How to Cite:
Alonazi, R. S. (2024). Next-generation sequencing in laboratory practice: Applications and challenges. *International Journal of Health Sciences*, *8*(S1), 954–963.
https://doi.org/10.53730/ijhs.v8nS1.15017

Next-generation sequencing in laboratory practice: Applications and challenges

Reem Saleh Alonazi
KSA, National Guard Health Affairs

***Abstract*---Background** _ The COVID-19 pandemic has underscored the critical role of next-generation sequencing (NGS) in diagnostic testing and surveillance-based screening. Public health laboratories need to incorporate these advanced molecular technologies to enhance their capabilities. However, challenges such as a shortage of skilled personnel, lack of sequencing expertise, assay standardization issues, and workload management within turnaround times are common. **Aim of Work –** This perspective paper aims to shed light on the benefits and challenges of using laboratory automation for sequencing purposes. It explores strategies for implementation, including instrument selection, validation approaches, staff training, and troubleshooting. **Methods** – In order to locate journal publications, the Scopus, Web of Science, and PubMed academic databases were searched. The following selection criteria were utilized: Articles must be written in English, focus on normative reasons rather than merely empirical research, include an abstract for software analysis, and describe NGS technology. The paper reviews current literature on laboratory automation in the context of NGS, examines case studies of successful implementation, and discusses potential barriers and solutions. **Results** – The findings highlight the potential of workflow automation to address many of the challenges faced by laboratories. It can improve efficiency, reduce human error, and increase throughput. However, the transition to automation requires careful planning, investment in training, and ongoing support. **Conclusion** – Laboratory automation offers significant advantages for NGS, but successful implementation depends on overcoming various challenges. By addressing these issues, laboratories can enhance their diagnostic capabilities and better respond to public health needs.

***Keywords*---**Laboratory Automation, Next-Generation Sequencing, Public Health Laboratories, Workflow Optimization, COVID-19 Diagnostics.

Corresponding author *Email: akk29337@gmail.com*
Manuscript submitted: 9 Nov 2023, Manuscript revised: 10 Dec 2023, Accepted for publication: 15 Jan 2024

Introduction

The advent of readily available next-generation sequencing (NGS) technology has significantly transformed the field of clinical and public health microbiology. It provides the opportunity to enhance diagnosis, surveillance, and public health response. Sequencing is now often used to regularly aid epidemic investigations, hence assisting labs in promptly and accurately identifying disease clusters **[1–3]**. By substituting conventional microbiological techniques with culture-independent approaches for identifying pathogens, Next-Generation Sequencing (NGS) has the capability to provide more precise guidance for patient treatment **[4]**. As a result, it is unsurprising that there has been a significant effort to allocate resources towards genome sequencing in the last three years **[5]**. The use of Next-Generation Sequencing (NGS) data has proved important in the context of the COVID-19 pandemic. When used in conjunction with epidemiology, it provides a method to examine the patterns of transmission as the virus continues to propagate worldwide. Currently, clinical and public health institutions are facing the challenge of operating with a reduced workforce. Recently recruited individuals may possess insufficient expertise or experience to comprehend the intricacies of sequencing tests. Therefore, initially, the implementation of Next-Generation Sequencing (NGS) technologies may seem too complex and time-consuming for labs to adopt or even attempt to enhance their sequencing capabilities. Workflow automation offers a chance to overcome some obstacles.

Aim of Work

Laboratory automation has been widely implemented across many kinds of labs for some years. In recent times, its growth has significantly accelerated, leading to the emergence of a multi-billion dollar business. With the growing need for next-generation sequencing, it is logical to explore the possible use of automation to facilitate this kind of testing. In this discussion, we will explore many facets of automation in the preparation of sequencing libraries. We outline the primary advantages and difficulties associated with using automated liquid handlers. In addition, we elaborate on our methodology for verifying one of these systems.

The advantages of NGS automation

The preparation of specimens for next-generation sequencing is a laborious procedure that encompasses many stages, commencing with sample extraction. The procedure of creating the sequencing libraries is an essential component in achieving findings of superior quality. The process includes many time-critical stages, transferring tiny amounts using a pipette, and repeatedly cleaning and rinsing the samples **[6,7]**. The whole procedure might consume many hours of a laboratory researcher's time, and a single error can lead to the forfeiture of an entire day's worth of effort. Various firms have developed specialized automated liquid handlers exclusively for this intricate procedure. Various sizes and capacities of automated instruments have been developed and may be programmed to execute a whole library preparation procedure either as a single streamlined operation or as separate individual processes. Although automation

is not a panacea for all issues, it can provide some noteworthy advantages and may help labs overcome certain challenges associated with using NGS **[8]**.

Quality Enhancement

Automation is primarily beneficial because it improves the quality of samples, frequently achieving a higher level of consistency than what can be achieved manually by laboratory professionals. In Next-Generation Sequencing (NGS), several library preparation techniques include magnetic beads and multiple washing stages to purify the samples and select fragments based on their size. Proficient training and expertise are essential for manual preparation in order to prevent the loss, contamination, or inadequate quality of samples, all of which may have a negative impact on subsequent studies. Automated platforms are specifically engineered for performing accurate pipetting procedures, resulting in consistently high-quality libraries in a shorter duration compared to manual preparation. Based on our observations, we have seen improvements in quality via several means, such as achieving more uniformity in nucleic acid fragment lengths and reducing the need for repeated testing of samples. In the end, a reduction in unsuccessful attempts conserves time, reagents, and resources.

Intuitive Interface

While the backend algorithms for automating a sequencing library preparation technique may be complex, several devices are equipped with a computer that has user-friendly control software pre-installed. Minimal familiarity with NGS or a comprehensive grasp of the scientific process is not required for scientists to set up or operate these liquid handlers. Standard procedures often use simple visuals to precisely show the correct placement of consumables, provide visual prompts to indicate the current stage of the process, and allow the instrument to calculate the necessary reagent volumes for the number of samples being processed. Consequently, the amount of time required for training the network is decreased, and scientists should not have to possess specialist programming skills in order to resolve simple problems.

Enhanced Adaptability

Laboratories can adjust their capacity as necessary using automated equipment. There are instruments available that provide different degrees of throughput while yet ensuring fast turnaround times. The laboratory has the capability to handle a range of 4 to 384 samples in a single run, depending on the specifications of their system and the desired result. Furthermore, several solutions include modular workflow choices that include safe stopping points, allowing laboratories to make necessary adjustments. Laboratories have the option to choose use the instrument for certain processes, such as library clean-up, instead of using it for the whole end-to-end procedure. For those requiring more than the conventional library preparation procedure provided by commercial vendors, firms like as Agilent and Beckman Coulter provide graphical or simplified software interfaces that simplify the process of developing bespoke protocols. In addition, they provide training programs in procedural programming using their software.

Hamilton and Beckman Coulter provide decks that may be easily modified to accommodate different processes. Nevertheless, this may not hold true for every platform. Certain systems have protocols that are not easily changed and need the maker to create new processes.

Efficient

Automated systems for library preparation include capabilities beyond just liquid transfer and mixing. On-deck thermocyclers, shakers, and heat blocks may be added to instruments to create a completely automated system, eliminating the need for operator intervention. The manual preparation of the Illumina DNA Prep methodology takes roughly 3 hours to produce a library that is ready for sequencing. Although the total duration of the automated workflow is comparable, the amount of time spent actively working on it is significantly decreased. Setting up the instrument takes around 30 minutes, and the automated run time is 2.5 hours. In contrast, the manual methodology requires almost 3 hours to process 8 samples. A notable advantage is that the setup of an instrument requires just one scientist, regardless of the quantity of samples being processed. After loading the samples and starting the application, the scientist is able to leave and concentrate on other duties.

The Difficulties Associated With NGS Automation

Although automated workflows provide several advantages, as previously said, it is crucial to carefully evaluate the key obstacles before opting to use these systems. Acquiring automated instruments can be quite costly, with price estimates ranging from $45K to $300K for low and high throughput platforms. It is important to carefully evaluate whether obtaining such instruments is feasible or necessary for the current and future workload. These systems have gotten more intricate, typically including additional features like as on-deck thermocyclers, bulk pipettor attachments, and robotic arms. These features are designed to accommodate varying laboratory capacity requirements and to carry out various test techniques. Before selecting the most suitable system design, it is important to thoroughly comprehend the basic elements of a method or product. The choice of instruments available for usage may be limited based on the beginning sample material, reagent kit type, and sequencing platform to be used. Our estimation is that the cost per sample is around $40. Therefore, there is likely little opportunity to reduce the real cost. However, the reduction in hands-on technician time may be valuable. In addition, the increasing prevalence of automation may lead to a reduction in prices for consumables and future systems.

Diagnostic And Instructional Support

The manufacturer will most likely give first on-site training, which aims to familiarize users with the installed instrument and provide an overview of its fundamental operation. Nevertheless, it is quite probable that practical experimentation will be necessary in order to acquire expertise and a more comprehensive comprehension of the intricacies of the system. Conducting clean

water runs and test runs will aid in evaluating any operational mistakes or other problems that might affect the quality of testing samples downstream. Modifications to the software program operating the workflow may be required to enable precise and smooth execution of stages in the user's laboratory, with minimum errors and interruptions. It is important to mention that the manufacturer may impose limitations on alterations. Based on our experience, it is quite unlikely that we will have the opportunity to get training from external vendors to customize software or protocol procedures. Training of testing workers is necessary for each new instrument. Training senior staff members, such as upper management or division supervisors, as "super users" may be beneficial in preventing the loss of knowledge due to employee turnover or restricted availability of competent workers. We advise ensuring that there are always at least two individuals designated as "super users." These "super users" should possess expertise in resolving complex issues that may need remote support from the manufacturer, as well as the capability to readjust deck placements (known as "deck teaching"), among other proficiencies.

Regular Execution And Upkeep

One advantage of using automated processes is the ability to "walk-away" without any disruption to testing. However, the actual experience may be more intricate than that. Adhering to the manufacturer's recommendations for daily, weekly, and long-term maintenance plans is of utmost importance to ensure the instrument operates smoothly. Regular maintenance involves calibrating the channels, adjusting the spacing, aspiration, and dispensing, and cleaning the surface to eliminate dust or other contaminants. The instrument will most likely suggest for this automatically, but if not, it is advisable to establish a regular timetable (weekly) for these actions. The manufacturer often offers annual preventive maintenance as part of special contracts (at an extra cost of $15K–30K per year) to minimize the chances of larger issues arising. These scheduled preventive maintenance appointments often involve coordinating with the on-site person, resulting in a potential delay before the service is carried out. Similarly, this applies to any additional service requests that may be necessary when the instrument encounters an error or problem that the user is unable to handle alone. Based on our experience, it is typical to have direct connection with field engineers and applications experts. This practice effectively minimizes instrument downtime and eliminates the need for a hierarchical response system via the main customer support line. While our current configuration does not provide remote access, it is possible for others to build their system in a way that allows this capability. This would help reduce the need of on-site visits for addressing minor faults and issues. It is advisable to be proficient in a manual preparation technique to prevent any interruption in activity in case the equipment has to be repaired or serviced.

Continuous monitoring of quality controls (QC) is essential for ensuring that the equipment and technique used consistently provide trustworthy and accurate findings, as is the case with all assays. In sequencing, there are many quality control "checkpoints" to ensure that the integrity of each sample is preserved throughout the process. These checkpoints are often located at crucial stages of the operation, such as after DNA extraction, after library creation, and after

sequencing. Automated systems may have limits in measuring sample quality at certain specified points, depending on the library preparation kit utilized. Occasionally, it may be essential to adjust the suitable moments for doing system checks, and to use innovative approaches in determining when and how to assess quality. For instance, certain techniques used to extract or break down cells may result in the formation of small beads. Consequently, conventional methods of measuring the quantity of the extracted material may not be feasible after these procedures. When instances like these occur, it is crucial to set quality criteria as soon as possible to minimize loss of time, samples, and reagents. Whether the extracted specimen cannot be quantified because of the presence of beads, it is crucial to use the QC checkpoint to quantify DNA after completing the library preparation. This will help determine whether each sample satisfies the required quality for sequencing. Failure to achieve the sequencing threshold may result in the wastage of reagents and samples, necessitating a full re-extraction of the sample. This has been a minor inconvenience in our experience, occurring seldom and not more often than in other techniques.

Several automated technologies are available in the market specifically designed for next-generation sequencing library preparation. Prior to making a commitment, labs should evaluate their financial resources, infrastructure, and sequencing process in order to determine the most suitable approach for achieving their sequencing objectives. While a clinical laboratory may give priority to doing a large number of tests, a research facility may need a system that has an adaptable workflow. In this brief, we will specifically address three primary aspects: system compatibility, system capability, and system capacity.

Although NGS is increasingly used, particularly in public health laboratories, it is important to carefully assess the design and execution of the assay to ensure regulatory compliance. Several regulatory initiatives, including as CLIA and CAP, have recently provided more precise advice. Additionally, there are other valuable tools accessible to help develop an effective strategy **[9,10]**.

The validation of automated liquid-handling equipment has been established by incorporating recommended methodologies from several sources. This validation process has been customized to suit the specific instrument being utilized (Hamilton Microlab STAR) and the DNA library preparation kit (Illumina DNA Prep Kit) **[11]**. It is important to mention that laboratory developed tests (LDT) designed for surveillance purposes have less stringent requirements for regulatory compliance compared to those intended for diagnostic reasons. It is important to consider how the findings will be used while implementing sequencing tests and platforms throughout the on-boarding process. Further extensive illustrations using alternative approaches may be located to facilitate the creation, advancement, and execution in various environments and distinct laboratory configurations **[12, 13]**.

Precision

Here, agreement between the tested sample and a reference is measured and evaluated for the following:

Wet lab - DNA sequencing platforms such as Illumina MiSeq, Oxford Nanopore, PacBio, etc. Bioinformatics pipelines for dry lab experiments.

Accuracy

Consistency, in this context, refers to the degree of similarity seen in the tested sample when it is run numerous times under varying circumstances such as various days, operators, and sample preparations. The determination of the number of samples needed to fulfill this requirement should be made under the guidance and authorization of the director of each respective laboratory. We used 5 samples to assess precision, since this number was the minimal need to evaluate the whole spectrum of organisms we usually test, while also considering the expenses associated with supplies, reagents, and instrument utilization.

Comparison Of Methods (Manual Vs. Automated Processes)

We included a technique comparison to ascertain any disparities between the outcomes acquired from the newly automated procedure and the presently validated manual preparation methodology. This was primarily used to evaluate the library preparation component of the protocol, since the extraction process and the bioinformatics pipeline for analysis were similar for both approaches.

Normal Range

The term "defined" in this context refers to the standard value that is often used to accurately identify the genus and species of a certain Gram-negative bacterial panel. Nevertheless, this measurement may be characterized in several ways depending on the specific objective and intended purpose for reporting the outcomes. An example may be the existence or nonexistence of a certain target gene.

Measurable Range

Here, the term "output result" refers to the information obtained from a process, which is often utilized for reporting purposes. This information usually includes the identification of the genus and species, but it may also include other details such as the serotype or other relevant information. Additional factors with stringent thresholds, such as coverage, Q30 scores, and read duration, may be necessary depending on the intended use of the outcome.

Discussion

Emerging from the COVID-19 pandemic, it is evident that public health laboratories must be prepared to manage future outbreaks. The rise of new infections and the spread of existing antibiotic resistant threats will probably increase the workload in public health in the next several decades. The use of sequencing, particularly via automation, is in its early stages of addressing public health requirements and assisting in clinical diagnostic and therapy determinations. Collaborating with research, commercial, and clinical labs is crucial to enable a smooth progression from discovery and design to diagnosis,

practice, and expansion. Continued progress in NGS automation is anticipated, leading to increased prevalence of new and improved systems and instruments, particularly as they become more efficient and cost-effective. Hence, it is vital for several public health labs to contemplate the platforms and technologies that would be most suitable for their employees, patients, and financial resources.

Conclusion

In conclusion, automation is crucial for developing testing capabilities and decreasing the burden of manual testing methods. While automation is dependable and efficient, it may also be intricate and introduce novel learning difficulties in order to be used effectively. We advise public health labs to demonstrate patience throughout the process of purchasing new apparatus. It is important to be flexible and generous with the time and resources needed for successful implementation. Additionally, effective communication with others is crucial for problem-solving and troubleshooting. The use of automation is becoming more prevalent, and there is a developing network of labs and public health facilities that may collaborate to assure the continued effectiveness and success of automation.

References

1- **Chen, X, Kang, Y, Luo, J, Pang, K, Xu, X, Wu, J, et al.** Next-generation sequencing reveals the progression of COVID-19. Front Cell Infect Microbiol. (2021) 11:632490.
2- **Dougherty, CE, and Graf, E.** Next generation sequencing for outbreak investigation in the clinical microbiology laboratory. Clin Lab Sci. (2019) 32.
3- **Malek, A, McGlynn, K, Taffner, S, Fine, L, Tesini, B, Wang, J, et al.** Next-generation-sequencing-based hospital outbreak investigation yields insight into Klebsiella aerogenes population structure and determinants of carbapenem resistance and pathogenicity. Antimicrob Agents Chemother. (2019) 63:e02577-18.
4- **Morash, M, Mitchell, H, Beltran, H, Elemento, O, and Pathak, J.** The role of next-generation sequencing in precision medicine: a review of outcomes in oncology. J Pers. Med. (2018) 8:30.
5- **The White House. (2021).** Available at: https://www.whitehouse.gov/briefing-room/legislation/2021/01/20/president-biden-announces-american-rescue-plan/ (Accessed March 22, 2023).
6- **Antonios, K, Croxatto, A, and Culbreath, K.** Current state of laboratory automation in clinical microbiology laboratory. Clin Chem. (2022) 68:99–114.
7- **Laboratory Automation Systems Global Market Report.** The business research company. (2022). Available at: https://www.reportlinker.com/p06280847/Laboratory-Automation-Systems-Global-Market-Report.html (Accessed March 22, 2022).
8- **Karakoyun HK, Sayar C, Yararbaş K.** Challenges in clinical interpretation of next-generation sequencing data: Advantages and Pitfalls. Results in Engineering. 2023 Dec 1;20:101421.
9- **Aziz, N, Zhao, Q, Bry, L, Driscoll, DK, Funke, B, Gibson, JS, et al.** College of American Pathologists' laboratory standards for next-generation sequencing clinical tests. Arch Pathol Lab Med. (2015) 139:481–93.

10- **Gargis, AS, Kalman, L, and Lubin, IM.** Assuring the quality of next-generation sequencing in clinical microbiology and public health laboratories. J Clin Microbiol. (2016) 54:2857–65.

11- **Centers for Disease Control and Prevention. T**he next generation sequencing quality initiative. (2022). Available at: https://www.cdc.gov/labquality/ngs-quality-initiative.html (Accessed March 22, 2023).

12- **Socea JN, Stone VN, Qian X, Gibbs PL, Levinson KJ.** Implementing laboratory automation for next-generation sequencing: benefits and challenges for library preparation. Frontiers in Public Health. 2023 Jul 13;11:1195581.

13- **Lepuschitz, S, Weinmaier, T, Mrazek, K, Beisken, S, Weinberger, J, and Posch, AE.** Analytical performance validation of next-generation sequencing based clinical microbiology assays using a K-mer analysis workflow. Front Microbiol. (2020) 11:1883.

التسلسل من الجيل التالي في الممارسة المعملية: التطبيقات والتحديات

الملخص

الخلفية - أبرزت جائحة COVID-19 الدور الحاسم للتسلسل من الجيل التالي (NGS) في الاختبار التشخيصي وعمليات الفحص القائمة على المراقبة. تحتاج مختبرات الصحة العامة إلى دمج هذه التقنيات الجزيئية المتقدمة لتعزيز قدراتها. ومع ذلك، فإن التحديات مثل نقص الموظفين المهرة، ونقص الخبرة في التسلسل، وقضايا توحيد المقايس، وإدارة عبء العمل ضمن أوقات التحول شائعة.

هدف العمل - يهدف هذا المقال إلى إلقاء الضوء على الفوائد والتحديات المرتبطة باستخدام أتمتة المختبرات لأغراض التسلسل. يستكشف استراتيجيات التنفيذ، بما في ذلك اختيار الأجهزة، ونهج التحقق، وتدريب الموظفين، وإصلاح الأعطال.

الطرق - للبحث عن المنشورات العلمية، تم البحث في قواعد البيانات الأكاديمية Scopus و Web of Science و PubMed.تم استخدام المعايير التالية للاختيار: يجب أن تكون المقالات مكتوبة باللغة الإنجليزية، وتركز على الأسباب المعيارية بدلاً من البحث التجريبي البحت، وتحتوي على ملخص للتحليل البرمجي، وتصف تقنية NGS. يستعرض المقال الأدبيات الحالية حول أتمتة المختبرات في سياقNGS ، ويحلل دراسات الحالة الناجحة، ويناقش الحواجز المحتملة والحلول.

النتائج - تسلط النتائج الضوء على إمكانات أتمتة سير العمل في معالجة العديد من التحديات التي تواجهها المختبرات. يمكن أن يحسن الكفاءة، ويقلل من الأخطاء البشرية، ويزيد من الإنتاجية. ومع ذلك، يتطلب الانتقال إلى الأتمتة التخطيط الدقيق، والاستثمار في التدريب، والدعم المستمر.

الاستنتاج - تقدم أتمتة المختبرات مزايا كبيرة لـNGS ، ولكن التنفيذ الناجح يعتمد على التغلب على مجموعة متنوعة من التحديات. من خلال معالجة هذه القضايا، يمكن للمختبرات تحسين قدراتها التشخيصية والاستجابة بشكل أفضل لاحتياجات الصحة العامة.