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Developing a next level integrated genomic surveillance: Advances in the molecular epidemiology of HIV in Germany

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ABSTRACT

Advances in the molecular epidemiological studies of the Human Immunodeficiency Virus (HIV) at the Robert Koch Institute (RKI) by laboratory and bioinformatic automation should allow the processing of larger numbers of samples and more comprehensive and faster data analysis in order to provide a higher resolution of the current HIV infection situation in near real-time and a better understanding of the dynamic of the German HIV epidemic. The early detection of the emergence and transmission of new HIV variants is important for the adaption of diagnostics and treatment guidelines. Likewise, the molecular epidemiological detection and characterization of spatially limited HIV outbreaks or rapidly growing sub-epidemics is of great importance in order to interrupt the transmission pathways by regionally adapting prevention strategies. These aims are becoming even more important in the context of the SARS-CoV2 pandemic and the Ukrainian refugee movement, which both have effects on the German HIV epidemic that should be monitored to identify starting points for targeted public health measures in a timely manner. To this end, a next level integrated genomic surveillance of HIV is to be established.

1. Introduction

For HIV, UNAIDS - leading the global effort to end AIDS as a public health threat - has announced the "95–95-95 goals", meaning that 95% of people living with HIV (PLWH) know their HIV status, 95% of PLWH and know their HIV status, will receive antiretroviral treatment (ART), and 95% of PLWH receiving ART will have been virally suppressed by the year 2025 (Frescura et al., 2022). These goals should be implemented by as many countries as possible, including Germany, in order to achieve global effects. At the end of 2021, the number of PLWH in Germany was estimated at 90,800 (An der Heiden et al., 2022). Of these, around 8600 are HIV infections which have not yet been diagnosed. Thus, the proportion of diagnosed HIV infections is around 90% - meaning that the first UNAIDS goal has not been achieved in Germany yet. In contrast, the proportion of PLWH on ART is estimated to be 96%, of which around 96% are also virally suppressed. Reasons for not yet meeting the first goal are multifactorial, e.g. vulnerable groups at risk for HIV can be hard to reach for testing or people can be unaware of being at risk for HIV (Del Rio, 2016; Lampejo et al., 2018; Pantke et al., 2022). In addition, the behavior and composition of risk groups in Germany may also be affected by recent global events which requires monitoring.

New HIV diagnoses must be reported directly to the RKI in accordance with the German Infection Protection Act. The RKI received a

Abbreviations: HIV, Human Immunodeficiency Virus; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; HIVdb, HIV database; TDR, transmitted drug resistance; SARS-CoV2, Severe Acute Respiratory Syndrome–related coronavirus 2; PLWH, people living with HIV; ART, antiretroviral treatment; IGS-HIV, Integrated Genomic Surveillance of HIV new diagnoses; InzSurv-HIV, Incidence surveillance of HIV new diagnoses; ECDC, European Center for Disease Prevention and Control; WHO, World Health Organization; PrEP, Pre-Exposure Prophylaxis; UNAIDS, the Joint United Nations Program on HIV/AIDS; NGS, Next Generation Sequencing; WGS, Whole Genome Sequencing; DEMIS, Deutsches Elektronisches Melde- und Informationssystem für den Infektionsschutz/ German electronic reporting and information system for infection protection; AmpliSeq, amplicon sequencing; TSI, time since infection; KOKPIT, Klinische und molekulare Surveillance von HIV/HCV-Koinfektionen sowie HCV-Monoinfektionen vor dem Hintergrund der Paradigmenwechsel in der HCV- und HIV-Therapie / Clinical and molecular surveillance of HIV/HCV co-infections and HCV mono-infections against the background of paradigm shifts in HCV and HIV therapy; RKI, Robert Koch Institute.

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total of 3111 confirmed new HIV diagnoses for 2019 (Marcus et al., 2021). In comparison, 2468 newly confirmed HIV diagnoses were reported for 2020 and 2258 for 2021 during the SARS-CoV2 pandemic (An der Heiden et al., 2022; Marcus et al., 2021, 2023). This corresponds to a decrease of 21% between 2019 and 2020 followed by a further decrease of 9% between 2020 and 2021. For 2022, 3239 new HIV diagnoses have been reported, equating to an increase of 43% from 2021 to 2022 (Marcus et al., 2021, 2023). Among the 3239 new diagnoses in 2022, 724 people originated from Ukraine. These data show that both the SARS-CoV2 pandemic and the Ukraine war have, and will continue to, strongly impact the German HIV epidemic.

The effects of the SARS-CoV2 pandemic on the decline in reported new HIV diagnoses have already been described elsewhere (Marcus et al., 2023). In summary, there are three different contributing factors: 1.) a decline in the number of sexual partners during the lockdowns, 2.) a temporary decline in national and international mobility, and 3.) the temporary decline in testing availability and demand (Marcus et al., 2023). The last point in particular raised the risk that new HIV infections will only be detected late, which could, among other things, lead to spread within risk groups who were tested more frequently before the pandemic. The increase in newly reported HIV diagnoses in 2022 is mainly due to the high proportion of Ukrainian refugees among the newly diagnosed PLWH. The HIV prevalence in Ukraine in 2019 was estimated at 0.9 - 1.0% of the total population, although the prevalence in certain vulnerable groups is likely to be significantly higher (e.g. sex workers: 22.5%, intravenous drug users: 5.2%, men who have sex with men [MSM]: 7.5%) (Koppe et al., 2023; unaids.org, 2019). The HIV epidemics in Ukraine and Germany also differ at the molecular epidemiological level. In Ukraine, subtype A6 has higher prevalence (>80%) (Vasylyeva et al., 2019, 2018), while in Germany subtype B is dominant, with 58.7% majority (2017–2020) (Fiebig et al., 2023).

It can be concluded that it is important to both continuously monitor the German HIV epidemic and to determine the impact of crises on its dynamic at a molecular epidemiological level in order to obtain additional information on virus specifics such as transmitted drug resistance mutations, virus variants, and transmission dynamics from sampled HIV sequences. One of the public health initiatives to measure and evaluate the molecular epidemiological situation in Germany in the context of HIV is a joint action of two studies: Integrated Genomic Surveillance of new HIV diagnoses (IGS-HIV) and Incidence Surveillance of new HIV diagnoses (InzSurv-HIV). Both studies are based the German Infection Protection Act (IfSG) and provide insights in different aspects of the German HIV epidemic. The necessity and description of these studies, combined with outlining ongoing improvements, are included in this article.

2. Conceptualizing integrated genomic surveillance and incidence surveillance of new HIV diagnoses

The **InzSurv-HIV** study has been designed to estimate the proportion of people with recent HIV infections among newly diagnosed PLWH. Because the duration of infection can be several years prior to HIV diagnosis, additional information beyond the notification data is needed to obtain insights on current HIV transmission dynamics. Since 2008, serum or plasma samples are collected from around 45–60% of all newly reported HIV diagnoses. These samples are then serologically tested to differentiate between recently acquired (approx. < 6 months) and long-standing HIV infections (Hauser et al., 2019, 2014). By continuously recording new HIV diagnoses and linking serological data to epidemiological data, vulnerable populations and regions with increased levels of recent transmission can be identified (Hofmann et al., 2017; Pantke et al., 2022). In addition, by continuously investigating recently acquired HIV infections, temporal trends can be observed within different risk groups. In 2022, for example, new diagnoses within MSM continued to decrease, although there was a significant increase in new diagnoses overall.

The **IGS-HIV** study contributes pertinent molecular information for monitoring the German HIV epidemic. Since 2013, HIV sequences are derived from the same sample material as in the InzSurv-HIV study to analyze the current spread of HIV from a molecular point of view. The main study objectives include (I) the continuous analysis of transmitted HIV drug resistance, (II) the monitoring of the circulating HIV variants, (III) the identification and characterization of transmission networks, (IV) and the detection, investigation and assessment of regional and national HIV outbreaks. A schematic overview on the processes can be seen in Fig. 1. In a semi-automated process, the viral RNA is isolated and the genomic regions encoding the HIV protease, reverse transcriptase, integrase and envelope are amplified by RT-PCR. The amplicons are sequenced using Illumina-based Next Generation Sequencing (NGS) (Hauser et al., 2018b). The resulting HIV sequences cover the relevant positions for genotypic determination of drug resistance and tropism and are suitable for determining the HIV subtype.

2.1. Continuous analysis of transmitted HIV drug resistance

Transmitted drug resistance (TDR) can affect the effectiveness of individual antiretroviral drugs and entire drug classes, which may limit the choice of medications for first-line ART of newly diagnosed PLWH. Furthermore, new drug classes or novel drugs in established drug classes for ART or pre-exposure prophylaxis (PrEP) are constantly developed and approved (Paredes et al., 2017). Therefore, it is crucial to monitor newly emerging drug resistance profiles and known TDR. This will ensure that drug resistance algorithms and treatment guidelines remain effective and up-to-date. (Fiebig et al., 2023; Hauser et al., 2017, 2018a). Moreover, the continuous monitoring of the emergence and transmission of drug resistant HIV in Germany is an official task of the Robert Koch Institute and also part of the German “Strategy to contain HIV, hepatitis B and C and other sexually transmitted diseases” from the Federal Ministry of Health (BMG). Identification of drug resistance mutations in the HIV sequence and genotypic prediction of TDR are performed using web tools (e.g. Stanford HIVdb Genotypic Resistance Interpretation Algorithm (Tang et al., 2012), HIV-GRADE algorithm (Obermeier et al., 2012)). TDR prevalence is continuously reported to the European Center for Disease Prevention and Control (ECDC) of the World Health Organization (WHO).

2.2. Monitoring of circulating HIV variants

Regionally differing HIV epidemics internationally are dominated by varying subtypes and recombinant forms. As a result, monitoring HIV diversity provides important information on infection dynamics and the endemic spread of certain viral variants in Germany (Fiebig et al., 2023; Hanke et al., 2019; Hauser et al., 2017, 2018a). Additionally, some HIV subtypes show reduced susceptibility to certain antiretroviral drugs and subtype-specific properties in terms of transmission or pathogenesis in certain populations and risk groups have been reported (Touloumi et al., 2013; Wainberg and Brenner, 2012). The continuous observation of HIV diversity is therefore of great importance for the coordination of diagnostic and treatment guidelines. Web tools (e.g. COMET HIV-1 (Struck et al., 2014), REGA HIV Subtyping Tool v3 (Pineda-Pena et al., 2013), and the Geno2Pheno Virus Detection and Subtyping Tool (https://subtyping.geno2pheno.org/)) are used to determine the HIV subtype or recombinant form from the HIV sequences. If the subtyping of a specific HIV sequence is indistinguishable, maximum likelihood phylogenetic analyses utilizing HIV reference sequences are performed.

2.3. Identification and characterization of HIV transmission networks

The reconstruction of related transmission events leads to a deeper understanding of the HIV epidemic and its dynamics. This helps to derive targeted recommendations for prevention or the evaluation of previously implemented measures. The identified HIV transmission

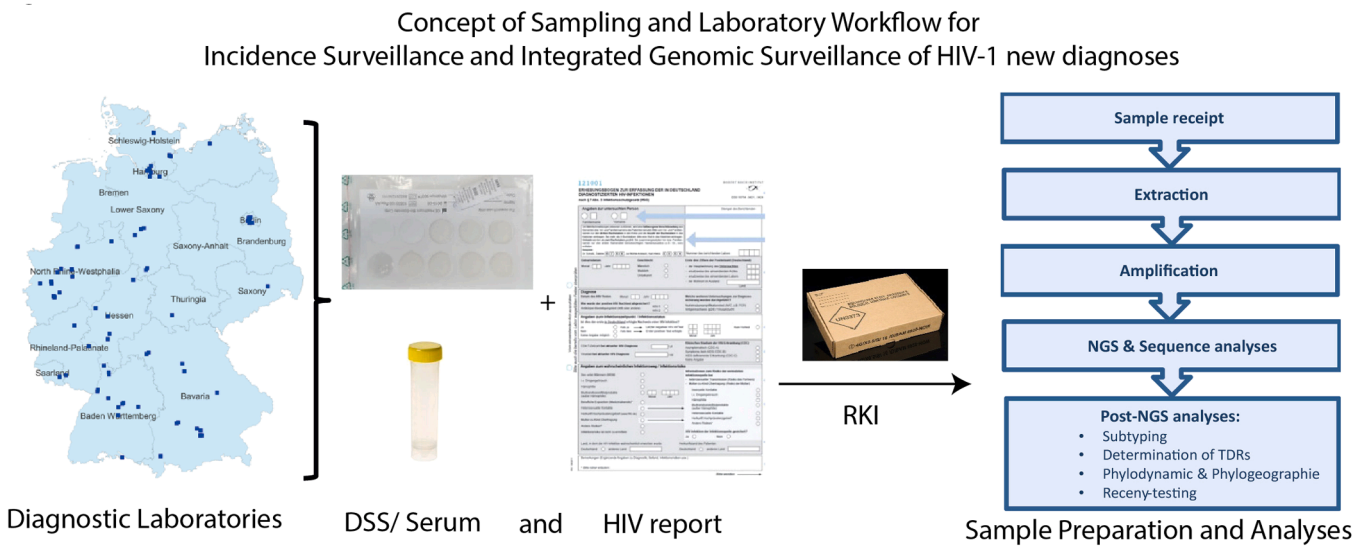

Fig. 1. Current InzSurv-HIV/IGS-HIV workflow for sample collection and processing: Samples (dried serum spots (DSS) or serum) from new HIV diagnoses are sent to the Robert Koch-Institute (RKI) by 70 diagnostic laboratories distributed representatively all over Germany. Mandatory HIV reports containing pseudonymized epidemiological data are sent separately to the RKI. After receiving the sample and viral RNA extraction, amplification of the HIV *pol* and *env* regions and sequencing using Illumina NGS follows for sequence database curation. Sequences are used to analyze subtype, transmitted drug resistances (TDRs), transmission clusters, and to estimate the duration of infection (recency testing).

chains and clusters are postulated based on the genetic distance of the analyzed sequences and the statistical support for the branches (bootstrap and posterior values). The results are displayed as a tree structure. The phylogenetic analyses are carried out using both maximum likelihood and Bayesian statistical methods. The latter, for example, enables the linking of sequence and epidemiological data, aiding in reproduction number (Re) calculation, and ultimately the estimation of the distribution dynamics in defined risk groups. Furthermore, Bayesian analyses can be used to reconstruct the temporal and phylogeographic dynamics of the HIV epidemic (Hanke et al., 2019).

2.4. Detection, investigation and assessment of HIV outbreaks

HIV outbreaks or sub-epidemics are high-frequency transmission events from a particular strain, which therefore appear as unique, very compact clusters in phylogenetic analyses. Once an outbreak has been detected, the responsible public health actors are informed so that public health measures can be initiated. These can include, for example, low-threshold testing and counseling services or interventions such as the provision of condoms or sterile injection materials (Hanke et al., 2020).

3. Achieving the next level of IGS-HIV and InzSurv-HIV - increasing the significance and public health benefit by laboratory and bioinformatic automation

The IGS-HIV study is being further developed in order to continuously assess the German HIV epidemic and to enable an evidence-based design of public health measures in the areas of prevention, diagnostics and therapy. For this purpose, various laboratory and bioinformatic automations and extensions will be implemented gradually (see Fig. 2, marked in green).

3.1. Goal 1: Achieving a near, comprehensive molecular recording of all new HIV diagnoses in Germany

In order to further extend the informative value of the IGS-HIV, the existing sample collection should be supplemented by the direct transmission of existing HIV sequences from the diagnostic laboratories' genotypic drug resistance testing via the digital reporting system in Germany (DEMIS). This would further increase the speed of data analyses by eliminating the need for laboratory processing and would lower laboratory costs. Moreover, the coverage of included sequences would be increased as laboratories beyond the sampling network are also invited to provide their sequences from HIV new diagnoses.

In parallel, the current laboratory process needs to be replaced as it is only partially automated. The labor- and time-consuming processing of the samples allows neither an increase in sample throughput nor an extension to whole HIV genome analysis. Therefore, an automation of the laboratory process has started for the IGS-HIV study, enabling both the complete sequencing and analysis of approximately 3.000–3.500 new HIV diagnoses in Germany each year. Through this process, we aim to achieve consistent and informative sequencing results, leading to a reduction in manual workload and an increase in cost-effectiveness. For this purpose, two methods for amplicon sequencing (AmpliSeq) from Thermo Fisher Scientific and Illumina are compared and benchmarked in terms of accuracy, sensitivity, specificity, cost efficiency and automatability. Furthermore, an approach of viral RNA capturing with Illumina sequencing is evaluated. All methods can be implemented on laboratory robots following the automated extraction of viral RNA and quantification of viral load measurements. The bioinformatic pipeline for automated NGS data analysis is to be adapted accordingly.

3.2. Goal 2: Delivery of data and interpretations to improve the scientific basis of decisions with regard to targeting public health measures

Fast and reliable sample processing protocols are of paramount importance for HIV surveillance, but they will be of little value without a comprehensive data analysis. Reproducible and well-structured bioinformatic workflows will allow for simplified data interpretations, and as a result, quicker decision-making to control the ongoing HIV epidemic. Until 2025, four automated bioinformatic pipelines are to be set up for subsequent analyses with the determined HIV sequences. Resulting from these pipelines, the results on transmitted drug resistance, diversity, transmission clusters and outbreaks as well as recency estimations are to be automatically evaluated and presented to the public via a newly established HIV dashboard.

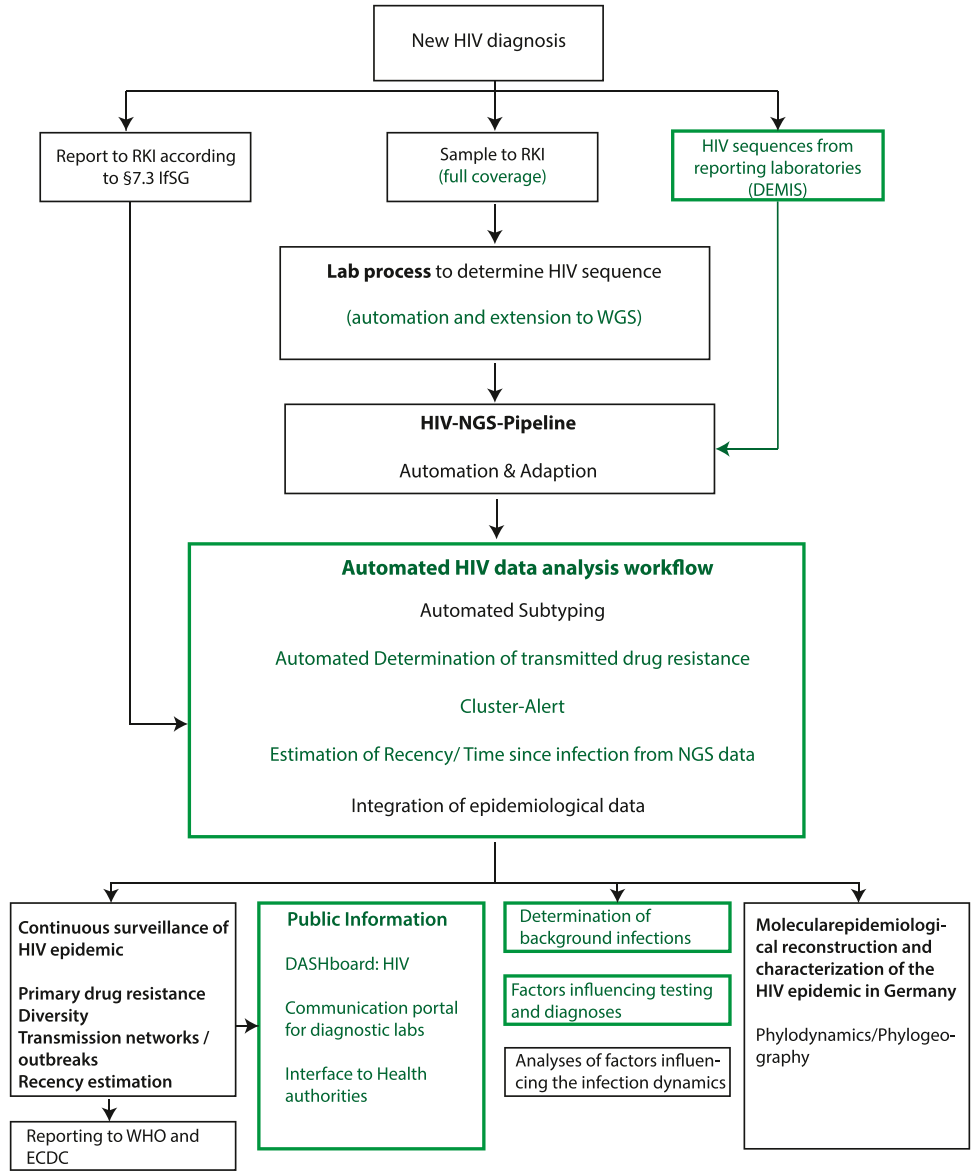

Fig. 2. Schematic overview of the intended further development of InzSurv-HIV/IGS-HIV: Existing structures are displayed in black. Improvements and novel developments that are actively being implemented (including prospective implementations) are displayed in green.

3.2.1. *Automated HIV variant detection*

One of the post-NGS data analysis systems already established in our department is an open-source bioinformatics pipeline developed to automatically report all necessary subtyping information (publication in preparation). The pipeline enables the output comparison from four field-accredited subtyping tools (Stanford HIVdB (Tang et al., 2012), COMET HIV-1 (Struck et al., 2014), REGA HIV Subtyping Tool v3 (Pineda-Pena et al., 2013), and the Geno2Pheno Virus Detection and Subtyping Tool (https://subtyping.geno2pheno.org/), and based on its own decision-making process, classifies samples into two categories: samples with a distinguished subtyping assignment and those which require further investigation. All unclear cases are marked and undergo a subsequent phylogenetic maximum likelihood analysis using IQ-TREE (Nguyen et al., 2015) with the GTR+F+R6 evolutionary model. These subtyping results are not only used to analyze circulating HIV variants, but also to define suitable datasets which are further investigated for the detection of fast-growing transmission clusters. In addition, the determined subtypes are linked to epidemiological data and will be presented on the public dashboard.

3.2.2. *Automated detection of transmitted drug resistance mutations*

In a separate attempt, we are planning to set up a drug resistance mutation reporting pipeline, which automatically characterizes incoming sequences for drug resistance mutations via e.g. the Stanford HIVdb interface (Tang et al., 2012) or HIV-GRADE (Obermeier et al., 2012). This information is linked to epidemiological data, ultimately counting and categorizing noticeable accumulations of TDRs. These results will also be visualized on the public dashboard.

3.2.3. *Rapid and automated detection of transmission clusters and outbreaks*

Spatially limited outbreaks or rapidly growing sub-epidemics can always occur, but often cannot be seen in the national surveillance data due to their relatively small number of cases. These clusters and sub-epidemics are often only noticed at later points during targeted analyses, delaying potential preventive measures which may no longer be applicable. These include, for example, clusters of people who inject drugs (PWID) in which the virus spreads locally over a short period of time (Hanke et al., 2020). The early, automated detection of these fast-growing infection clusters would be insurmountable to initiate

preventive measures, interrupting transmission chains at an early stage. To quickly detect new cases of fast-growing clusters, we are currently instituting a pipeline with an inclusive, presorting mechanism based on subtype and sub-subtype—an internal SQLite Cluster database linking new, incoming data with predefined clusters of HIV and ultrafast automated cluster detection based on the Transmission Cluster Engine (HIV-TRACE, (Kosakovsky Pond et al., 2018)). Subsequently, incoming sequences will be examined for membership of known transmission clusters. Lastly, newly established clusters are identified and reported—both within the pipeline and to the public dashboard.

3.2.4. Estimation of the time since infection (TSI) directly from HIV sequence data

Finally, in addition to the core automation projects, additional developments include a seamless system to estimate TSI for new HIV diagnoses. Our recency estimation pipeline provides an end-to-end TSI solution and entirely relies on the HIV-phyloTSI model (Golubchik et al., 2022). Although preliminary results on HIV full-length sequencing data are quite promising, it is still required to adjust the default model settings to the virus population in Germany. Moreover, we believe that the HIV-phyloTSI model can demonstrate unsurpassed performance if retrained on additional and internal datasets with increased accuracy. Evidently, time since infection is valuable, and as such, it further extends our understanding of the national HIV epidemic.

4. Conclusions and future perspectives

Full automation of laboratory and data analysis processes within the HIV integrated genomic surveillance system will enable IGS-HIV to enhance efficiency, effectiveness, and accuracy of the entire HIV operational workflow and reveal new potentials for its further development and expansion. In addition, it will shorten the time between sampling and final analyses, guiding the viewpoint on the most relevant issues within the epidemic, and avoiding these issues being overlooked or detected with long delay. In addition to the other diverse possibilities achieved by expanding the IGS-HIV, the goal to inform the public, the government, and local health authorities is paramount. Presenting the results on public dashboards will raise the awareness for HIV in expert groups and associations, allowing public health authorities to promptly initiate and adapt prevention strategies for vulnerable population groups.

A yet unaddressed issue is the linking of HIV diagnoses and samples to relevant co-infections. It is known that people with HIV are also commonly diagnosed with other infections, such as Hepatitis C virus (HCV), Hepatitis B virus (HBV), *Treponema pallidum*, *Neisseria gonorrhoeae* or *Mycobacterium tuberculosis*. Testing of HIV samples for blood-borne co-infections such as HCV, HBV and *Treponema pallidum* has been proven in the past to be feasible in the context of molecular-epidemiological studies such as the German HIV-1 seroconverter study and pilot projects such as the KOKPIT study (Hanke et al., 2020; Jansen et al., 2015; Wang et al., 2019). The automation of sequencing processes, for example as part of AmpliSeq or virus RNA capture, could be technically extended to other blood-borne diseases. However, the legal basis must first be examined and determined in terms of data privacy and data protection frameworks.

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CRediT authorship contribution statement

Meixenberger Karolin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. **Heuer Dagmar:** Resources, Validation, Writing – review & editing. **Gunsenheimer-Bartmeyer Barbara:** Funding acquisition, Project administration. **Koppe Uwe:** Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Rykalina Vera:** Formal analysis, Methodology, Software, Writing – original draft. **Hanke Kirsten:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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