variants with different characteristics. For instance, depending on the situation, samples from travelers coming from a specific country could be sequenced, samples from specific population groups (young or school-age children, nursing home residents) etc…

Additional RAG recommendations

While the importance of following variant circulation through a genomic surveillance is not questioned, the RAG stresses once more that diagnostics for severe respiratory infections are crucial. The RAG has previously made recommendations regarding the use of multiplex testing for respiratory viruses (see advice 19/08/2020, 14/12/2020 and 02/05/2023): multiplex PCR tests, which simultaneously detect multiple respiratory pathogens, are useful in patients with severe respiratory symptoms and provide information for surveillance purposes. These recommendations remain valid, and clear guidance and indications for reimbursement are still needed.

Table 1: Overview of surveillance systems available - strengths and limitations regarding genomic surveillance

Surveillance system	Strengths	Limitations
SARS-CoV-2 Sequencing Consortium	- *Baseline genomic surveillance*: detailed and timely overview of circulating variants - *Targeted genomic surveillance*: possible detection of emerging variants - Upscaling possible	- Dependent on testing strategy (currently not representative of general population; insufficient amount of samples if number of positive tests decreases further)
SARI sentinel surveillance	- Detection of circulating variants - Independent of testing strategy	- Currently delays in the surveillance (but problem being addressed) - Bias towards severe infections - No upscaling possible - No detection of new emerging variants
ILI sentinel surveillance	- Provides a picture of circulating variants in the community (thus including mild cases) - Independent of testing strategy	- Sentinel surveillance system disrupted during the pandemic (no comparison yet possible) - Number of samples available for sequencing limited due to legal aspects (patient informed consent) - No detection of new emerging variants
NH sentinel surveillance	- Provides a picture of circulating variants in vulnerable group of the population - Independent of testing strategy	- Not implemented yet - Targeted genomic surveillance
Environmental surveillance	- Provides a picture of circulating variants in the community, - Independent of health seeking behavior - Independent of testing strategy	- Only possible when sufficient viral concentration available in samples which is the case when the virus is circulating already at an important level in the population. - Wastewater sequencing currently limited to SARS-CoV-2

Figure 8: Overview of samples available for the sentinel genomic surveillance (Source KULeuven)

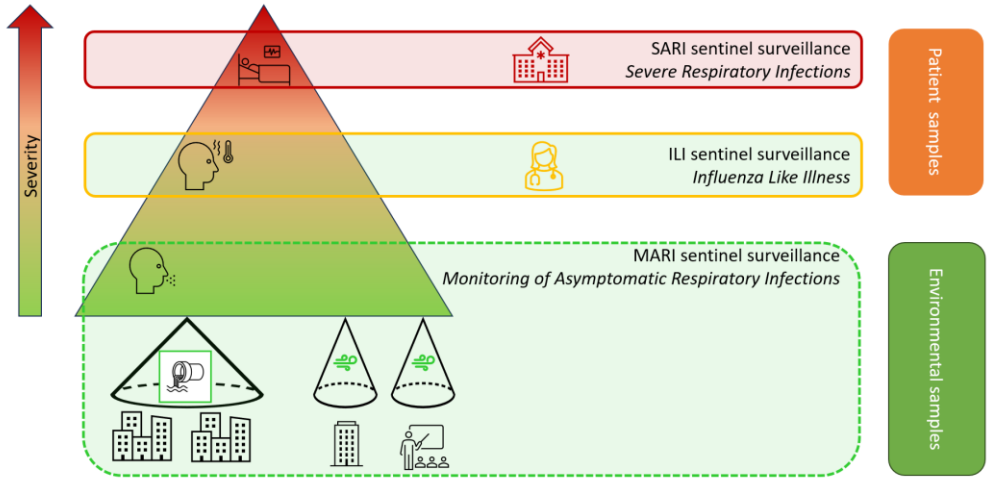
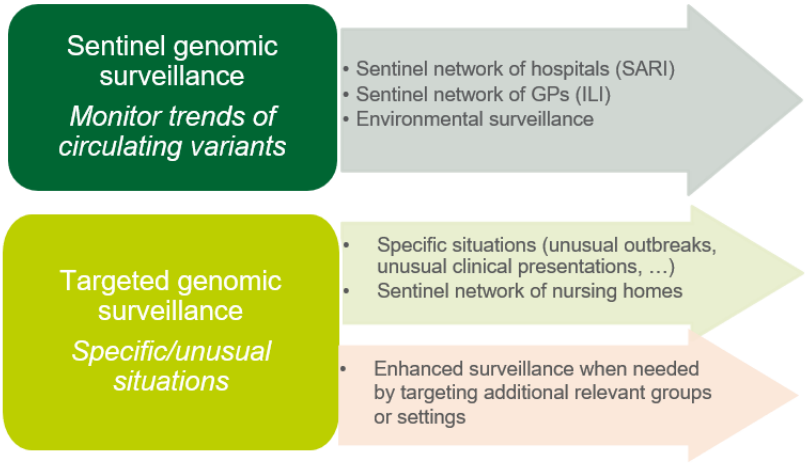


Figure 9 Summary of the proposed genomic surveillance approach



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