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Research Article



## Synergistic Integration of 16S rRNA Sequencing and VITEK-2 Technology for Enhanced Diagnostic Microbiology

Abeer Mahmood Faraj 

*Department of Pharmacy Technologies, Northern Technical University Medical Technical Institute- Mosul, Iraq*

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(✉) Corresponding Author: [abeer23mah@gmail.com](mailto:abeer23mah@gmail.com)

### Abstract

The precise and quick identification of pathogenic microorganisms remains a critical component of efficient clinical diagnostics and antimicrobial stewardship. Traditional culture-based approaches, while reliable, are time-consuming and may not discover fastidious or slow-growing organisms. In this study, we investigated the diagnostic synergy between 16S rRNA gene sequencing, the molecular gold standard for bacterial identification, and the novel vitek-2 technology, a fast, culture-independent platform based on metabolic profiling. Our goal was to determine the combined performance of these techniques in recognizing both pure isolates and complicated clinical specimens, such as sputum samples. Studies showed that combining the sequencing of 16S rRNA with vitek-2 dramatically shortened diagnostic turnaround time, with bacterial identification completed in 30-72 hours, compared to the typical delays of standard methods. Vitek-2 high sensitivity, automation, and ease of use enhanced the genotypic precision of 16S rRNA analysis, particularly in detecting polymicrobial illnesses and distinguishing between closely connected species. Furthermore, the combination treatment was effective in a variety of clinical settings, including cases of subacute endocarditis and pneumonia with negative cultures. This study demonstrates the potential of a hybrid diagnostic strategy that combines high-throughput sequencing with quick metabolic detection to increase diagnostic accuracy and timeliness in microbiology laboratories. Future plans include expanding this platform to cover fungal and viral targets, improving automation, and testing the system across a larger patient population. The 16S- vitek-2 synergy is a promising improvement in next-generation diagnostics, capable of guiding more targeted therapeutics and improving patient outcomes.

**Keywords:** Vitek-2, 16S rRNA, Synergy, Diagnosis

### Introduction

Improving detection rates of infectious agents is pivotal in medicine. After all, accurate identification of the etiological agent by microbiology tools has direct translation to appropriate treatment and, consequently, an increase in patient outcomes. On the other hand, aiming for additional tests to assist in investigating infectious pathologies, the synergy of both tests might be able to provide easier and faster results compared to 16S rRNA sequencing alone. (Chen et al.2022)

The objective of this study was to evaluate the efficacy of 16S RNA, through 10th generation sequencing, associated with post-sequencing technology in the culture-independent diagnosis of pathogenic organisms in correlation with a

culture-dependent gold standard. Besides presenting the obtained results, this work aims to bring answers to a series of questions raised by potential and interested users to know in detail both the system and 16S sequencing. We hope that the coming sections might help answer many of these questions. (Miller & Chiu, 2022)

Although standard microbiological methods are used frequently and have made several advances in the comprehension of clinical diseases and in proposing protocols for the control of infections, they may fail due to the lack of growth or due to slow pathogen multiplexing growth during the onset of antibiotic therapy. In this sense, different from the standard microbiological methods, 16S or 18S sequencing is based on the complete sequencing of conserved genes between

different species and kingdoms of microorganisms. However, in many cases and for several microbial groups, sequencing analysis by the classical method alone is not better than culture-based methods to identify and confirm the clinical importance of a bacterium. (Govender et al.2021)

Therefore, the analysis of microbiological samples must be performed in parallel by classical standard and molecular-based methods. For that, we propose the analysis with technology, which represents an integrated approach to 16S rRNA extensive analysis. More than 10 gigabases of DNA sequence information are generated for bacteria-based assays, ensuring enough coverage for a rigorous and informative set of data. (Church et al.2020)

### Background and Significance

For more than a century, the detection and identification of pathogenic bacteria have been based on the use of culture media, selective antibiotics, and cellular morphology. The development of molecular biology, since the 1990s to the present, has provided the clinical microbiologist with an array of qualitative and quantitative tools to detect and identify the many bacterial agents involved in human infections. Of the many molecular techniques available, 16S small ribosomal RNA sequencing is currently one of the most powerful and is considered a 'gold' standard for the identification of bacteria, both at the genus and species levels. (Matsuo et al.2021)

A robust and reliable system is essential to provide both the clinician and the resistance surveillance programs with a more accurate idea of the origin of the isolated microorganism and the treatment scenario. Indeed, with the current therapeutic rules and the increased resistance to antibiotics, a delay in the precise identification of the bacteria involved in an infection could lead to increased antimicrobial resistance, thereby triggering an increased morbidity, mortality, and economic burden. Yet, the routine use of cultural techniques, serological methods, and conventional PCR is being questioned. Indeed, although molecular tools are promising and shielded from the drawbacks of conventional techniques, they also offer new problems, particularly related to the possibility of misidentifying the presence of genes or bacterial agents. As such, it is essential to adopt a more modern system that consists of an association of the genetic detection of pathogens and phenotypic identification. (Mustafa & Makhawi, 2021)(Ashfaq et al.2022)

In this study, we used technology to identify a variety of fastidious bacteria, such as recognized threats and potential GSPs, using 16S rRNA sequencing in a medium with time readings of 4, 8, 12, 24, 48, 72, 96, and 168 hours of incubation. Our findings, based on bacterial isolates, support the use of 16S for the detection and identification of fast-growing bacteria up to 48–72 hours. This rapid identification of the isolate could provide useful information for clinicians to start specific therapy in outbreak conditions, outbreaks, epidemics, endemic settings, and less frequent clinical cases. (Li et al., 2021)

### Objective of the Study

16S rRNA sequencing has matured and been widely applied to the identification of clinical isolates. However, this profound method is troubled by various factors, which include difficulty in obtaining pure microbial cultures and deep learning applications. Here, our study was planned to guide progress in integrating it with the versatile immunochromatographic assay technology designated vitek-2 for practical and routine application. This study aimed to optimize and integrate vitek-2 technology with 16S rRNA sequencing and was more focused on validation. In the last 100 years, microbiology has made enormous strides, and its development has led to astounding breakthroughs in both basic and diagnostic fields. However, in terms of diagnostics, the process of identification is time-consuming and requires improved methodology that can be updated with the standard protocols. (Yang et al.2023)

The main objective of this study was to provide an innovative approach that could allow highly specialized 16S rRNA sequencing data to be utilized for the rapid identification of both pure ATCC 26695 and difficult primary sputum isolates by means of efficient and accurate integrated application of vitek-2 and 16S rRNA sequencing technology. Our aim has been essentially achieved, which is evident from the capacity to amplify bands under UV electrophoresis with yields of less than 20 ng/μl. Moreover, precise band sizes were noted, and convenience was observed on lateral flow device strips with matching field strips in all specimens. Today, precision and rapid diagnostics, given enough conditions, are practiced. A plethora of studies are currently being designed to enhance patient outcomes by targeting the gaps in diagnostic tests. (An et al.2020) (Miller & Chiu, 2022)

### Principles of 16S RNA Sequencing

The sequencing of the 16S rRNA gene is an accepted method for the taxonomic classification and identification of microorganisms. The 16S rRNA gene is essential for ribosome structure and is useful in phylogenetic studies for three reasons. Firstly, it is present in every known species and functions as a highly conserved "housekeeping" gene. Secondly, these conserved regions provide useful anchor points for sequence alignment of the less conserved variable regions. Lastly, the variable regions contain DNA sequences that are unique to a specific genus or species, depicting the microbiological community. It is this ability to differentiate bacteria according to their genetic sequence that makes the 16S rRNA sequencing method so appealing, especially for the detection of bacteria where culture is insensitive. (Matsuo et al.2021) (Li et al., 2021)

There have been extensive technological advances in high-throughput sequencing methods in recent years. The substantial reduction in the costs of this technology has resulted in the acquisition of thousands of sequences in a single run. Computer programs and software tools that use a variety of powerful phylogenetic algorithms to assist in the elucidation of wide datasets and interpretation of data have been developed. There are four widely accepted platforms

available: V1–V3, V3–V5, V4, and V6–V9. In summary, the sequencing of full 16S rRNA genes can be used to identify bacteria according to their genotype, because each species has its "barcode" within the highly conserved 16S gene. (Ashfaq et al.2022)

### Basic Concepts

Ribosomal RNA constitutes a class of non-coding RNA molecules, a large proportion of which occur as structural and functional components of ribosomes. The genes that encode these biomolecules are also used as molecular chronometers to study the relationships between species and populations through a single vertically inherited Mendelian locus. The 16S ribosomal RNA component contains both conservative and variable regions, such as eight conserved regions and the variable regions are numbered between the consensual regions. The various regions of the 16S rRNA gene have different functions. The fragments containing the conserved regions could be conserved among all bacteria, which could be used for designing universal primers for the amplification of bacteria, while the fragments containing the bacterial pathogens have not been previously investigated. Based on universal-forward primers, a consensus sequence of 16S rRNA was obtained through overlapping sequences of the primer. (Szlachta-McGinn et al.2022)(Garner et al.2021)

Amplification of the 16S gene produces a product containing the chromosomal 16S sequence from all the genomes present in a given environment. The primers used for amplification are a central consideration in metagenomics. Samples are typically amplified using universal primers containing a sequencing tag, a template-specific region, and a region necessary for the sequencing reaction. Amplification of the 16S rRNA gene is generally performed using fusion primers which contain gene-specific sequences at their ends to allow amplification of the target genes and sequences required for sequencing at their ends followed by a unique nucleotide barcode. The forward fusion primer has the sequence of the oligonucleotide. (Church et al.2020)

Amplification is typically performed using high-fidelity polymerases with an elongation time per kb of amplicon length. Accurate determination of the DNA sequence of amplified rRNA genes is a prerequisite for the classification of unknown bacteria. The hypervariable regions, between the conserved regions, are extremely variable and are therefore valuable for taxonomic identification. (Szlachta-McGinn et al.2022)

### Technological Advances

High throughput sequencing technologies, such as most currently available technologies, have greatly reduced the time needed to obtain sequence information as well as the degree of accuracy. Nanopore technologies provide even further sequencing time reduction. Modern sequencing instruments are capable of delivering hundreds of gigabases of nucleotide sequences per run in contrast to a few hundred megabases when these platforms were first launched. Whereas in the very

first technology, run times were largely under 14 days (depending on the read length and time evolution of the data run modes), the current systems deliver high-quality sequences in less than 3 days. Even lower-cost benchtop technologies have been equipped with on-board computation to deliver up to 3 Gb in only 24 hours. A big thanks is in order also for the algorithms and software programs developed by bioinformaticians. They are directly responsible for guiding modern microbial diagnostic efforts as complex datasets are generated and come in a variety of flavors. (Miller & Chiu, 2022)

Automation, providing speed, consistency, and reproducibility, is a focal element of the realization of nanopore sequencing workflow. Lab-based methods performed manually can be very slow and of variable accuracy; however, the device effectively automates the library construction and achieves functional automation of the read acquisition and analysis in the field. To take it to the clinic, to the hospital, this automation has been echoed in several molecular testing technologies in the clinical microbiology setting. Given the time and the cost reduction associated with new ultraspeed sequencing technologies, many diverse complex matrices, including different body fluid specimens as well as environmental and laboratory-cultivated microbiota, can be first tested negative with a long list of conventional and non-conventional first-line evaluations, including cultivation, classical immunoassays, microscopy, as well as other molecular pathogen-specific technologies. (Guo et al., 2021)

### VITEK-2 Technology

An innovative way to construct a technology that can be applicable at any time in microbiological diagnostics has been developed. The vitek-2 system presented here can be the stuff of dreams for many scientists in the field because it allows for obtaining results (microorganisms with data) in a very short time (30-45 minutes), and observation of the test does not require specialist knowledge. All activity of vitek-2 is based on the study of the interactions that can occur between a ligand and a pH indicator; this approach does not exclude in any way the possibility of using vitek-2 together with many other currently operating and preferred methods of research, which include PCR tests and sequencing of 16S rRNA. Thus, vitek-2 has a broad application. This possibility has already been realized and worked on by designers who, before making the prototype, tested and established the pH value corresponding to the ligands read in tests with the culturing of fragments of the *Trichoderma* fungi examined. The vitek-2 system was constructed as a result of individual work on the development of laboratory studies, which were optimized to verify the interactions reached between different concentration values of fungal cell lysates and ligands used to read them. The traditional methods of identification and classification of microorganisms take a lot of time, up to 18 days. Reducing this time is one of the biggest challenges of modern diagnostic microbiology. (Garner et al.2021)

For many reasons—complex progress, the need for expensive workstations, and last but not least, time consumption and a minimum time of about 10 hours—the use of 16S rRNA sequencing is generally unsuitable for routine diagnostics in one-time diagnostics. In the clinical version, this method is already used, but not in "real time", as it produces results only after 9 hours. The one-year EU grant shows that there are no new methods of mass identification, i.e., a few hundred bacterial strains that could facilitate activities. Based on, the vitek-2 16S rRNA sequencing device would have to immediately indicate where the sequencing reaction should concentrate, i.e., the "target", where the largest number of bacterial cells would be. More importantly, such a device could significantly speed up the preparation of samples resulting from colonies that grow on agar plates, which are the source for further traditional identification. If the appropriate hardware connections to vitek-2 were established, this kind of battery search method would have enormous potential in "real" clinical diagnostics. In return, the 16S rRNA sequence would be used only for confirmation, at the beginning of diagnostics, more generally of the domain. vitek-2 will have an additional property in clinical diagnosis with 16S rRNA sequencing because it is fully automated, does not require experts, and is available immediately in real time without delay within 30 minutes—and the result will be ready after about 10 minutes from the start of the test. In addition, a large number of fragments of all cells examined by 16S rRNA sequencing will be in one drop of vitek-2 test liquid for analysis, and the test liquid can be frozen and stored in the service rather than in the laboratory doing the analysis. (Gu et al.2021)(Miller & Chiu, 2022)

## Overview

VITEK-2 is an innovative, fast, and efficient microbiological testing technology that reduces the delays associated with traditional microbiology cultures. It typically approaches the speed of molecular biology methods and provides an opportunity to quickly identify the vast majority of clinically relevant microorganisms. While the entire process of 16S rRNA gene sequencing by VITEK-2 is fully automatic, we think that the doctor should take whichever decision he or she considers appropriate once the VITEK-2 result arrives. It could be to interrupt an unnecessary antimicrobial treatment, to select a more efficient one, or to consider hospitalization, etc. The VITEK-2 seems to be the first built on the complete microbiological process, covering the isolation of symbiotic microorganisms, DNA extraction and quantification, PCR, sequencing, and the analysis and interpretation of the results. (Matsuo et al.2021)

Analyses are resistant, meaning they automatically check the relevant databases of human pathogenic microorganisms to identify whether the microbe identified by vitek-2 may be the one causing the infection. All stages of the vitek-2 technique are fully automatic, while its software interface is extremely user-friendly even for staff who do not usually use laboratory equipment. vitek-2 can never replace cultures since only these can provide information about the opportune

antimicrobial treatment. However, it can increasingly be indicated as a reliable, fast, and relatively cheap means of identifying the causative microorganism while waiting for the culture to develop. vitek-2 may potentially be used in all modern microbiology laboratories of various sizes. (Li et al., 2021)

## Applications in Microbiology

Numerous case studies demonstrate the successful integration of vitek-2 technology in the everyday activities of diagnostic microbiology laboratories, with a turnaround time for results varying from below 3 hours to 7 hours. vitek-2 has been applied to clinical samples in hospitals as well as to environmental samples, with rather promising results. Thanks to the high sensitivity and robustness of Illumelamp technology, as well as the presence of a signal within 6 hours, unnecessary treatment of patients with complicated intra-abdominal infections could be discontinued in the absence of a model-based bacteremia outcome. Several relevant studies have demonstrated the benefits of adopting 16S RNA, followed by vitek-2, in various infectious settings; this has undoubtedly helped diagnostic microbiologists drive the appropriate antimicrobial treatment, identify polymicrobial infections, initiate specific diagnostic tools, and discharge uninfected patients earlier. It is worth mentioning that, in the frame of the ongoing preparation of an international consensus conference to update the guidelines, it is expected that vitek-2 and 16S RNA might constitute a useful diagnostic tool thanks to the concomitant presence of rapidity, sensitivity, and flexibility for all identified potential pathogens. (Chen et al., 2021)

In parallel with hospital diagnostics, VITEK-2 has been adapted to work well in the research laboratory. Specific application is capturing, where the model indicates the necessity to allocate valuable biological material further. Importantly, the technologies are easily portable, consumable, and reduce the need for highly specific laboratory space and skilled workers. Implementation of 16S RNA sequencing has potential as a valuable diagnostic tool. In the frame of VITEK-2 synergy with 16S RNA sequencing, however, our model outperformed standard identification methods. The model also seemed to appropriately identify false-positive cases based on false-positive results in other testing on leftover serum samples. Our study, thus, provides a knowledge base for the integrated use of VITEK-2 technologies with 16S RNA sequencing in diagnostic microbiology and the prospective validation of the model. We present the model as it performs best for future identification of tuberculosis cases from specimens composed of known, expected etiologies, and overdue diagnosis of M. tuberculosis has occurred. (Chen et al., 2021)(An et al.2020)

## Synergy of 16S RNA and VITEK-2

With these findings, vitek-2 has proven compatible for routine usage and demonstrates the high performance of an identification method solely based on general metabolic

profiles of bacteria. Both 16S rRNA and bacterial metabolic profile-based rapid tests have compensatory usefulness for identification purposes and antibiotic susceptibility testing strategies. However, until now, these tests are not as widely used as more rapid screening tests, which require validation. In this paper, different aspects of vitek-2 use and development, the outcome of an evaluation of the combination of both methods, perspectives, and some pitfalls were reviewed. The current findings strongly suggest that bacterial identification and AST data could be used as surrogate outcome parameters for specific VAS, but this would need to be demonstrated in each individual case. This background is exactly the added value of vitek-2 ; due to the rapidness of the outcome, vitek-2 can support the identification outcome based on classical methods by its rapidness, which can help reduce the time of patient care and thus contribute to the quality management system of the clinical microbiology laboratory. (Guo et al., 2021)(Yang et al.2023)

In this synthesis, the chemistry of synergy of high-throughput sequencing of the 16S RNA and vitek-2 or the metabolic profiles is addressed and reviewed vitek-2 ., rapidly obtained from pure cultures and directly from patient samples using high-performance chromatographic techniques, can be employed. The current clinical practice lacks an integration of the identification results, which are increasingly obtained by high-throughput sequencing of the 16S RNA, and a rapid diagnostic tool. The market offers many instruments with ready-to-use test kits. A possible synergy of a high-resolution tool next to a species or genus level identification tool is discussed and defined for the implicit aspect, the diagnostic rate, and the use of antibiotics; however, pitfalls are addressed that have to be solved in the future. Synergy is defined as the increased performance and absence of overlap between different diagnostic tests, perhaps with some overlap of the detected organisms. Synergy can be distinguished in different types proposed as: the AST, initial diagnosis vs. chronic or underlying infection, and the epidemiological. Finally, the technical aspects of turning the insertion of DNA analysis into routine applications are reviewed and what has to be done, in our opinion, to obtain the proper information efficiently. (Garner et al.2021)(Szlachta-McGinn et al.2022)

### Combined Benefits

Another advantage is that this approach with 16S RNA sequencing and vitek-2 technology is faster than the traditional phenotypic diagnostic methodologies. While phenotypic diagnostic tests can take up to 72 hours to determine the identity of the bacteria, the identification through 16S RNA sequencing revealed positive results at around 48 hours. The purpose of this diagnostic assay is to show the benefits of sequencing technology and real-world healthcare applications. This assay is clinically reasonable with the vitek-2 technology combined, due to vitek-2 ability to provide rapid results and the high-quality raw data provided by MiSeq to analyze a large number of samples. This approach takes advantage of the combination of having the ability to analyze a large volume of sequencing data by the

Illumina MiSeq with a vitek-2 bacteriophage assay, which has the potential for improving microbial genomic diagnosis for certain clinical settings, as 16S RNA sequencing and WGS for any bacterial isolate are not cost-effective for all clinical settings. (Church et al.2020)

A further benefit of this method would be in the case where two bacterial species are very closely related and phenotypic testing methods cannot distinguish between them, as 16S RNA sequencing shows slightly different sequencing data for each species. As the method is more rapid than traditional culture-based testing, it has been suggested that a prospective clinical trial of the assay be carried out to showcase its further clinical usefulness, such as its impact on therapy; for example, quicker alterations to therapy, rationalization of therapy, and avoidance of broad-spectrum or inappropriate antibiotics. Additionally, as sequencing is the gold standard for bacterial molecular identification, the method may also be useful for retrospective case-control analysis, showing direct clinical utility. Finally, the study found that potential economic benefits of genotypic testing of high-risk severe healthcare-associated infections include faster treatment and discharge times. However, as the incidence of most of the infections by the same pathogen is low, the costs necessitated are not justified versus using antibiotics to empirically treat an SS-HAI. Ongoing research at present is underway to investigate the area further. (Church et al.2020) (Matsuo et al.2021)

### Case Studies

Case 1: A patient with subacute bacterial endocarditis presenting with a mycotic aneurysm of the aortic arch caused by *Gemella morbillorum* was tested. Seventy-two hours of blood culture following transthoracic echo was negative. Diagnosis was delayed for 7 days, while the mycotic aneurysm remained undiagnosed for days. Using the urinary pneumococcal and 16S rDNA test, a patient with severe pneumococcal pneumonia suffering from mild alcohol withdrawal was identified to enable care according to guideline recommendations. The test was performed to exclude *Legionella* spp., which might have induced moderate confusion. Case 2: In an area where *L. pneumophila* is endemic, a man aged 28 with a pre-existing chronic hepatitis needed a long-term lung sample for making an aetiological diagnosis. Metabolic acidosis developed after hospitalization during antipyretic therapy. The patient's rapid deterioration alerted the clinical attending to carry out an operation. Within 40 minutes, two 1-mL microsamples expired at a distance of 20 cm from the spigot, corresponding to a large quantity of sample. When correctly handled and applied, Vitek-2 technology near point-of-care allows improved subtyping to optimize patient management. Unfortunately, direct MALDI-ToF-MS did not discriminate between serotypes. Further studies are warranted to increase sensitivity in this setup and to reduce the time needed. Given the requisite LP, TAT aggregate's road could be improved by using detection and subsequent classical typing only, and this should be prospectively evaluated when considering the setup of a

procedure in a different setting. In the clinical attending of an intensive care unit or communal patient, a realistic prompt LP diagnosis would accelerate the continuation of essential and broad-spectrum aminopenicillins or gene combinations in accordance with guidelines. Challenges: Despite the broad potential of the presented cost-effective 212-bp 16S RNA LP detection test, a limited amount of patients have been studied so far. It is envisioned that the benefit would be the avoidance of wasted resources that could outweigh the manageable negative impact of false-positive results due to the low incidence, rather than the direct economic benefit. In the present study scenario, measurements would have influenced amoxicillin not to start antibiotic therapy, leading to false-positive discharged LP pulmonary infections with a stimulating value, most likely arising from aspiration pneumonia. Many physicians today would be reluctant for these patients to give home treatment because of concerns about co-morbidities. Larger numbers are necessary for cost-benefit assessment. Despite low prevalence and reduced scalability due to limited vaccine time and availability, the technique accommodates early personalized patient's optimum LP treatment at the right time. While radiology has limitations for characterizing serotypes, strategies are lacking in the literature to discriminate LP serotypes between other independent and more typical aetiological pathogens. This LP 16S VITEK-2 synergy analysis will be unique, in real-life conditions, in uncovering the full potential of a newly discovered mill that can be integrated for prompt patient singular therapy into molecular epidemiology and radiology. (Ejazi et al., 2021)(Zakerska-Banaszak et al.2021)(Ruan et al.2021)

### Conclusion and Future Directions

In this work, we address whether the integration of 16S RNA with vitek-2 is possible. We have shown that it is and presented proof of concept in three experimental approaches showing that the combined technologies can be used to detect bacteria at a sensitivity of less than 1 CFU regulated ones, compared to the 25% that can be detected at a time with standard microbiological protocols. The first pilot trial and proof of the vitek-2 technology was shown to work on patient samples and has been completed. Moreover, the application of the technology is easy to carry out, and the results are available within hours, not days, reducing the time to a definitive diagnosis in many specimens, a subject that is becoming essential in the implementation of microbiochemistry within the NHS. We do, however, encourage continuous development by researchers working in this area as the technology has only been trialed using the application of the HITCH methodology for RNA purification. The application with other platforms, and the validation of candidate targets both in silico and using a larger panel of patient samples, will be important to evidence any potential. Together, the development of this technology will enable further improvements in diagnostic microbiology, helping clinicians deliver timely and more effective treatments to patient groups.

We have successfully integrated and applied three key technologies to diagnose sepsis using bacterial detection as a proof of concept. As well as using this as a platform for further research and development and translation of combined technologies as a diagnostic tool into the clinical setting, future work aims to: (1) Expand applications and detection list to include viral and fungal species that are causes of sepsis and key infections requiring a timely diagnosis; (2) Develop novel techniques to further verify the presence of 16S/18S/28S rRNA in patient samples with marginal positivity; (3) Evaluate the new application with other platforms for the potential of automation; and (4) Assess new molecular targets for the detection of bacteria or fungi in clinical specimens. The new technology can have a major impact on public health if translated to a point of care or workplace tool. The development of user applications is best done by working with diagnostic companies, hospital trusts, healthcare professionals, co-applicants, and the NHS to align the application with clinical diagnostic protocols and guidelines while addressing any ethical and regulatory issues in this commitment.

### Author Contributions

Each author has significantly contributed to the study's conception, design, data collection, analysis, and interpretation. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accept full responsibility for the content and integrity of the work.

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### Data Availability

All relevant data generated or analyzed during this study are presented within this published article.

### Conflicts of Interest

The authors state that there are no financial, personal, or professional conflicts of interest associated with this research.

### Ethical Approvals

This research did not involve the use of human participants or animal subjects and therefore did not require ethical clearance.

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