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Performance Evaluation of a Commercial Automated Library Preparation System for Clinical Microbial Whole-Genome Sequencing Assays

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Next-generation sequencing (NGS) has proven clinical utility on disease management and serves as an important tool for genomic surveillance. Currently, hurdles surrounding its implementation, namely the complex and demanding analytical workflows, have impeded its widespread use in many laboratories. To address this challenge, the UCLA Molecular Microbiology and Pathogen Genomics Laboratory evaluated the performance of the Tecan MagicPrep NGS system, a commercial automated solution for library preparation for clinical whole-genome sequencing assays, against the Illumina Nextera DNA Flex Library Prep. Using 35 unique organisms (28 bacteria and 7 fungi) for various clinical applications, including microbial identification and genomic characterization, we compared the quantity and quality of the prepared libraries and the resulting sequences, and concordance of the overall results. We also assessed the impact of its implementation on laboratory workflow. The MagicPrep NGS produced higher library concentrations with smaller sizes, and correspondingly, higher molarity. Quality metrics of the sequences, however, demonstrated no significant impact on the overall results, producing 100% concordance with the reference method. Importantly, workflow analysis showed 5 hours less hands-on time per run with more flexibility. This evaluation study indicates that performance of the MagicPrep NGS is comparable to the Nextera DNA Flex with the added benefit of improving workflow efficiency and reducing labor for performing routine clinical microbial whole-genome sequencing tests. *(J Mol Diagn 2024, 26: 719—726; https://doi.org/10.1016/j.jmoldx.2024.05.006)*

The clinical applications of next-generation sequencing (NGS) are rapidly becoming standard tools in laboratory medicine due to its unique ability to fulfill clinical needs unable to be accomplished by other available methods. In particular, the UCLA Molecular Microbiology and Pathogen Genomics (MMPG) Laboratory has developed numerous amplicon-based and whole-genome sequencing (WGS)-based assays by NGS for microbial identification, antimicrobial resistance (AMR) prediction, and genotypic characterization with proven clinical impact.[1–6] Notably, NGS has also contributed to significant investigational studies that elucidated important trends in the epidemiology and dynamics of emerging pathogens within the UCLA Health System and the greater metropolitan region it serves.[7–11] However, despite the exponential growth in the clinical applications of NGS, its burden on staffing and the requirement for technical expertise have barred its widespread adoption in many laboratories.

The workflow for NGS can be separated into two distinct sets of processes: one, the analytical wet lab protocols, and two, the post-analytical dry lab bioinformatics. Although historically, the complex and highly technical bioinformatic pipelines have been the most significant hindrance in the development of NGS-based assays in clinical labs, major advancements in commercially available software with graphical user interfaces, along with freely available cloud-based tools, are beginning to decrease the technical expertise required to perform sequencing-based analyses.

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In stark contrast, the wet lab procedures involved in employing NGS, particularly for microbiological applications, have remained largely unchanged from their initial development in research labs—long, manual protocols requiring expertly trained personnel with knowledge of techniques in both microbiology and molecular genetics. Library preparation, in particular, is arguably the most labor- and time-demanding process, requiring 5 to 6 hours of assay time with only 15 to 30 minutes of intermittent uninterrupted walk-away time.

To address this burden and maximize workflow efficiency, we evaluated the performance of a commercially available library preparation system for clinical NGS assays. The Tecan MagicPrep NGS System (Tecan Group Ltd., Morrisville, NC) is a fully automated benchtop platform that generates NGS libraries in a one-step process, allowing for approximately 5.5 hours of continuous walk-away time. We evaluated the performance of MagicPrep NGS in comparison to the current standard-of-care method, the Illumina Nextera DNA Flex Library Prep (Illumina, Inc., San Diego, CA), for our clinically validated assays, including microbial identification (bacterial and fungal), virulence profiling (hypervirulent *Klebsiella pneumoniae*), and AMR prediction (*Mycobacterium abscessus* and *M. tuberculosis* complex).

Materials and Methods

Quantitative Evaluation of MagicPrep NGS Libraries and Sequences

A method comparison was performed to evaluate the technical performance of the MagicPrep NGS using the Nextera DNA Flex, the authors' current standard-of-care method, as the comparator. Clinical samples were first heat inactivated at 95°C for 30 minutes followed by a bead beating step to disrupt the cell wall. Genomic DNA was extracted using the Qiagen EZ1 Tissue kits on the BioRobot EZ1 instrument (Qiagen, Germantown, MD). Libraries were then prepared in parallel with both methods using the extracted genomic DNA ranging from 0.26 to 59.0 ng/μL (median ± SD, 6.66 ± 15.93 ng/μL]. The samples were diluted to a target DNA input quantity according to the manufacturers' recommendations. Notably, the minimum input for the MagicPrep NGS is 50 ng of DNA, whereas the Nextera DNA Flex has a substantially lower limit of 1.2 ng of DNA. In cases where the concentrations were insufficient to satisfy the optimal recommendation for the MagicPrep NGS, the maximum volume allowed was utilized, and the final input DNA quantities were noted. In total, 70 libraries were prepared among the 35 unique organisms listed in Table 1. Library preparation metrics were compared for all samples. Additionally, the total sequence output, the sequences mapped to the relevant marker genes (ie, 16S rRNA, *rpoB*, and *groEL* for bacteria, ITS and 28S rRNA for fungi), the quality of *de novo* assembly (N50), and the average depth of coverage were also compared for the microbial identification samples for which the following metrics are routinely monitored.

Concordance Analysis of NGS Assay Results

The bioinformatic analyses of the NGS data were performed according to previously described protocols.[1–6] A concordance analysis was performed to evaluate the impact of MagicPrep NGS libraries on the clinically reportable results. For the WGS-based microbial identification workflows (bacterial and fungal), the results were analyzed according to both genus-level and species-level concordance; whereas for genotypic characterization assays (hypervirulent *K. pneumoniae* genotyping and *M. abscessus* and *M. tuberculosis* complex resistance prediction), the results were analyzed to the categorical-level and gene/mutation-level concordance. In cases where discrepant results were observed, the corresponding bioinformatic analyses were repeated and corroborated by an independent operator to confirm post-analytical discrepancies, and the results were reviewed by the UCLA MMPG Laboratory director.

Evaluation of Workflow Impact of MagicPrep NGS Automation

Impact on workflow was assessed using the average time to completion of a full sequencing run by an experienced user. A full WGS run using Illumina MiSeq at the UCLA MMPG Laboratory is optimized for 16 samples, with a minimum of 12 samples (including controls).

Statistical Analyses

All statistical analyses were performed using GraphPad Prism software version 10.2.1 (GraphPad Software, Boston, MA). For comparison of two groups, nonparametric *U*-test was performed. For comparison of greater than two groups, repeated measures one-way analysis of variance with Greenhouse-Geisser correction was performed. For analysis of correlation, two-tailed Spearman correlation was performed. For all analyses, $P < 0.05$ was considered statistically significant. All statistically significant results are noted in the figures.

Results

Technical Performance of the MagicPrep NGS Automation

The Nextera DNA Flex produced library concentrations ranging from 2.47 to 11.90 ng/μL (median ± SD: 6.92 ± 2.44 ng/μL), whereas MagicPrep NGS produced significantly higher quantities of libraries with over 3× the median concentration (median ± SD: 23.30 ± 35.42 ng/μL), although with a much wider range from 0.13 to 263.0 ng/μL (Figure 1A). The higher library concentration generated by

Table 1 List of Organisms Used in the Evaluation of the MagicPrep NGS

Purpose	Taxonomic classification	Category	Expected result
Microbial identification	Bacteria	Acid-fast *bacillus*	*Mycobacterium avium* subsp. *hominissuis*
Microbial identification	Bacteria	Acid-fast *bacillus*	*Mycobacterium lentiflavum*
Microbial identification	Bacteria	Acid-fast *bacillus*	*Mycobacterium simiae*
Microbial identification	Bacteria	Acid-fast *bacillus*	*Mycobacteroides abscessus*
Microbial identification	Bacteria	Acid-fast *bacillus*	*Mycolicibacterium cosmeticum*
Microbial identification	Bacteria	Acid-fast *bacillus*	*Mycobacterium avium* subsp. *hominissuis*
Microbial identification	Bacteria	Gram-negative rod, aerobe	*Capnocytophaga canimorsus*
Microbial identification	Bacteria	Gram-negative rod, aerobe	*Cupriavidus gilardii*
Microbial identification	Bacteria	Gram-negative rod, aerobe	*Herbaspirillum seropedicae*
Microbial identification	Bacteria	Gram-negative rod, aerobe	*Klebsiella pneumoniae*
Microbial identification	Bacteria	Gram-negative rod, aerobe	*Salmonella enterica* subsp. *enterica serovar Enteritidis*
Microbial identification	Bacteria	Gram-negative rod, anaerobe	*Akkermansia muciniphila*
Microbial identification	Bacteria	Gram-negative rod, anaerobe	*Dialister pneumosintes*
Microbial identification	Bacteria	Gram-negative rod, anaerobe	*Porphyromonas somerae*
Microbial identification	Bacteria	Gram-positive rod, aerobe	*Bacillus cereus*
Microbial identification	Bacteria	Gram-positive rod, aerobe	*Bacillus toyonensis*
Microbial identification	Bacteria	Gram-positive rod, aerobe	*Nocardia thailandica*
Microbial identification	Bacteria	Gram-positive rod, aerobe	*Pseudonocardia* species most closely related to *autotrophica*
Microbial identification	Bacteria	Gram-positive rod, aerobe	*Tsukamurella* species most closely related to *tyrosinosolvens*
Microbial identification	Bacteria	Gram-positive rod, anaerobe	*Alloscardovia omnicolens*
Microbial identification	Bacteria	Gram-positive rod, anaerobe	*Intestinimonas butyriciproducens*
Microbial identification	Bacteria	Gram-indeterminate, aerobe	*Mycoplasma hominis*
Microbial identification	Fungi	Thermally dimorphic fungi	*Histoplasma capsulatum*
Microbial identification	Fungi	Mold	*Cladorrhinum bulbillosum*
Microbial identification	Fungi	Mold	*Fusarium solani (Fusarium solani species complex)*
Microbial identification	Fungi	Mold	*Rhizopus microsporus*
Microbial identification	Fungi	Yeast	*Candida tropicalis*
Microbial identification	Fungi	Yeast	*Cystobasidium* species*
Microbial identification	Fungi	Yeast	*Preussia* species*
Klebsiella pneumoniae hypervirulence genotypic prediction	Bacteria	*K. pneumoniae*	Wild-type
Klebsiella pneumoniae hypervirulence genotypic prediction	Bacteria	*K. pneumoniae*	Hypervirulent
Mycobacterium abscessus genotypic resistance prediction	Bacteria	*M. abscessus* spp. *abscessus*	Clarithromycin resistant; amikacin non-resistant
Mycobacterium abscessus genotypic resistance prediction	Bacteria	*M. abscessus* spp. *massiliense*	Clarithromycin susceptible; Amikacin non-resistant
Mycobacterium tuberculosis genotypic resistance prediction	Bacteria	*M. tuberculosis*	Wild-type
Mycobacterium tuberculosis genotypic resistance prediction	Bacteria	*M. tuberculosis*	Pyrazinamide resistant

K. pneumoniae (Kp) isolates utilized for hypervirulent Kp (HvKp) genotypic characterization were also analyzed for microbial (bacterial) identification according to UCLA standard operating protocol.

*Identified only to genus level due to limitations in the NCBI database at the time of bioinformatic analysis.

the MagicPrep NGS was expected due to the higher loading DNA quantity as suggested by the manufacturer (>50 ng). Importantly, among the subset of 18 samples with input DNA quantities below the recommended lower limit for the MagicPrep NGS (13 to 33 ng), the median concentration of the prepared libraries was still greater than the corresponding concentrations using the Nextera DNA Flex (median ± SD: 10.28 ± 13.43 ng/μL versus 5.98 ± 1.54 ng/μL, respectively). Our data indicate that the MagicPrep NGS can accommodate DNA input that is much lower than recommended loading quantity and still produce libraries within the acceptable range for downstream sequencing.

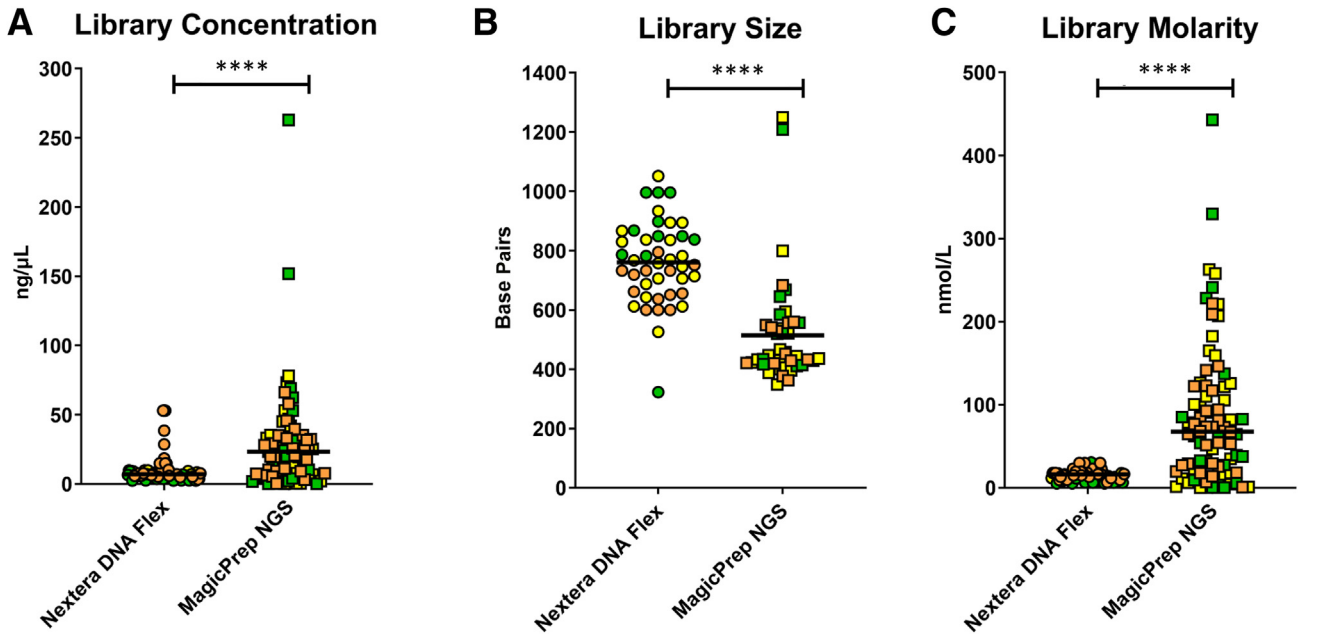

Figure 1 Comparison of technical performance of Nextera DNA Flex library prep and Tecan MagicPrep NGS. **A:** Concentration of prepared libraries. The line represents median concentration. **B:** Size of prepared libraries. The line represents the mean size. **C.** Molarity of prepared libraries. The line represents the median molarity. **A**–**C:** Color of each point corresponds to sample type. Yellow: bacterial identification; green: fungal identification; orange: genotypic characterization (*M. abscessus* antimicrobial resistance prediction and hypervirulent *Klebsiella pneumoniae* determination). $P < 0.05$ is considered statistically significant (*U*-test). ****$P < 0.0001$.

The sizes of the prepared libraries were also appreciably different between the two methods. Nextera DNA Flex produced libraries with a mean size of 760.62 bp (range ± SD: 323 to 1052 ± 138.78 bp), whereas MagicPrep NGS libraries were significantly smaller with a mean size of 514.44 bp (range ± SD: 349 to 1250 ± 182.02 bp) (Figure 1B). Accordingly, the higher concentration and the smaller library size equated to libraries with significantly greater median molarity with the MagicPrep NGS compared with the Nextera DNA Flex (median ± SD: 67.65 ± 83.56 nmol/L versus 16.16 ± 6.23 nmol/L, respectively) (Figure 1C). Lastly, libraries from a representative subset of the evaluation samples were prepared in triplicate runs to assess the technical precision of the MagicPrep NGS, which demonstrated highly comparable results among the runs (Supplemental Figure S1).

Taken together, the technical performance of the MagicPrep NGS, despite demonstrating significant differences from the Nextera DNA Flex, satisfied the library requirements for sequencing with the Illumina MiSeq system, and was determined to be equivalent to their current standard-of-care method.

Performance Characteristics of the Tecan MagicPrep NGS

To evaluate the effect of the noted differences in technical performance on assay results, the sequencing analytics of the two methods were first compared using data obtained from their microbial identification workflows for which the following metrics are routinely monitored. First, the MagicPrep NGS and the Nextera DNA Flex both produced comparably high counts of total reads, ranging between 10^6 to 10^7 reads per sample (Figure 2A). Moreover, counts of mapped reads, the length of the N50 (an indicator for the quality of *de novo* assembly), and the average coverage (depth, x) all had statistically significant correlation indicating the comparable qualities of the reads sequenced by both library preparation methods (Figure 2, B–D).

Next, to contextualize the overall performance of MagicPrep NGS and determine its impact on clinically reportable results, the qualitative outcomes were compared according to each NGS assay at two levels of resolution, designating the results of the Nextera DNA Flex as the comparator (expected) result. The microbial identification assays (bacterial and fungal) produced 100% concordance at both genus and species levels (Table 2). Notably, two fungal isolates that were identified only to the genus level using the Nextera DNA Flex were similarly identified to the same taxonomic level using the MagicPrep NGS, suggesting a limitation in the post-analytical bioinformatic pipeline and database, and not in the analytical phase of the assay. Similarly, all three genotypic characterization assays (hypervirulent *K. pneumoniae* determination, and *M. abscessus* and *M. tuberculosis* complex AMR prediction) also produced 100% concordance at the categorical and gene/mutation levels.

Assessment of post-analytical metrics of the MagicPrep NGS compared with the Nextera DNA Flex demonstrated significant correlation. Importantly, equivalence in the final

reportable results indicates no compromise in the quality of the provision of clinical care by the laboratory.

Impact of Tecan MagicPrep NGS on Wet Lab Workflow

After establishing equivalent performance compared with the current standard-of-care, the perceptible impact of the MagicPrep NGS was determined on the existing workflow. The wet lab process of NGS comprises several discrete procedures including genomic DNA extraction, library preparation, sample pooling, and quality assessment prior to loading onto the sequencing instrument. Currently, the UCLA MMPG Laboratory utilizes automated instruments for DNA extraction and clean-up, and for the final analyses of prepared libraries, with only the library preparation remaining as a largely manual method. Nextera DNA Flex is a continuous protocol requiring 5 to 6 hours of hands-on time in which the extracted genomic material is fragmented and tagmented, then amplified to optimize the sequencing yield. Due to the demands of this manual process, all samples (up to 16 isolates) are batched to optimize efficiency, which requires all samples to be extracted prior to the start of library preparation. As such, the genomic DNA extraction step, which uses an instrument that can only accommodate up to 14 samples per run, is relegated to a multi-day process for a 16-sample sequencing run (Figure 3).

The greatest potential for improvement by the MagicPrep NGS is its effect on streamlining workflow and optimizing wet lab efficiency. Although the MagicPrep NGS can only accommodate up to eight samples per instrument per run, its single-step operation requires only 15 to 20 minutes of hands-on time with 5.5 hours of walk-away time. As such, only the first set of eight samples needs to be batch-

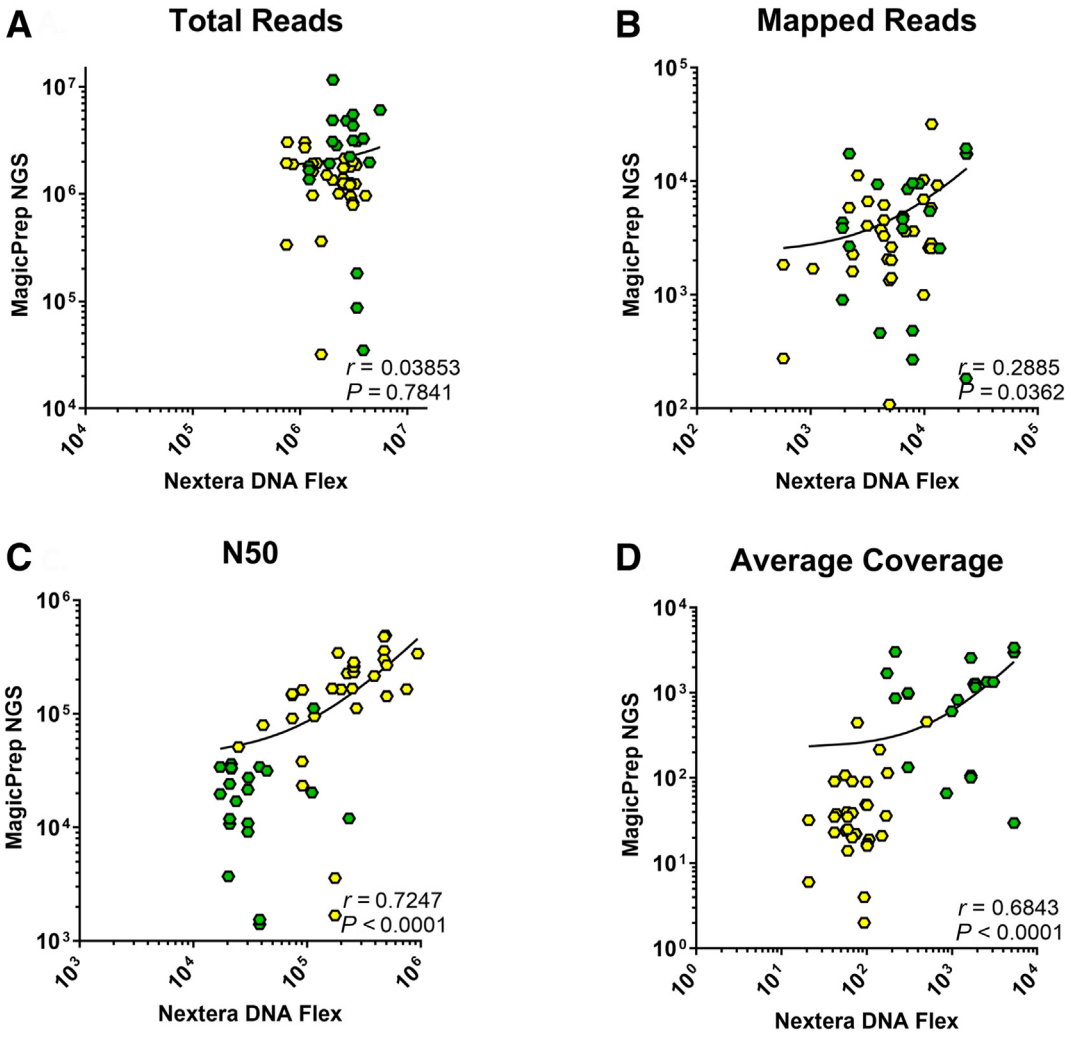

Figure 2 Correlation of post-sequencing performance metrics of Nextera DNA Flex library prep and Tecan MagicPrep NGS. **A:** Correlation of total reads measured in read counts. **B:** Correlation of mapped reads measured in read counts. **C:** Correlation of N50 measured in base pairs. **D:** Correlation of average coverage measured in depth of coverage per base. **A**—**D:** Color of each dot corresponds to sample type. Yellow: bacterial identification; green: fungal identification; the line represents the linear regression best fit line. $P < 0.05$ is considered statistically significant (two-tailed Spearman correlation). Spearman's rank-correlation of coefficient (r) is noted.

extracted prior to the first library preparation, followed by staggered processing in a single day. Specifically, the genomic DNA extraction for the second MagicPrep NGS run (second set of eight samples) can be performed during the first library preparation run. And the second library preparation only needs to be initiated before end of day, because the MagicPrep NGS has a closed, temperature-monitored system that allows the products to be kept refrigerated overnight until the following day (Figure 3). Altogether, these procedural differences can consolidate the multi-day operation into a single NGS preparation day with dedicated staffing, and the overall hours of hands-on time can be dramatically reduced to <45 minutes. In addition, it is worth noting that the instrument performs robustly without mechanical failures after more than 40 runs in a time period of over a year.

Discussion

NGS offered clinical laboratories a powerful diagnostic tool previously only available in research settings. Between amplicon-based sequencing, WGS, and metagenomic sequencing, NGS can be applied in various ways to meet specific diagnostics needs otherwise not feasible by other methods.[12] The reality of implementing NGS, however, has proven to be an extreme hurdle that has prevented its widespread adoption in many hospital laboratories.

The wet lab component of the NGS workflow is arguably the most prohibitive barrier for clinical laboratories that are already experiencing strenuous challenges with staffing. Library preparation, in particular, is composed of several manual protocols requiring highly technical skills in both microbiology and molecular genetics. Thus, the primary challenge for automation has been to develop a system that replaces hands-on interactions without compromising technical performance. Accordingly, NGS automation has largely revolved around open-source robotic platforms and liquid handlers that can emulate manual pipetting. However, given the tremendous cost of fully programmable, commercially available NGS instruments that allow for a high degree of adaptability toward individual needs, a balance between the investment in the technology and the benefits to the laboratory must be considered.[13]

The UCLA MMPG Laboratory is a lower-volume sequencing laboratory with typically up to 16 samples in a single sequencing run per week. Currently, due to the demands of manual library preparation using the Nextera DNA Flex, wet lab operation is a highly fragmented, multi-day workflow that has posed as a persistent challenge for laboratory staffing and scheduling. Thus, their primary considerations for implementing NGS automations were: i) performance, ii) ease of operation and maintenance (including space requirements), and iii) cost.

Table 2 Result Concordance between Nextera DNA Flex Library Prep and Tecan MagicPrep NGS

Microbial identification	Genus level	Species level
Total	100% (61/61)	100% (59/59)*
Bacterial identification	100% (40/40)	100% (40/40)
Fungal identification	100% (21/21)	100% (19/19)*
Genotypic characterization	**Categorical**	**Gene/mutation**
Total	100% (25/25)	100% (25/25)
Hypervirulent *Klebsiella pneumoniae* determination	100% (7/7)	100% (7/7)
Mycobacterium abscessus AMR prediction	100% (8/8)	100% (8/8)
Mycobacterium tuberculosis AMR prediction	100% (10/10)	100% (10/10)

For microbial identification assays (bacterial and fungal), results were analyzed for genus-level and species-level concordance. For genotypic characterization (hypervirulent *K. pneumoniae,* HvKp, determination, and *M. abscessus* AMR prediction), results were analyzed for categorical-level and gene/mutation-level concordance. Percent concordance is noted; counts of concordant results over the total analyzed is noted in parentheses. Acceptable criterion is ≥90%.

*Two fungal samples were identified only to genus level due to limitations in the NCBI GenBank database at the time of bioinformatic analysis.

The MagicPrep NGS is a fully automated solution to library preparation that replaces 5.5 hours of manual procedures with a 1-step walk-away instrument. The current evaluation of its technical performance has demonstrated comparability in all quantifiable metrics, including library analytics and overall result concordance against the Nextera DNA Flex. More importantly, objective workflow analysis demonstrated the potential for consolidation of the wet lab processes toward a more streamlined NGS workflow with a single day of dedicated personnel staffing, followed by less than half a day for loading onto the sequencing instrument.

With respect to instrument operation and maintenance, the MagicPrep NGS is a batched, closed-system instrument with preprogrammed protocols utilizing Illumina-compatible reagents. Although it still allows for certain parameters to be manipulated by the operator (ie, input DNA and amplification cycles), the instrument is immediately ready for use, allowing for rapid adoption into existing workflows. Moreover, the MagicPrep NGS is a benchtop instrument with a small footprint and minimal space requirements, and requires no routine maintenance from the manufacturer. Together, these features allow for ease of implementation and scalability in throughput to accommodate the specific needs of the laboratory.

The reagent (manufactured by Tecan, the same vendor of MagicPrep) cost of the automation is also estimated to be similar in comparison with the reagent cost of manual library preparation (manufactured by Illumina). However, it is important to point out the reagent cost neutrality is based on an increment of eight samples as each MagicPrep cartridge is designed for one-time use for eight samples. If a

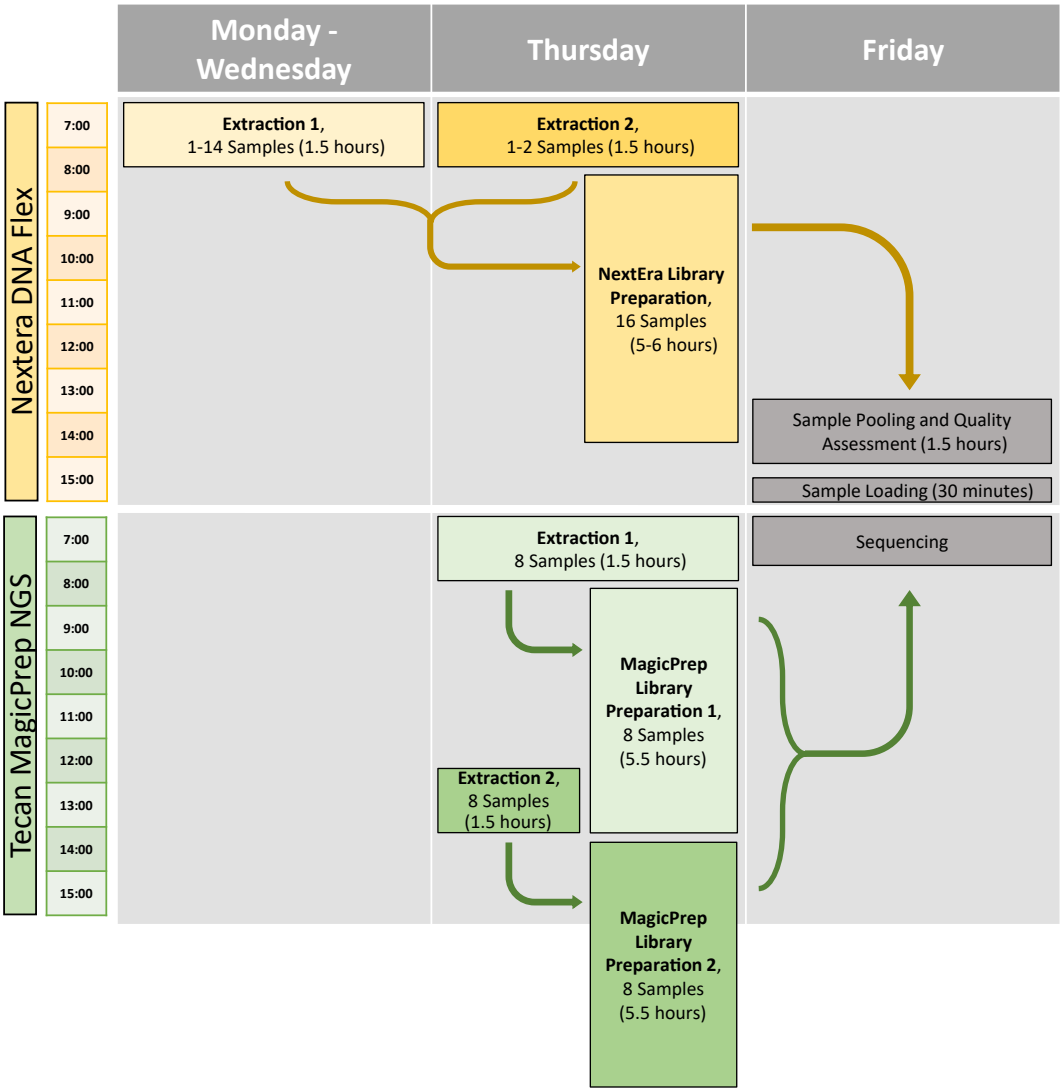

Figure 3 UCLA sequencing workflow. Nextera DNA Flex (yellow) is a batched protocol requiring DNA from all samples to be extracted prior to library preparation. Accordingly, DNA extraction is performed prior to the day dedicated to Nextera DNA Flex library preparation. MagicPrep NGS (green) can accommodate eight samples in a single step with 5.5 hours of walk-away time, allowing for stacked processing in a single day. The second run need only be initiated; the closed-system instrument allows for storage of the prepared libraries until the following day.

cartridge is partially filled, then the reagent cost per sample will increase. When labor cost is factored in, the overall cost is estimated to decrease by more than $200 per run, based on 5 hours of labor reduction per run for 16 samples. When the instrument cost is factored in, the overall cost is estimated to increase ~$100 per run, based on a weekly run schedule with 10 years of usage. Therefore, the overall cost is estimated to be ~$100 less per run after the library prep automation is implemented. In reality, the primary advantage of this automation in the clinical laboratory where the study was conducted lies less in cost savings but more in increased productivity.

The main limitation of this study is that it was only conducted in a low-throughput NGS laboratory (≤16 samples/week), and thus, the conclusion on the workflow assessment may not necessarily be generalizable to medium- or high-throughput operations. In addition, this study only focused on the performance of WGS of clinical bacterial and fungal isolates and not metagenomics. Further studies are undergoing to assess the capability of the MagicPrep NGS for amplicon-based metagenomic sequencing for pathogen detection directly from clinical samples.

In summary, we evaluated the performance and workflow impact of a commercial automated NGS library preparation platform for microbial WGS in a clinical laboratory setting and found that this platform provides equivalent and satisfactory performance with a more labor- and time-efficient workflow. The study demonstrates that an effective NGS library automated solution can be a key to overcome a burdensome challenge in implementing NGS in clinical laboratories.

Acknowledgments

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Disclosure Statement

None declared.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2024.05.006*.

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