# Practical Issues in Implementing Whole-Genome-Sequencing in Routine Diagnostic Microbiology

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Review

# Practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology

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ABSTRACT

*Background:* Next generation sequencing (NGS) is increasingly being used in clinical microbiology. Like every new technology adopted in microbiology, the integration of NGS into clinical and routine workflows must be carefully managed.
*Aim:* To review the practical aspects of implementing bacterial whole genome sequencing (WGS) in routine diagnostic laboratories.
*Sources:* Review of the literature and expert opinion.
*Content:* In this review, we discuss when and how to integrate whole genome sequencing (WGS) in the routine workflow of the clinical laboratory. In addition, as the microbiology laboratories have to adhere to various national and international regulations and criteria for their accreditation, we deliberate on quality control issues for using WGS in microbiology, including the importance of proficiency testing. Furthermore, the current and future place of this technology in the diagnostic hierarchy of microbiology is described as well as the necessity of maintaining backwards compatibility with already established methods. Finally, we speculate on the question of whether WGS can entirely replace routine microbiology in the future and the tension between the fact that most sequencers are designed to process multiple samples in parallel whereas for optimal diagnosis a one-by-one processing of the samples is preferred. Special reference is made to the cost and turnaround time of WGS in diagnostic laboratories.
*Implications:* Further development is required to improve the workflow for WGS, in particular to shorten the turnaround time, reduce costs, and streamline downstream data analyses. Only when these processes reach maturity will reliance on WGS for routine patient management and infection control management become feasible, enabling the transformation of clinical microbiology into a genome-based and personalized diagnostic field. **J.W.A. Rossen, Clin Microbiol Infect 2018;24:355**


## When and how to integrate NGS in the routine workflow?

The role of next generation sequencing (NGS) as a powerful tool in medical microbiology is increasingly being demonstrated [1]. Whole genome sequencing (WGS) can be used in the routine laboratory workflow should typing of pathogens by a method having the highest possible discriminatory power be required, for example during hospital outbreaks [2]. Apart from this, WGS of bacterial genomes may be used to reveal the presence of antimicrobial resistance (AMR) genes, or genes associated with virulence and pathogenicity as well as to discover new genetic mechanisms for the three previously defined important clinical features of a bacterium [3,4]. In addition, NGS allows for culture-free identification of a range of pathogens in complex polymicrobial samples, including clinical, environmental, and food samples in an unbiased fashion, also allowing the identification of fastidious and

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difficult-to-culture taxa [5,6]. This latter unbiased approach, also known as whole genome metagenomics, is beyond the scope of this review, which will focus on the application of WGS only.

The implementation of WGS in routine diagnostics will require several adaptations in the laboratory workflow. To ensure successful implementation, both parts of the process, including the 'wet' laboratory part (extraction, library preparation, sequencing), performed by the so called 'wet-lab' microbiology personnel as well as the 'dry' bioinformatics part performed by personnel who have been trained to use commercial or open source software tools, should be performed by dedicated staff members specialized in WGS. Therefore, integrating interventional genomics in clinical microbiology settings requires that development of NGS technology will go hand-in-hand with human resource development, for example by building capacity through educational workshops such as those organized by ESCMID (Fig. 1).

Currently, the use of WGS fits best in a batch-wise approach for analysing samples. While this approach is typical for academic or surveillance projects, it is far from ideal for routine diagnostics, which would benefit from a case-by-case approach as there is a clear need for generating speedy diagnostic results for individual cases. In addition, a balance should be kept between costs, quality, speed and complexity of the 'wet' and 'dry' processes. Costs decrease if more samples are tested in parallel, but this may negatively influence the quality of the sequencing results. Generating longer reads may facilitate the downstream analysis of genomes, but could result in longer running times and higher costs. Future developments, for example the introduction of platforms such as MinION, within the laboratory workflow may overcome such limitations, as such sequencers generate long reads in relatively short runtimes of just a few hours, compared with platforms such as Illumina sequencers. Other lower throughput sequencers may be entering the market in the near future. Altogether, such platforms may be used in a 'per demand' way, thereby increasing the influence of WGS technology in both patient and outbreak management, provided that sufficient sequencing quality is achieved (i.e. coverage, base error rates, and other quality scores).

Whereas individual patients may benefit from a low-throughput high complexity analytical approach, allowing extraction and inference of all clinically relevant information from the genome (such as virulence attributes or resistance determinants), battling hospital outbreaks, for example with a multidrug-resistant bacterium, may require a high-throughput low complexity approach that will mainly enable rapid phylogenomic analysis. Bacterial species consist of multiple comparable strains, each containing their own unique DNA signatures. Collation of all these different genomic 'types' (SNP variants or allelic profiles) obtained with WGS in a database, preferentially shared between different laboratories, could allow rapid identification of unique sequences of DNA in an (new) outbreak strain, in addition to genomic typing, which is reviewed elsewhere in this thematic series. Subsequently, knowledge of such markers can be used to design a tailor-made diagnostic or surveillance PCR specific for the outbreak strain. This can substantially reduce the timeframe of patient screening from several days down to several hours. Clearly, such time gain is important for the treatment of infected patients, but also for prevention of new infections [2].

## The place of NGS in the diagnostic hierarchy of microbiology

Another aspect of NGS being a disruptive technology relates to its potential impact on the diagnostic hierarchy in clinical microbiology (as in other laboratory medicine fields). Traditionally, frontline laboratories performed standard identification and antimicrobial susceptibility testing (ID/AST) for bacterial isolates, combined with a varying level of molecular detection/quantitation of pathogens in clinical samples and occasionally typing using methods such as pulsed-field gel electrophoresis (PFGE) or multilocus sequence typing (MLST). Isolates would then have been referred to a more specialized laboratory, mainly a regional or national reference laboratory, based on need (e.g. confirmatory testing) or for surveillance purposes, with traditional reference functions including confirmation of ID and AST using reference methods, advanced characterization (e.g. detection of virulence attributes) and surveillance/molecular epidemiology [7].

In many countries, WGS first found its way into microbiology in academic and/or reference laboratories, because of the need for capital investment, operational costs, and requirements for expertise in the 'wet' and 'dry' lab components. Depending on the nature of the health system, development of WGS capabilities at reference laboratory level may reflect a structure that favours a centralized microbiological surveillance and reference functions, that builds on the traditional concept of diagnostic hierarchy [8]. An example could be the characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) at the frontline using methods such as *spa* typing and detection of specific genes such as the pantone-valentine leukocidin (PVL), later complemented at the reference laboratory by WGS to establish the entire resistome and virulome of MRSA as well as high-resolution typing. However, the reduced costs of sequencing, especially bench-top and low-to-medium throughput devices, the growing availability of commercial or user-friendly bioinformatics tools and accumulating experience in pioneering clinical settings have all led to a wide and rapid introduction of NGS technology to frontline laboratories, facilitating a

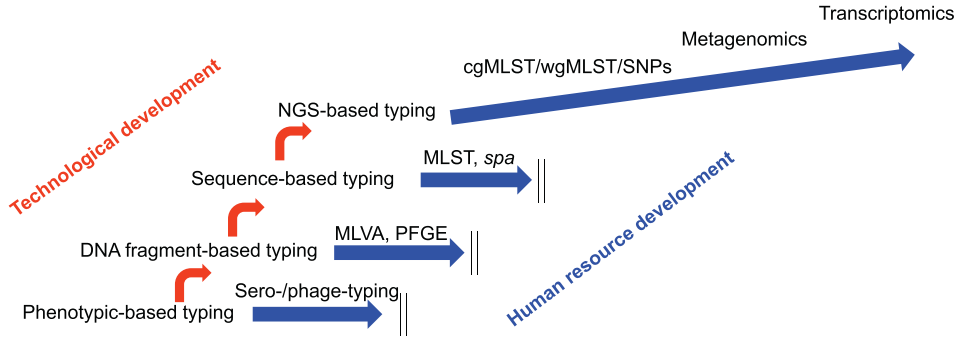


**Fig. 1.** Capacity building for interventional genomics in clinical microbiology. Integrating interventional genomics in clinical microbiology settings requires development of NGS technology going hand in hand with human resource development, for example by building capacity through educational workshops such as those organized by the ESCMID Study Group for Genomic and Molecular Diagnostics. MLVA, multi-locus variable number tandem repeat analysis; PFGE, pulse field gel electrophoresis; MLST, multi-locus sequence typing; NGS, next generation sequencing.

transition from a hierarchical to a network-like structure [1]. The increased use of NGS in clinical pathology and human genetics is another catalyst for this process [9,10].

The performance of WGS at the frontline may significantly reduce turnaround time, empower hospital-based microbiology, and positively impact local efforts such as infection control interventions [11]. On the other hand, this shift in weight towards routine WGS in a decentralized system, also carries risks, such as hampering national surveillance capabilities because of scattering of data and disrupting reference functions because of reduced referral of isolates to the reference centres. Ideally, health systems should devise a model for WGS integration [12] that will assure appropriate balance is maintained between meeting the needs of active clinical services and those of national surveillance efforts, taking into account factors such as geography or infrastructure. Different operational solutions with varying distribution of the 'weight' between frontline and reference laboratories could be contemplated. These may include: (1) maximizing WGS use and data analysis at the frontline while maintaining mandatory (and possibly automated) submission of isolates/sequences to a national repository; (2) performance of sequencing at the frontline but using centralized bioinformatics services for surveillance; or (3) having consolidated national sequencing and bioinformatics facilities. Maximizing WGS use at the frontline in a decentralized fashion facilitates the rapid development and implementation of new technologies but the optimal solution may differ between countries and settings.

## Quality control issues for using NGS in microbiology

Nowadays, diagnostic laboratories have to adhere to various national and international regulations and criteria for their accreditation. For example, ISO 15189:2012 specifies requirements for quality and competence in medical laboratories. The validation of WGS is far from being straightforward. WGS often is superior to other methods currently used within the laboratory; for example, the index of discrimination for WGS-based phylotyping is far higher than current methods. Also, WGS can generate data that otherwise can only be obtained by combining different separate technologies (e.g. taxonomical or resistance and virulence data). Nevertheless, guidelines on validation of NGS in oncology have been published and may serve as a model for the microbiological field [9]. One should also consider that both the wet- and dry-lab parts should be validated. As WGS can be used for all microbial species, it is nearly impossible to perform an extended validation for all known species. Therefore, one may consider choosing several indicator species for the validation of the WGS workflow.

Concerning the wet-lab part, several criteria can be used to assess the quality of the sequence itself. To control the entire procedure, one can consider spiking the samples with an internal control, such as the PhiX Control - a ready-to-use control library for Illumina sequencing runs. Furthermore, identification of a single marker, such as a housekeeping gene can be used as an internal control for the quality of the obtained WGS data [13].

For the validation, the repeatability and reproducibility of both the wet- and dry-lab parts of the analyses can be determined by performing both the complete procedure of sequencing several strains and the subsequent downstream analyses several times by different technicians on different occasions. The precision and accuracy can be determined by comparing generated results with results of already established technologies such as PFGE in the case of typing, and real-time PCR and/or DNA microarrays for identification of individual genes and/or pathogens.

With respect to typing, one should keep in mind that WGS is currently the method with the highest discriminatory power. Therefore, clusters identified with another typing method may be split into different sub-clusters when using WGS. It will only become clear after building extensive experience with the WGS typing method and taking the specific research question and descriptive epidemiological data into account whether such sub-clustering makes sense, as is discussed in another review within this thematic issue. Criteria for typing methods such as those developed by the ESCMID Study Group for Epidemiological Markers (ESGEM) and future guidelines on WGS-based typing interpretation, will play an important role in guiding these processes.

In addition, identification of pathogens may be hampered by incomplete databases being available. Furthermore, previously the taxonomy of bacterial species changed dramatically by the introduction of 16S rRNA gene sequencing. Nowadays, WGS is also used in laboratories to identify and describe new species. By comparing the whole genome sequences of different species with each other, the limited taxonomic resolution of using the sequence of the 16S rRNA gene for certain bacterial genera can be overcome [14]. Common examples include differentiating between species of the *Acinetobacter baumannii* complex or *Shigella* from *Escherichia coli*. Notably, it was proposed by Thompson et al. that with the availability of WGS, descriptions of new taxa should also include a draft genome sequence, with at least 20 X coverage [15]. Thus, validation of WGS in routine microbiology may be hampered by different factors and remains a practical and a regulatory challenge.

## Proficiency testing for WGS in microbiology

Ensuring the quality of WGS may be a bottleneck for the transition from research use of WGS (with occasional *ad hoc* use in urgent situations like outbreak investigations) to routine implementation [16]. External quality assurance (EQA) and proficiency testing (PT) have become a cornerstone in laboratory medicine and have enabled the wide use of molecular diagnostics in diverse settings. A survey performed recently by the Global Microbial Identifier (GMI) initiative has highlighted the great diversity of sequencing and bioinformatics approaches used across institutions and the need for implementing EQA [17]. Performing EQA for WGS may be especially complicated because of the need to control for high-quality DNA extraction, library preparation steps, sequencing reactions (taking into account the increasing availability of different platforms), and the bioinformatics analysis [18]. Thus, a comprehensive EQA programme for WGS should at least involve the submission of query isolates, query DNA, and query sequences [17].

The field of EQA for WGS is rapidly evolving and there are several examples for successful EQA schemes developed in clinical pathology. Pilot PT has been also delivered by GMI, involving strains of high priority organisms such as *S. aureus* and *S. enterica*. While such piloting holds promise with respect to the feasibility of carrying out PT, it has highlighted many technical challenges associated with distributing and analysing PT data which should be addressed [19]. Also encouraging is the successful performance of a ring trial involving the distribution of *S. aureus* strains across five laboratories. The concordance between typing results generated by participating laboratories, for several different typing schemes of the query pathogen (*S. aureus*) is very reassuring with respect to both EQA and implementation of the methods [20]. Similar to molecular diagnostics in clinical microbiology, there should be a wide selection of EQA schemes (be it commercial or not-for-profit) from which laboratories may be able to choose to use schemes that meet their professional and regulatory requirements.

## Maintaining backwards compatibility with WGS

As WGS offers much greater discriminatory power than current reference standard typing tools (e.g. PFGE, MLST, MLVA), it will

inevitably become the new reference standard. However, maintaining backwards (reverse) compatibility between WGS and current methods is extremely important for several reasons. First, the transition to WGS-based typing is gradual, especially on a global scale and it is likely that we will be seeing a mixture of methods used in the short-to-medium term. Maintaining backwards compatibility will help prevent a 'Tower of Babel' effect, that is use of incompatible and non-harmonized typing results and languages by different stakeholders. Second, there are molecular subtypes for which a genotype-to-phenotype correlation has been established (e.g. a type or lineage associated with severe disease or increased transmissibility). Until genomic markers are identified and validated, maintaining backwards compatibility may assist in assessing and communicating risks, especially to stakeholders not proficient in WGS. Notably, backwards compatibility has been shown for important pathogens, for which analysis of the WGS allows not only the calling of SNPs and core-genome MLST types, but also extracting MLST data and single locus typing such as *spa* type as well as lineage-specific markers [13,21,22]. Contrary to that, an example for backwards compatibility challenges is the difficulty in calling the 7-loci sequence-based type in *L. pneumophila* from WGS data. The creation of an *in silico* solution for this required much bioinformatics development work but was considered mandatory to facilitate the introduction of WGS for the global network of Legionnaires' disease surveillance [23].

Finally, there exist large repositories that build on current typing schemes for comparison of strains and for national or international surveillance, and maintaining backwards compatibility will ensure the ability to compare newly sequenced strains to existing and historical strains [24]. That said, reverse compatibility could also prove to be a limiting factor; over-reliance on reverse compatibility may have a negative effect on the wide implementation of WGS-based typing as it requires the investment of resource (re-sequencing historical isolates, modifying pipelines), may halt stakeholders from taking a 'leap' towards WGS, and may even lead to futile attempts to engineer bioinformatics solutions for extracting data from genomes that will soon become obsolete.

## Management of WGS in microbiology

Like every new technology that is being adopted in microbiology, the integration of NGS into clinical and routine workflows should be carefully managed. As a complete transition to WGS-based diagnostics will not be feasible in the foreseeable future for most laboratories, a careful needs assessment is a mandatory first step to consider the optimal implementation of WGS in a given setting. There are myriad factors that should be considered in the process of assessing, procuring, and integrating WGS in a routine setting [11].

Some key factors include: (1) defining the clinical or public health applications for which WGS is considered and the existing evidence concerning the utility and added value of NGS; (2) comparing technologies and platforms to satisfy diagnostic needs and also considering future needs; (3) the identification of (and possibly partnership with) other NGS users in an institution, availability of funding and consideration of operational models and related cost modelling (e.g. in house sequencing vs. internal outsourcing vs. external outsourcing); (4) manpower considerations, especially with respect to bioinformatics expertise; (5) quality control/quality assurance considerations and compliance with local and international regulations as appropriate; (6) infrastructure considerations, especially with respect to existing information technology infrastructure, high-performance computing, data storage, data protection, and data sharing arrangements; (7) careful planning of the integration of WGS in laboratory workflows, and identifying pathogens, syndromes, or diagnostic processes for which WGS implementation would be worthwhile and cost-effective.

While quite a number of position papers and policy documents have examined the various aspects of WGS integration in laboratory medicine, and specifically in clinical microbiology [8], there is a paucity of studies actually exploring decision-making and evaluating management aspects of WGS integration. Such evidence is increasingly needed to enable the democratization of WGS in clinical microbiology.

Two very important considerations affecting the integration of WGS in routine laboratories are turnaround time and costs. A summary of typical costs and turnaround times for the various components of the WGS process is given in Table 1. It cannot be overstressed that many factors influence time and cost. We estimate that the performance of WGS for 16-20 bacterial isolates in-house in a routine setting would cost around 200 euros per isolate and last around 2.5-3 days. The cost and duration of the downstream analysis is much more difficult to estimate.

**Table 1**
Turnaround time and cost implications for routine WGS

| Step | Turnaround time | | Cost implications | |
|---|---|---|---|---|
| | Estimated time (hours) | Determinants | Estimated cost per sample[a] (euros) | Determinants |
| DNA extraction | 1–2h | Choice of kit, additional steps (e.g. enrichment), automation | 10 | Kits vs. reagents, technician hands-on time vs. automation |
| Library preparation | 4–6h | Method (enzymatic vs. shearing), choice of kit, automation | 30 | Choice of kit, automation |
| Sequencing[b] | 50h | Platform, chemistry, read length, run protocol | 75 | Platform, chemistry, read length, number of samples per run/coverage |
| Initial analysis[c] | 1–2h | Depending on number of samples, computing power, available software and pipelines | NA | Commercial vs. free software, availability of bioinformaticians, computer infrastructure |
| Specific analysis[d] | 4h | | | |

[a] For performing all steps in house; direct costs and consumables not including personnel.
[b] Assuming Illumina Miseq 250×2, 16–24 samples in one run.
[c] Quality control, read trimming, assembly, annotation.
[d] Specific analysis dependent on the clinical/epidemiological circumstances.

## Conclusion and outlook

Clearly, WGS has the potential to become a dominant technology used in the routine diagnostic microbiology laboratory, as it will allow detailed characterization of pathogens in all kinds of samples originating from humans, animals, food, and the environment using standardized laboratory protocols ("one test fits all" approach). Whether or not it can entirely replace routine microbiology is a matter of debate. This certainly depends on several conditions and future (technological and bioinformatics) developments. As mentioned, the time from sample to result should be dramatically reduced to obtain the result within a clinically relevant timeframe. Moreover, the correlation between genotype and phenotype is still surrounded by controversy. In particular, revealing the presence or absence of AMR genes does not always guarantee a respective phenotypic resistance or susceptibility to a specific antimicrobial drug. In a recent review from EUCAST, Ellington et al. conclude that for most bacterial species there is currently insufficient evidence to support the use of WGS-inferred antibiotic susceptibility testing to guide clinical decision making. However, the authors emphasized that WGS-AST should be a funding priority as soon as it becomes a rival to phenotypic AST, in its ability to guide treatment [25]. Moreover, inferring the MIC of bacteria from the WGS is still highly ambitious, although preliminary studies have reported promising results [26].

Another common argument for WGS never entirely replacing routine microbiology is the need to ensure pathogens are isolated from clinical samples to allow phenotypic testing (e.g. AST), inform surveillance, and maintain strain collections. With respect to this there is clear evidence from virology that real-time PCR and sequencing almost fully replaced virus culture. Therefore, it is only a matter of time before this will happen in bacteriology as well. If sufficient whole genome sequences become available from which adequate and comprehensive knowledge will accumulate on known mutations in the bacterial genome and their effect on virulence and resistance, bacterial culture would in turn be minimized and may end up as a 'lost art' performed only in national reference laboratories to maintain a culture collection or for special cases. Obviously, costs of sequencing should have dropped to the price of culture at that time making it available also at low resource countries.

In conclusion, further studies are required to improve the workflow for WGS, in particular to shorten the turnaround time for the library preparation and the runs on the WGS platforms, and, at the same time, further reduce costs. Next, automated pipelines for data analyses and easy-to-use software for analysis must be developed. Additionally, more established typing schemes for pathogens and cut-off values for interpretation within these typing schemes have to be established, coupled with reference databases having sequences and related metadata, underpinning (inter)regional and international collaborations. Importantly, external quality controls for proficiency testing have to be developed. Only when these processes reach maturity, will reliance on WGS for routine patient management and infection control management become feasible, enabling the transformation of clinical microbiology into a genome-powered and personalized diagnostic area. Having said that, WGS will be a bridge between Sanger sequencing and metagenomics-based diagnostics and clinical microbiology laboratories should invest in this technological gift to make sure they will be able to implement future applications of WGS.

## Transparency declaration

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