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Comparison evaluation of bacterial DNA extraction methods for improved molecular diagnostic accuracy of sepsis-causing pathogens in clinical whole blood samples

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Sepsis, a leading cause of mortality, requires rapid and accurate pathogen identification to ensure effective treatment. Current diagnostic methods such as blood cultures are time-consuming, whereas molecular diagnostic techniques represent a promising alternative for faster pathogen detection. Therefore, the aim of this study was to evaluate different DNA extraction methods for the improved detection of infectious pathogens in the bloodstream. Specifically, we compared one column-based DNA extraction method (QIAamp DNA Blood Mini Kit) with two magnetic bead-based DNA extraction methods (K-SL DNA Extraction Kit and GraBon™ system). Real-time PCR was performed using specific primers to assess the efficiency of each method. The K-SL DNA Extraction Kit and GraBon™ system exhibited higher accuracy rates of 77.5% (22/40) and 76.5% (21/40), respectively, compared to the QIAamp DNA Blood Mini Kit, which had an accuracy rate 65.0% (12/40) for *Escherichia coli* detection, whereas the GraBon™ system demonstrated higher accuracy rate of 77.5% (22/40) than the other two methods, which had an accuracy rates of 67.5% (14/40) for *Staphylococcus aureus* detection. All methods displayed high specificity for negative samples (100%). These findings highlight the superior performance of magnetic bead-based methods, particularly when automated, for extracting bacterial DNA from whole blood samples. Such methods may enable the more rapid and accurate diagnosis of bloodstream infections, potentially improving patient outcomes.

Keywords Sepsis, DNA extraction, Molecular diagnostic technique, Magnetic bead, Whole blood

Sepsis is a leading cause of mortality[1], whose successful treatment necessitates rapid and accurate identification of the causative pathogen. However, the prevalence of microorganisms involved in sepsis varies by region, with many pathogens responsible for bloodstream infections (BSI), including *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Klebsiella* spp., and *Acinetobacter* spp[2,3].

BSI is primarily diagnosed through a combination of patient factors, such as body temperature, pulse rate, respiratory rate, blood pressure, basic blood tests, and blood culture tests[4]. Blood cultures can confirm the causative pathogen; however, obtaining the final results often takes several days. Recent advances in molecular diagnostic methods, such as real-time multiplex PCR, have enabled the rapid and accurate detection of various BSI pathogens, and these methods now being introduced in clinical laboratories[5]. Molecular diagnostic tests have a substantially shorter turnaround time than blood cultures and achieve high sensitivity and specificity,

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Pathogen	Method	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Accuracy (Positive/Total)
E. coli	QIAamp DNA Blood Mini Kit	30 (16.56–46.53)	100 (91.19–100.0)	65.0% (12/40)
	K-SL DNA Extraction Kit	55 (38.49–70.74)	100 (91.19–100.0)	77.5% (22/40)
	GraBon™	52 (36.13–68.49)	100 (91.19–100.0)	76.5% (21/40)
S. aureus	QIAamp DNA Blood Mini Kit	35 (20.63–51.68)	100 (91.19–100.0)	67.5% (14/40)
	K-SL DNA Extraction Kit	35 (20.63–51.68)	100 (91.19–100.0)	67.5% (14/40)
	GraBon™	55 (38.49–70.74)	100 (91.19–100.0)	77.5% (22/40)

Table 1. Detection sensitivity, specificity, and accuracy of QIAamp DNA blood Mini kit, K-SL DNA extraction kit, and GraBon™ methods for *Escherichia coli* and *Staphylococcus aureus*.

Pathogen	Method	Results	K-SL DNA Extraction Kit			*p* value*	GraBon™			*p* value*
			Positive	Negative	Sum		Positive	Negative	Sum	
E. coli	QIAamp DNA Blood Mini Kit	Positive	8	4	12	0.031	10	2	12	0.022
		Negative	14	14	28		11	17	28	
		Sum	22	18	40		21	19	40	
S. aureus	QIAamp DNA Blood Mini Kit	Positive	8	6	14	>0.999	11	3	14	0.057
		Negative	6	20	26		11	15	26	
		Sum	14	26	40		22	18	40	

Table 2. Comparison of pathogen detection results among QIAamp, K-SL, and GraBon™ bacterial DNA extraction methods. **p* values were calculated using McNemar's test for discrepant results.

making them a promising diagnostic tool for BSI. In particular, the ability to directly detect pathogens in blood samples is crucial for rapid diagnosis[2,3].

Blood samples contain numerous PCR inhibitors (such as human DNA and hemoglobin), which can lead to various complications in the molecular diagnostic results[6]. Thus, the selection of an effective DNA concentration method and an efficient extraction platform that can isolate and purify DNA with high yield and purity is important for improving the results of molecular diagnostics[7–10]. DNA extraction methods can be broadly categorized into boiling, column extraction, and magnetic bead purification, each of which has its own advantages and limitations depending on the sample type and experimental objectives[11]. Many commercial DNA extraction kits and automated devices have been developed to detect pathogens in different types of sample. Column-based extraction methods such as the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) are widely used and often serve as benchmarks in research because of their high sensitivity[12]. Other notable products include the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, South Korea), a Korean product capable of rapidly extracting genomic DNA from various samples including whole blood (WB), and the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA), which is popular internationally[13,14]. Recently, magnetic bead-based extraction methods have gained attention owing to their ease of automation and their ability to yield high-purity DNA. Based on this technology, KingoBio, Inc. developed the K-SL DNA Extraction Kit (KingoBio, Inc., Seoul, South Korea) for bacterial DNA extraction from WB, incorporating bacterial isolation technology to enhance the extraction efficiency. Subsequently, KingoBio, Inc. developed GraBon™ (KingoBio, Inc., Seoul, South Korea), an automated platform that takes the process to the next level, utilizing the same reagents as the K-SL DNA Extraction Kit but employing robotic handling to increase throughput and consistency[15].

While molecular diagnostic techniques for BSI have advanced, there is limited research comparing magnetic bead-based extraction technologies, including those with bacterial isolation capabilities, to established column-based methods. This study aims to compare the molecular diagnostic accuracy of the K-SL Extraction Kit and GraBon™ with the QIAamp DNA Blood Mini kit, examining their potential advantages and clinical applicability.

Results

We determined the diagnostic sensitivity, specificity, and accuracy of each DNA extraction method for both *E. coli* and *S. aureus* in WB samples. For *E. coli*, the K-SL DNA Extraction Kit and GraBon™ demonstrated higher accuracy than the QIAamp DNA Blood Mini Kit (Table 1). The K-SL DNA Extraction Kit exhibited the highest accuracy at 77.5%, followed closely by GraBon™ at 76.5%, whereas the QIAamp DNA Blood Mini Kit showed lower accuracy of 65.0%. Statistical analysis demonstrated significant differences in accuracy between the two methods and that of the QIAamp DNA Blood Mini Kit ($p = 0.031$ and $p = 0.022$, respectively; Table 2).

For *S. aureus*, GraBon™ outperformed the other methods with an accuracy of 77.5%, whereas the K-SL DNA Extraction Kit and QIAamp DNA Blood Mini Kit both achieved an accuracy of 67.5% (Table 1). However, the differences in *S. aureus* detection accuracy among the three methods were not statistically significant (Table 2).

To assess the specificity, we evaluated 20 negative blood culture samples and 20 samples from routine health checkups. All three extraction methods consistently yielded negative results for bacterial detection in these samples, indicating 100% specificity for all tested methods (Supplementary Table 1).

Discussion

In this study, we evaluated three DNA extraction methods (QIAamp DNA Blood Mini Kit, K-SL DNA Extraction Kit, and GraBon™) according to their detection performance for *E. coli* and *S. aureus* in WB. The results demonstrated significant differences in performance, particularly regarding the ability to extract bacterial DNA from WB.

Both the K-SL DNA Extraction Kit and GraBon™ demonstrated significantly higher accuracy than the QIAamp DNA Blood Mini Kit for *E. coli*. This can be explained by differences in the bacterial lysis procedure between the methods. The K-SL DNA Extraction Kit and GraBon™ employ magnetic beads to isolate bacteria from the WB before lysis, providing a cleaner sample for DNA extraction. In contrast, the QIAamp DNA Blood Mini Kit performs bacterial lysis directly within the WB; thus, bacteria, blood proteins, enzymes, and other components are processed together, resulting in lower sensitivity and accuracy[7–10].

In the case of *S. aureus*, the superior performance of GraBon™, which was not statistically significant, may be explained by the structural differences between Gram-positive and Gram-negative bacteria. Gram-positive *S. aureus* possesses a thicker peptidoglycan cell wall which shows greater resistance to lysis than Gram-negative *E. coli*. This structural characteristic requires a powerful lysis method to efficiently extract DNA from *S. aureus*[16]. To address this, the GraBon system uses a unique lysis method based on a motor-driven rotating plastic tip for vigorous vortexing. This provided more effective disruption of the *S. aureus* cell wall. In contrast, the K-SL DNA Extraction Kit uses a gentle tube-mixing lysis method. The more aggressive lysis technique of the GraBon™ method contributes to its enhanced efficiency in extracting DNA from *S. aureus*.

GraBon™ can process 500 µL of sample and elute in 100 µL, effectively concentrating the DNA. In contrast, the K-SL DNA Extraction Kit and QIAamp DNA Blood Mini Kit handle 200 µL samples, eluting 100 µL and 200 µL respectively. This difference allows GraBon™ to achieve higher sensitivity. The ability to concentrate DNA from a larger initial sample volume (500 µL) to a smaller elution volume (100 µL) significantly improves the detection of low bacterial loads, which is crucial for clinical applications such as sepsis diagnosis. This concentration effect potentially leads to improved sensitivity in downstream applications like qPCR, which is critical for accurate pathogen detection.

Our study has several notable limitations. Primarily, the use of residual complete blood count (CBC) samples often results in insufficient blood volume for comprehensive testing across all three extraction methods. This limitation may have compromised the statistical power of our analyses, which was particularly evident in the *S. aureus* detection results. That is, although we observed differences in *S. aureus* detection performance among the methods, these differences did not reach statistical significance. Furthermore, our focus on only one species each of Gram-positive and Gram-negative bacteria may not fully represent the diverse spectrum of bacterial pathogens encountered in clinical settings. These limitations underscore the need for future studies with larger sample sizes and a more diverse range of bacterial species to provide a more comprehensive evaluation of extraction methods in clinical microbiology.

Despite these limitations, this study provides valuable insights into the performance of different DNA extraction methods for bacterial detection in WB samples. The findings highlight the advantages of magnetic bead-based methods, particularly when automated. The K-SL DNA Extraction Kit and GraBon™ showed superior performance for *E. coli* and *S. aureus* detection, emphasizing the importance of appropriate method selection in molecular diagnostics. These results have important implications for clinical microbiology, particularly for rapid and accurate BSI detection in WB samples.

Methods

Samples

A total of 120 WB samples were collected from January to December 2023 for this study. For samples in which *S. aureus* or *E. coli* were detected in blood cultures, additional residual samples from CBC tests collected on the same day were obtained. The sample set included 40 residual CBC samples of *S. aureus* and 40 residual CBC samples of *E. coli*. The sample set also included 20 CBC samples confirmed negative after blood culture and 20 samples from routine health checkups that were assumed to be free of bacterial contamination.

Bacterial DNA extraction was performed on all collected samples using three different methods: the QIAamp DNA Blood Mini Kit, the K-SL Extraction Kit, and GraBon™ system. All tests were conducted with approval from the Institutional Review Board of Ewha Womans University Seoul Hospital and Ewha Womans University Mokdong Hospital (IRB 2022-12-051 and 2023-05-005). The study was performed in accordance with relevant guidelines and regulations set by the same Institutional Review Board. Due to the use of residual samples and the absence of patient personal information, the IRB waived the requirement for informed consent. Throughout the study, patient confidentiality was maintained, and all samples were de-identified before analysis.

Bacterial DNA extraction methods

In this study, we compared three DNA extraction protocols for the detection *S. aureus* or *E. coli* in WB samples (Table 3). The following protocols were used.

The QIAamp DNA Blood Mini Kit is a manual bacterial DNA extraction kit designed for use with WB samples. In brief, 200 µL of WB was mixed with protease and a lysis buffer. After incubation at 56 °C for 10 min, ethanol was added to the cell lysates. The mixture was then transferred to a spin column and centrifuged. The

	QIAamp DNA Blood Mini Kit	K-SL DNA Extraction Kit	GraBon™
Sample volume (whole blood)	200 µL	200 µL	500 µL
Method	Manual	Manual	Automatic
DNA isolation method	Silica spin-column binding (centrifugation)	Magnetic beads + magnet rack	Magnetic beads binding + magnetic bar
Elution volume	200 µL	100 µL	100 µL
Concentration Factor	1 x	2 x	5 x

Table 3. Description of bacterial DNA extraction methods used for the detection of *S. aureus* and *E. coli*.

Bacteria (gene, amplicon size)	Primer orientation	Sequence (5'-3')	Reference
S. aureus (nuc, 207 bp)	F	ACACCTGAAACAAAGCATCC	[17]
	R	TAGCCAAGCCTTGACGAACT	
E. coli (eaeA, 150 bp)	F	GGCGGATTAGACTTCGGCTA	[18]
	R	CGTTTTGGCACTATTTGCCC	

Table 4. Nucleotide sequences of primers for real-time PCR amplification.

column was washed twice with two wash buffers to remove contaminants. Finally, the purified DNA was eluted with 200 µL of elution buffer.

The K-SL DNA Extraction Kit is a manual kit that isolates bacterial DNA using magnetic beads and a magnetic rack. In brief, 200 µL of WB was mixed with GraBeads™ to capture bacterial cells. The GraBeads™ -bound bacteria were then separated from the supernatant using a magnetic rack. Lysis buffer was added to disrupt the bacterial cells and release the DNA. A binding buffer was added to facilitate the attachment of DNA to the magnetic beads. Multiple washing steps were performed on the beads with bound DNA to remove impurities. Finally, purified DNA was eluted from the beads with 100 µL of elution buffer.

GraBon™ automates the K-SL DNA Extraction Kit process and can handle up to four samples simultaneously, utilizing a pre-filled well plate containing all required buffers. In brief, 500 µL of WB was initially mixed with GraBeads™ to capture bacteria. The system employs magnetic rods covered with plastic tips that move both vertically and horizontally to transfer GraBeads™ -bound bacteria through different wells. In the lysis buffer, the plastic tips rotate, creating vortices in the solution to enhance bacterial cell disruption. The magnetic rods then move the DNA-bound magnetic beads through the subsequent wells for automated washing to remove impurities. Finally, the beads were transferred to the elution well, where purified DNA was eluted in 100 µL of elution buffer.

Real-time PCR assay

For real-time PCR analysis, we employed 2 µL of the eluate obtained from each of the three extraction methods. Specific primers for *S. aureus* and *E. coli* were used to identify the DNA, and the results were compared with those from blood culture samples to confirm the presence of each bacterial species. Primers were designed using PrimerQuest, and the sequences of each primer are listed in Table 4.

The real-time PCR reaction mixture had a total volume of 20 µL, comprising 2 µL of sample DNA, 10 µL of iQ SYBR Green Supermix, 7 µL of distilled water, and 50 pmol of each specific primer. The amplification conditions were set for 40 cycles of 15 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C. Real-time PCR was performed in duplicate for both samples and controls using the QuantStudio 1 system (Applied Biosystems). RNase- and DNase-free water were used as non-template controls. The presence of target bacteria was confirmed by analyzing the melting temperatures, which are 80 °C and 88 °C for *S. aureus* and *E. coli*, respectively.

Statistical analysis

The sensitivity, specificity, and accuracy of the three methods (QIAamp DNA Blood Mini Kit, K-SL DNA Extraction Kit, and GraBon™) were analyzed at 95% confidence intervals using MedCalc. The sample size for each pathogen (*E. coli* and *S. aureus*) was $n = 40$. Positive and negative detection rates were compared using McNemar's test, which is appropriate for binary paired data. Comparisons of interest were: QIAamp DNA Blood Mini Kit vs. K-SL DNA Extraction Kit and QIAamp DNA Blood Mini Kit vs. GraBon™ for both *E. coli* and *S. aureus*. Data suitability was assessed, confirming no additional assumptions, such as normality, were required. Commercial software (MedCalc 23.0.5; MedCalc Software, Mariakerke, Belgium; SPSS 29.0; IBM Corp., Armonk, NY, USA) was used for the statistical analysis. All statistical tests were two-tailed, with an alpha level set at 0.05 for determining significance.

Data availability

The datasets generated during the current study are available from the corresponding author upon request.

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Author contributions

Conceptualization, B.N., J.P., J.L. and H-S.C.; Methodology, B.N., Y-H.K., E.P., J.L. and H-S.C.; Validation, B.N., J.L. and H-S.C.; Formal analysis, B.N., J.P. and S.P.; Investigation, J.P. and S.P.; Resources, J.P., J.J., J.L. and H-S.C.; Data curation, J.P. and S.P.; Writing – original draft, B.N., J.P, E.P., J.L. and H-S.C.; Writing – review & editing, Y-H.K., J.L. and H-S.C.; Project administration, J.L. and H-S.C.; Funding acquisition, J.L. and H-S.C.; Supervision, J.L. and H-S.C. All authors reviewed and approved the manuscript.

Declarations

Competing interests

This research was funded by KingoBio, Inc. and is a collaboration between KingoBio, Inc. and the Ewha Education and Research Center for Infection. B.N, S.P, E.P, J.J are employees of KingoBio, Inc., and J.L is the CEO of KingoBio, Inc. H-S Chung received research funding from KingoBio, Inc. This study was conducted in compliance with ethical guidelines under the supervision of Hospital-based Business Innovation Center and Ewha Institute of Convergence Medicine at Ewha Womans University Mogdong Hospital, and all authors ensured transparency and impartiality throughout the research process.

Additional information

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