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Review

Evaluation of the diagnostic effectiveness of next generation sequencing in sepsis etiology: a systematic review and meta-analysis

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Abstract

Introduction: Systematic evaluation of the diagnostic value of next generation sequencing (NGS) in sepsis etiology.

Methodology: We conducted a systematic search on four databases (Web of Science, Cochrane, PubMed, and Embase) and compiled diagnostic experiments using NGS to evaluate sepsis etiology. Two researchers conducted research and obtained data independently.

Results: Nine documents were included comprising 747 patients, 988 blood samples, 175 bronchoalveolar lavage fluid (BALF) samples, 16 cerebrospinal fluid samples, and one urine sample. The combined sensitivity of each study was 0.89 (95% CI: 0.82-0.95). The combined specificity was 0.40 (95% CI: 0.25-0.55). The combined positive likelihood ratio was 1.51 (95% CI: 1.18-1.98). The combined negative likelihood ratio was 0.28 (95% CI: 0.11-0.48). The diagnostic odds ratio (DOR) was 6.38 (95% CI: 2.53-15.32) and the area under the curve (AUC) was 0.84, (95% CI: 0.62-0.94).

Conclusions: Based on the data we collected, we found that compared with the blood culture technology, NGS has the advantages of high sensitivity and wide detection range, but its specificity was low. Further study is needed to confirm the value of NGS in the etiological diagnosis of patients with sepsis.

Key words: Sepsis; bloodstream infection; next generation sequencing; high-throughput nucleotide sequencing.

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Introduction

Sepsis is a life-threatening disorder characterized by organ dysfunction that is caused by a dysregulated host response to infection [1]. Sepsis and septic shock affect millions of people around the world every year, with one-third to one-sixth of patients dying [2-4]. Kumar *et al.* have shown that the delayed initiation of antimicrobial therapy is closely correlated to a higher mortality in patients with sepsis, with the mortality rate increasing with the delay in the use of antimicrobial drugs [5]. Therefore, the timely and accurate identification of pathogens is crucial to the clinical management and prognosis of patients with sepsis [6]. Currently, diagnostic procedures based on medical examination are recognized as the gold standard for diagnosing sepsis pathogens. However, the low positive rate, antibiotic contamination, and excessive use of antibiotics has greatly reduced their effectiveness [7]. In addition, despite biomarkers playing a certain role in the rapid identification of pathogens, it is currently difficult to accurately diagnose sepsis through the use of a single biomarker [8].

Since Sanger sequencing was first developed in 1977, DNA sequencing technology has gained momentum [9]. The development of next generation sequencing (NGS) technology, also known as high-throughput sequencing, improved the low throughput problem of the first generation of this sequencing technology [10]. Since 2000, massive parallel sequencing technology has made high-throughput sequencing possible. The development of pyrosequencing, reversible terminator sequencing, and oligonucleotide sequencing have greatly improved the throughput. Concurrently, the cost of sequencing has also been greatly reduced over time [11]. At present, the most common applications of NGS technology in diagnostic microbiology laboratories include targeted NGS and metagenomic next generation sequencing.

These techniques can quickly detect and comprehensively identify bacteria, fungi, and viruses without presuming the cause in advance [10]. Although this technology has the potential to become a powerful tool for the etiological diagnosis of sepsis, the efficacy of NGS for etiological diagnosis in patients with sepsis has yet to be precisely evaluated [12,13]. In this study, we conducted a systematic review of existing literature on the use of NGS for etiological diagnosis in patients with sepsis to provide a basis for subsequent clinical applications.

Methodology

Search strategy

This systematic review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement. We conducted a systematic search on four databases (Web of Science, Cochrane, PubMed and Embase) from inception to August 22, 2022. Two researchers (Dong Li and Yinghao Yang) independently searched the four databases. The search terms were "High-Throughput Nucleotide Sequencing, Next-Generation Sequencing, etc." (keyword 1) and "Sepsis; Bloodstream Infection, etc." (keyword 2). A complete list of the search terms and search strategy is provided in Supplementary Table 1. Two researchers (Dong Li and Ying Xie) independently screened the titles and abstracts of all retrieved records to exclude irrelevant studies. The remaining studies were assessed by reading the full text. Any disagreement was resolved by consensus or by involving an arbiter (Xiaofeng Hang).

Inclusion and exclusion criteria

The inclusion criteria for the articles were as follows: (i) in terms of subjects, patients with confirmed sepsis; (ii) the test group was evaluated using NGS, the control group was evaluated using conventional sequencing, and conventional sequencing was the gold standard; (iii) direct true positive, false negative, false positive, true negative rates, or sensitivity and specificity data were provided; (iv) articles written in English.

The exclusion criteria were as follows: (i) animal experiments; (ii) studies unable to provide relevant data in calculating sensitivity, specificity, and other relevant indicators; (iii) meta, reviews, meeting summaries, case reports, and letters.

Data extraction

The data collected independently by the two researchers (Dong Li and Yinghao Yang) included the author name, the year of publication, the country, the type of research, the number of research centers, the sequencing platform, whether samples were submitted for inspection at the same time, the criteria used for the diagnosis for sepsis, the source of the test subjects, the type and quantity of samples, and the type of microbiological test.

Study quality assessment

QUADAS-2 was used to assess the quality of the diagnostic accuracy of the studies. Study quality was evaluated based on the risk of bias and clinical applicability. The risk of bias included case selection, experiment to be evaluated, gold standard, case process, and progress. The clinical applicability included case selection, experiment to be evaluated, and the gold standard. Each result was expressed as high (risk), low (risk), or unclear.

Statistical analysis

R, version 4.2.2 (available at https://www.r-project.org/) and R packages of "meta," "mada," and "meta4diag" were used for meta-analysis. A bivariate model with random effects was applied to summarize the sensitivity and specificity of the NGS with 95% confidence intervals and plotted forest plots of sensitivity and specificity. The pooled prevalence of sepsis was calculated by pooling positive and negative predictive values, and Fagan's plot was provided. In addition, the threshold effect and heterogeneity were assessed. The Spearman correlation coefficient between sensitivity and false-positive rate was calculated, and a Spearman test of $p < 0.05$ indicated the presence of a significant threshold effect. The heterogeneity index (I^2) was used to determine the degree of heterogeneity of all studies. The analysis indices included sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio (DOR), and area under the curve (AUC). To explore the heterogeneity among the studies, we conducted subgroup analysis on the country, sequencing platform, whether samples were submitted for inspection at the same time, and sample type. Stata (version 15.0) was used to evaluate the publication bias of the literature using the Deeks' test. RevMan 5.3 was used to design the literature quality evaluation chart. Differences were considered to be statistically significant if $p < 0.05$.

Results

Literature search

Searching for English-language studies on the selected databases (Web of Science, Cochrane,

Table 1. Study details.

First author	Year	Country	Study type	Single-center / multicenter	Sequencing platform	Whether the samples were submitted at the same time	Gold standard	Patients
Yun Long [14]	2016	China	Prospective	Single	BGISEQ-100	Not mentioned	Not mentioned	ICU patients
Limin Sun [15]	2022	China	Prospective	Single	Not mentioned	Yes	sepsis 3.0 diagnostic criteria [16]	ICU patients
Jung-Yien Chien [17]	2022	China	Prospective	Single	Not mentioned	No	Not mentioned	ICU patients
Lili Wang [18]	2021	China	Prospective	Single	BGISEQ-50	Yes	sepsis 3.0 diagnostic criteria	ICU patients
Di Ren [6]	2021	China	Retrospective	Single	BGISEQ-50	Yes	sepsis 3.0 diagnostic criteria	ICU patients
Wang-Da Liu [19]	2022	China	Prospective	Single	MGISEQ-200	No	sepsis 3.0 diagnostic criteria	ICU patients with hematologic malignancies
Madiha Fida [20]	2021	USA	Prospective	Single	Illumina	Yes	sepsis 3.0 diagnostic criteria	Not mentioned
Silke Grumaz [21]	2019	Germany	Prospective	Single	Illumina	Yes	sepsis 3.0 diagnostic criteria	ICU patients
Ying Li [22]	2018	China	Prospective	Single	BGISEQ-100	Yes	sepsis diagnosis standards in 2012 International Guidelines for Management of Severe Sepsis and Septic Shock	ICU patients

PubMed, and Embase) according to the search strategy resulted in the identification of a total of 3067 documents. Next, the documents were screened according to the inclusion and exclusion criteria. As a result, a total of nine documents were included in this study. The corresponding search flowchart is presented in Supplementary Figure 1.

Documents

A total of nine articles were included in the analysis, comprising 988 blood samples from 747 patients, 175 bronchoalveolar lavage fluid (BALF) samples, 16 cerebrospinal fluid samples, and one urine sample. Further details are provided in Table 1 and 2.

Study quality

The quality and risk of bias were evaluated using QUADAS-2. As a result, a total of two studies were found to have a high risk of bias. The corresponding "high-risk" studies were found to use inappropriate exclusion criteria during patient selection. Further details have been provided in Supplementary Figure 2.

Inspection quality

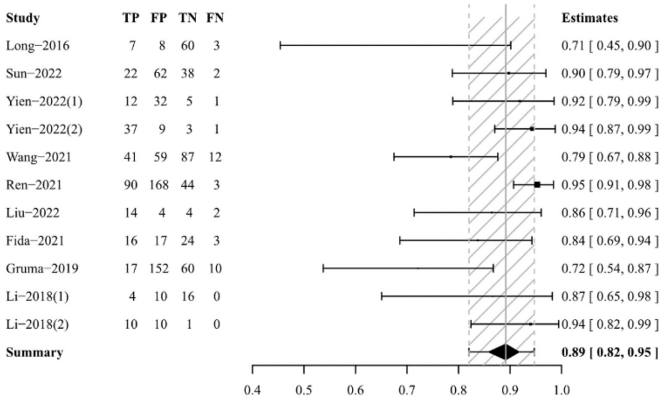
Assessment of threshold effect and heterogeneity between studies was performed using Spearman correlation coefficient and heterogeneity index (I^2). The Spearman correlation coefficient of sensitivity showed a false positive rate = 0.33 and p = 0.3176,

Table 2. The data of the included studies.

First Author	Year	Country	Sample size and type	Correlation and Concordance Between NGS and Blood Culture	TP	FP	FN	TN
Yun Long	2016	China	19 patients (78 blood specimens)	Not mentioned	7	8	3	60
Limin Sun	2022	China	124 patients (124 blood specimens)	Not mentioned	22	62	2	38
Jung-Yien Chien (1)	2022	China	50 patients (50 blood specimens)	12 double-positive cases, 0 case completely matched,9 cases partially matched，3 cases unmatched	12	32	1	5
Jung-Yien Chien (2)	2022	China	50 patients (50 BALF specimens)	37 double-positive cases, 1 case completely matched,27 cases partially matched，9 cases unmatched	37	9	1	3
Lili Wang	2021	China	199 patients (199 blood specimens)	41 double-positive cases, 16 case completely matched,13 cases partially matched，12 cases unmatched	41	59	12	87
Di Ren	2021	China	193 patients (184 blood specimens,104 BALF specimens, 16 CSF specimens, 1 urine specimen)	90 double-positive cases, 49 case completely matched,41 cases partially matched	90	168	3	44
Wang-Da Liu	2022	China	24 patients (24 blood specimens)	14 double-positive cases, 5 case completely matched,7 cases partially matched，2 cases unmatched	14	4	2	4
Madiha Fida	2021	USA	60 patients (60 blood specimens)	16 double-positive cases,11 case completely matched,5 cases partially matched	16	17	3	24
Silke Grumaz	2019	Germany	48 patients (239 blood specimens)	Not mentioned	17	152	10	60
Ying Li (1)	2018	China	30 patients (30 blood specimens)	4 double-positive cases,3 case completely matched,1 cases partially matched	4	10	0	16
Ying Li (2)	2018	China	30 patients (21 BALF specimens)	10 double-positive cases,1 case completely matched,9 cases partially matched	10	10	0	1

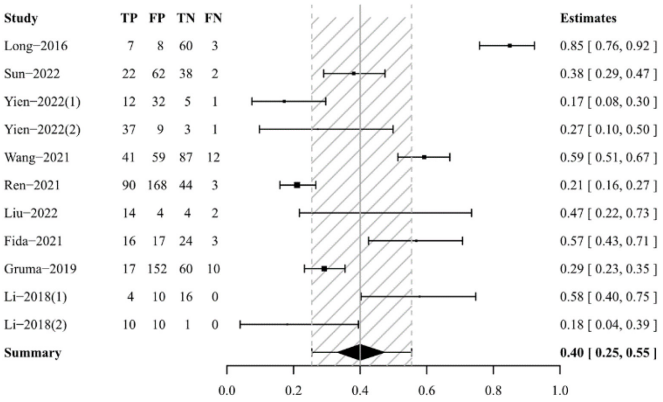
TP: True positive; TN: true negative; FP: false positive; FN: false negative.

Figure 1. The forest plot of pooled sensitivity of NGS.



The pooled sensitivity was 0.89 (95% CI: 0.82-0.95). TP, True positive; TN, true negative; FP, false positive; FN, false negative.

Figure 2. The forest plot of pooled specificity of NGS.



The pooled specificity was 0.40 (95% CI: 0.25-0.55). TP, True positive; TN, true negative; FP, false positive; FN, false negative.

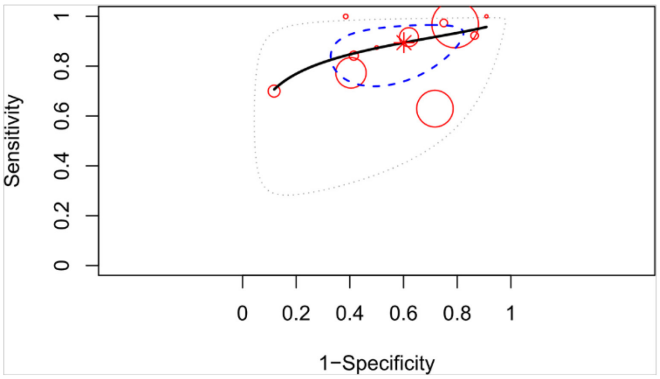
corresponding to the lack of a strong positive correlation, and there was no threshold effect. The calculated I^2 of DOR was 61.9% (p = 0.0034), suggesting that there was heterogeneity between studies, thus the random effect model was applied.

Diagnostic effectiveness of NGS in sepsis etiology

The pooled sensitivity of each study was 0.89 (95% CI: 0.82-0.95) (Figure 1), the specificity was 0.40 (95% CI: 0.25-0.55) (Figure 2), the positive likelihood ratio (LR+) was 1.51 (95% CI: 1.18-1.98), the negative likelihood ratio (LR-) was 0.28 (95% CI: 0.11-0.48), the area under the curve (AUC) was 0.84 (95% CI: 0.62-0.94) (Figure 3). This suggests that next generation sequencing technology has a high diagnostic efficiency in sepsis etiology, DOR was 6.38 (95% CI: 2.53-15.32). To evaluate the clinical utility of NGS, we constructed a Fagan nomogram (Figure 4).

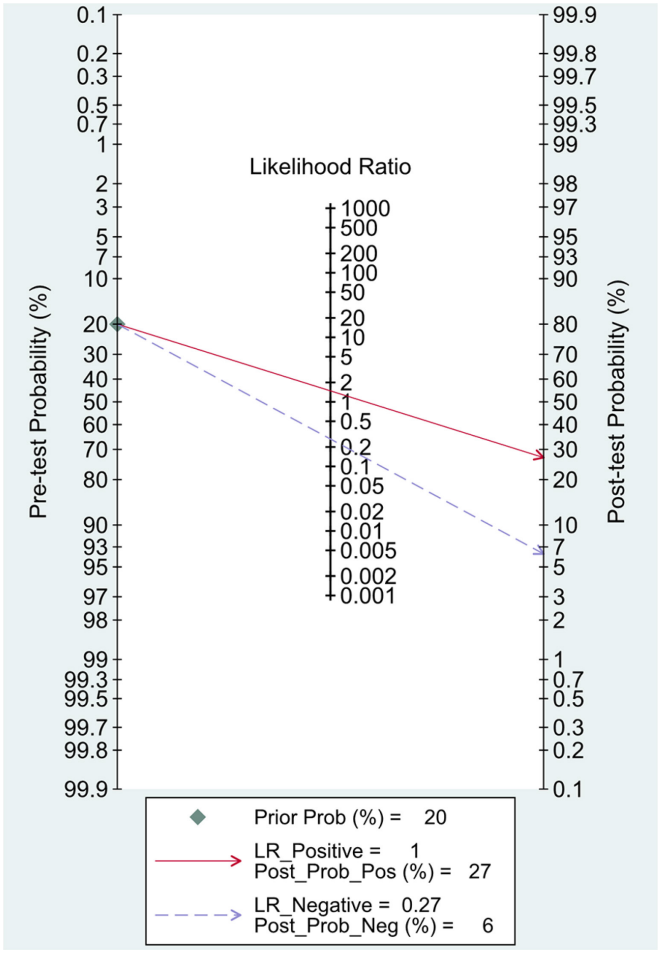
Under the assumption that the prior probability is 20%, the results indicate that if the test result is positive, the posterior probability of a positive sepsis etiology test was 27%, and if the test result is negative, the posterior probability was 6%.

Figure 3. SROC curve of the NGS for sepsis etiology.



The ellipse represents the 95% confidence region. SROC, Summary receiver-operating characteristic.

Figure 4. Fagan's plot to evaluate the clinical utility.



LR Positive, Positive likelihood ratio; LR Negative, negative likelihood ratio.

Subgroup analysis

To determine the source of heterogeneity, we separately conducted subgroup analysis on the country, sequencing platform, and sample type, as well as whether the samples were submitted for inspection at the same time. Further details on the corresponding data are provided in Supplementary Table 2.

From the analysis, we found that the sensitivity and specificity of the studies from China were 0.92 (95% CI: 0.85-0.96) and 0.40 (95% CI: 0.23-0.57), respectively, with an AUC of 0.84 (95% CI: 0.58-0.93). The sensitivity and specificity of studies that were not from China were 0.73 (95% CI: 0.5-0.9) and 0.42 (95% CI: 0.17-0.73), respectively, with an AUC of 0.74 (95% CI: 0.21-0.95). Both had high sensitivities.

The sensitivity of using the BGISEQ platform was slightly higher than that of the Illumina platform. Specifically, the combined sensitivity of two studies using BEISEQ-100 reached 100%.

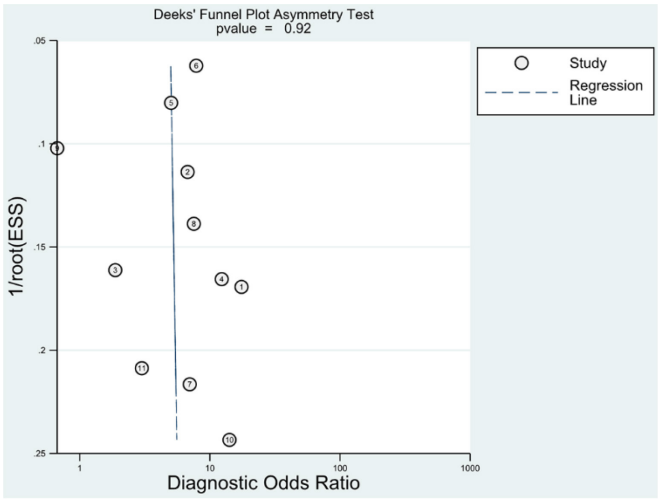
Regardless of whether the samples of conventional sequencing and NGS were submitted for inspection at the same time, both had high sensitivities. However, when the samples were not sent for inspection at the same time, the combined specificity was low, at only 0.25 (95% CI: 0.10-0.48).

Although the sensitivity of BALF was slightly higher than that of blood samples, its specificity was only 0.18 (95% CI: 0.05-0.39).

Publication bias analysis

According to Deeks' test, T = 0.10 and p = 0.92, suggesting that there was no publication bias (Figure 5).

Figure 5. The funnel plot.



Discussion

The detection technologies currently used for the clinical diagnosis of sepsis etiology have several disadvantages, including long detection cycles, the time-limited detection of pathogens, and the limited detection of pathogen types. Furthermore, rapid detection is not possible for unknown or rare pathogen infections [23]. At present, traditional bacterial culture is the gold standard for the diagnosis of sepsis etiology. However, the detection of fastidious bacteria and atypical pathogens using this method is hindered by technical limitations, in addition to overall low detection rates and longtime requirements, which in turn pose challenges to clinical diagnosis and accurate treatment. Studies have shown that approximately 60% of infectious diseases currently have unclear pathogen diagnoses [24]. The factors mentioned above may increase the rate of missed diagnosis, as well as the misdiagnosis of sepsis patients, and result in missing the time window for treatment, leading to life-threatening situations [25]. Therefore, there is an urgent need for the rapid and accurate detection of pathogens in the early stages of sepsis to reduce the mortality of sepsis and improve the prognosis of patients [26]. NGS can mitigate the limitations of traditional sequencing by directly sequencing the whole genome of the sample without bias, obtaining all the relevant information of the pathogen in the sample. At present, many studies have confirmed the feasibility of applying this technology to the etiological diagnosis of patients with sepsis and septic shock [27,28]. However, this study is the first to systematically evaluate the efficacy of NGS in the diagnosis of sepsis etiology.

Nine eligible studies were selected in this study, which included a total of 747 patients. The meta-analysis results showed that the sensitivity of NGS for the diagnosis of sepsis was 0.89, with a specificity of 0.40. The positive likelihood ratio was 1.51 and the negative likelihood ratio was 0.28, which indicated that there was a high possibility of excluding infection when the diagnosis result was negative. The DOR was 6.38 and the AUC was 0.84, indicating that the next generation diagnostic techniques had a high diagnostic value.

The analysis indicated that the most commonly detected microorganisms through NGS were symbiotic bacteria in the human microbiome. The top five pathogenic microorganisms with the highest detection rates were cytomegalovirus, *Klebsiella pneumoniae*, EB virus, *Candida parapsilosis*, and *Pseudomonas aeruginosa*. In both NGS and traditional sequencing, the top three pathogenic bacteria were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. In the 9 studies included, as

compared to blood culture, NGS was able to detect more types of pathogens, which means that NGS has a wider detection range, but at the same time, it also means low specificity.

By analysing the subgroups by country, the sequencing platform used, whether the samples were submitted for inspection at the same time, and the sample type, we found that NGS had a high sensitivity and low specificity in the diagnosis of sepsis etiology. This may be due to the low positive rate in the body fluid culture of patients with sepsis, leading to a significant increase in the false positive rate of next generation sequencing [29]. This resulted in a low specificity, especially with the BALF sample type, which only had a specificity of 0.18. However, considering that there were relatively few studies using BALF as the sample type, more studies will be needed to confirm the difference in the specificity between the different sample types.

Theoretically, NGS can detect the nucleic acid sequence of all pathogens. However, due to constraints in its clinical application, current technologies are not yet able to replace traditional sequencing and other detection technologies. For example, in the process of sample collection, storage, and transportation, if NGS is not handled properly, it can easily lead to sample contamination and nucleic acid degradation, as well as false positives and false negatives. To extract the nucleic acid sequence from the sample, it is necessary to distinguish the contamination bacteria from the colonization bacteria, as well as the host's nucleic acid sequence from the background nucleic acid. Moreover, the cost of NGS is higher than that of conventional detection technologies [30,31].

This study has some limitations. Firstly, all of the studies included in our analysis were single-center studies, and there was a lack of multi-center comparisons. Among the nine selected studies, seven were from China, and a comparison with more diverse populations would be needed. Furthermore, most of the study samples were small. As such, there is a need to conduct further studies in larger clinical settings.

To summarize, NGS was found to have a high sensitivity for the diagnosis of sepsis etiology. However, further high-quality, large-scale, and prospective studies are needed from multiple centers and different populations to verify the value of this technology in clinical applications.

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Informed Consent

Data analyzed in this meta-analysis were a re-analysis of previously existing data published in several studies. For such a meta-analysis, no formal ethical court vote was required according to our ethical court.

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Annex – Supplementary Items

Supplementary Table 1. The complete list of the search terms and search strategy.

Keyword1：	**Keyword2:**
High-Throughput Nucleotide Sequencing	Sepsis
High Throughput Nucleotide Sequencing	Bloodstream Infection*
Nucleotide Sequencing, High-Throughput	Infection, Bloodstream
Sequencing, High-Throughput Nucleotide	Pyemia*
Next-Generation Sequencing	Pyohemia*
Next Generation Sequencing	Pyaemia*
Sequencing, Next-Generation	Septicemia*
Illumina Sequencing	Poisoning, Blood
Sequencing, Illumina	Blood Poisoning*
Ion Torrent Sequencing	Poisonings, Blood
Sequencing, Ion Torrent	Severe Sepsis
Ion Proton Sequencing	Sepsis, Severe
Sequencing, Ion Proton	
Deep Sequencing	
Sequencing, Deep	
High-Throughput RNA Sequencing	
High Throughput RNA Sequencing	
RNA Sequencing, High-Throughput	
Sequencing, High-Throughput RNA	
Massively-Parallel Sequencing	
Massively Parallel Sequencing	
Sequencing, Massively-Parallel	
Pyrosequencing	
High-Throughput Sequencing	
High Throughput Sequencing	
Sequencing, High-Throughput	
High-Throughput DNA Sequencing	
DNA Sequencing, High-Throughput	
High Throughput DNA Sequencing	
Sequencing, High-Throughput DNA	

Search Strategy Used in PubMed 2022/08/22

No.	**Search items**	**Items found**
#1	"High-Throughput Nucleotide Sequencing"[Mesh]	49132
#2	((((((((((((((((((((((((((((High Throughput Nucleotide Sequencing) OR (Nucleotide Sequencing, High-Throughput)) OR (Sequencing, High-Throughput Nucleotide)) OR (Next-Generation Sequencing)) OR (Next Generation Sequencing)) OR (Sequencing, Next-Generation)) OR (Illumina Sequencing)) OR (Sequencing, Illumina)) OR (Ion Torrent Sequencing)) OR (Sequencing, Ion Torrent)) OR (Ion Proton Sequencing)) OR (Sequencing, Ion Proton)) OR (Deep Sequencing)) OR (Sequencing, Deep)) OR (High-Throughput RNA Sequencing)) OR (High Throughput RNA Sequencing)) OR (RNA Sequencing, High-Throughput)) OR (Sequencing, High-Throughput RNA)) OR (Massively-Parallel Sequencing)) OR (Massively Parallel Sequencing)) OR (Sequencing, Massively-Parallel)) OR (Pyrosequencing)) OR (High-Throughput Sequencing)) OR (High Throughput Sequencing)) OR (Sequencing, High-Throughput)) OR (High-Throughput DNA Sequencing)) OR (DNA Sequencing, High-Throughput)) OR (High Throughput DNA Sequencing)) OR (Sequencing, High-Throughput DNA)	136261
#3	("High-Throughput Nucleotide Sequencing"[Mesh]) OR ((((((((((((((((((((((((((((High Throughput Nucleotide Sequencing) OR (Nucleotide Sequencing, High-Throughput)) OR (Sequencing, High-Throughput Nucleotide)) OR (Next-Generation Sequencing)) OR (Next Generation Sequencing)) OR (Sequencing, Next-Generation)) OR (Illumina Sequencing)) OR (Sequencing, Illumina)) OR (Ion Torrent Sequencing)) OR (Sequencing, Ion Torrent)) OR (Ion Proton Sequencing)) OR (Sequencing, Ion Proton)) OR (Deep Sequencing)) OR (Sequencing, Deep)) OR (High-Throughput RNA Sequencing)) OR (High Throughput RNA Sequencing)) OR (RNA Sequencing, High-Throughput)) OR (Sequencing, High-Throughput RNA)) OR (Massively-Parallel Sequencing)) OR (Massively Parallel Sequencing)) OR (Sequencing, Massively-Parallel)) OR (Pyrosequencing)) OR (High-Throughput Sequencing)) OR (High Throughput Sequencing)) OR (Sequencing, High-Throughput)) OR (High-Throughput DNA	136261

	Sequencing)) OR (DNA Sequencing, High-Throughput)) OR (High Throughput DNA Sequencing)) OR (Sequencing, High-Throughput DNA))	
#4	"Sepsis"[Mesh]	136983
#5	((((((((((Bloodstream Infection*) OR (Infection, Bloodstream)) OR (Pyemia*)) OR (Pyohemia*)) OR (Pyaemia*)) OR (Septicemia*)) OR (Poisoning, Blood)) OR (Blood Poisoning*)) OR (Poisonings, Blood)) OR (Severe Sepsis)) OR (Sepsis, Severe)	260205
#6	("Sepsis"[Mesh]) OR ((((((((((Bloodstream Infection*) OR (Infection, Bloodstream)) OR (Pyemia*)) OR (Pyohemia*)) OR (Pyaemia*)) OR (Septicemia*)) OR (Poisoning, Blood)) OR (Blood Poisoning*)) OR (Poisonings, Blood)) OR (Severe Sepsis)) OR (Sepsis, Severe))	260205
#7	(("High-Throughput Nucleotide Sequencing"[Mesh]) OR ((((((((((((((((((((((((((((((High Throughput Nucleotide Sequencing) OR (Nucleotide Sequencing, High-Throughput)) OR (Sequencing, High-Throughput Nucleotide)) OR (Next-Generation Sequencing)) OR (Next Generation Sequencing)) OR (Sequencing, Next-Generation)) OR (Illumina Sequencing)) OR (Sequencing, Illumina)) OR (Ion Torrent Sequencing)) OR (Sequencing, Ion Torrent)) OR (Ion Proton Sequencing)) OR (Sequencing, Ion Proton)) OR (Deep Sequencing)) OR (Sequencing, Deep)) OR (High-Throughput RNA Sequencing)) OR (High Throughput RNA Sequencing)) OR (RNA Sequencing, High-Throughput)) OR (Sequencing, High-Throughput RNA)) OR (Massively-Parallel Sequencing)) OR (Massively Parallel Sequencing)) OR (Sequencing, Massively-Parallel)) OR (Pyrosequencing)) OR (High-Throughput Sequencing)) OR (High Throughput Sequencing)) OR (Sequencing, High-Throughput)) OR (High-Throughput DNA Sequencing)) OR (DNA Sequencing, High-Throughput)) OR (High Throughput DNA Sequencing)) OR (Sequencing, High-Throughput DNA))) AND (("Sepsis"[Mesh]) OR ((((((((((Bloodstream Infection*) OR (Infection, Bloodstream)) OR (Pyemia*)) OR (Pyohemia*)) OR (Pyaemia*)) OR (Septicemia*)) OR (Poisoning, Blood)) OR (Blood Poisoning*)) OR (Poisonings, Blood)) OR (Severe Sepsis)) OR (Sepsis, Severe)))	710

Search Strategy Used in EMBASE 2022/08/22

No.	Search items	Items found
#1	'high throughput sequencing'/exp	138168
#2	'high throughput nucleotide sequencing':ab,ti OR 'nucleotide sequencing, high-throughput':ab,ti OR 'sequencing, high-throughput nucleotide':ab,ti OR 'next-generation sequencing':ab,ti OR 'next generation sequencing':ab,ti OR 'sequencing, next-generation':ab,ti OR 'illumina sequencing':ab,ti OR 'sequencing, illumina':ab,ti OR 'ion torrent sequencing':ab,ti OR 'sequencing, ion torrent':ab,ti OR 'ion proton sequencing':ab,ti OR 'sequencing, ion proton':ab,ti OR 'deep sequencing':ab,ti OR 'sequencing, deep':ab,ti OR 'high-throughput rna sequencing':ab,ti OR 'high throughput rna sequencing':ab,ti OR 'rna sequencing, high-throughput':ab,ti OR 'sequencing, high-throughput rna':ab,ti OR 'massively-parallel sequencing':ab,ti OR 'massively parallel sequencing':ab,ti OR 'sequencing, massively-parallel':ab,ti OR pyrosequencing:ab,ti OR 'high-throughput sequencing':ab,ti OR 'high throughput sequencing':ab,ti OR 'sequencing, high-throughput':ab,ti OR 'high-throughput dna sequencing':ab,ti OR 'dna sequencing, high-throughput':ab,ti OR 'high throughput dna sequencing':ab,ti OR 'sequencing, high-throughput dna':ab,ti	127711
#3	#1 OR #2	179579
#4	'sepsis'/exp	318804
#5	'bloodstream infection*':ab,ti OR 'infection, bloodstream':ab,ti OR pyemia*:ab,ti OR pyohemia*:ab,ti OR pyaemia*:ab,ti OR septicemia*:ab,ti OR 'poisoning, blood':ab,ti OR 'blood poisoning*':ab,ti OR 'poisonings, blood':ab,ti OR 'severe sepsis':ab,ti OR 'sepsis, severe':ab,ti	50409
#6	#4 OR #5	336669
#7	#3 AND #6	1160

Search Strategy Used in Cochrane 2022/08/22

No.	Search items	Items found
#1	MeSH descriptor:[High-Throughput Nucleotide Sequencing]explode all trees	95
#2	(High Throughput Nucleotide Sequencing):ti,ab,kw OR (Nucleotide Sequencing, High-Throughput):ti,ab,kw OR (Sequencing, High-Throughput Nucleotide):ti,ab,kw OR (Next-Generation Sequencing):ti,ab,kw OR (Next Generation Sequencing):ti,ab,kw OR(Sequencing, Next-Generation):ti,ab,kw OR (Illumina Sequencing):ti,ab,kw OR (Sequencing, Illumina):ti,ab,kw OR (Ion Torrent Sequencing):ti,ab,kw OR (Sequencing, Ion Torrent):ti,ab,kw OR (Ion Proton Sequencing):ti,ab,kw OR (Sequencing, Ion Proton):ti,ab,kw OR (Deep Sequencing):ti,ab,kw OR (Sequencing, Deep):ti,ab,kw OR (High-Throughput RNA Sequencing):ti,ab,kw OR (High Throughput RNA Sequencing):ti,ab,kw OR (RNA Sequencing,	2603

	High-Throughput):ti,ab,kw OR (Sequencing, High-Throughput RNA):ti,ab,kw OR (Massively-Parallel Sequencing):ti,ab,kw OR (Massively Parallel Sequencing):ti,ab,kw OR (Sequencing, Massively-Parallel):ti,ab,kw OR (Pyrosequencing):ti,ab,kw OR (High-Throughput Sequencing):ti,ab,kw OR (High Throughput Sequencing):ti,ab,kw OR (Sequencing, High-Throughput):ti,ab,kw OR (High-Throughput DNA Sequencing):ti,ab,kw OR (DNA Sequencing, High-Throughput):ti,ab,kw OR (High Throughput DNA Sequencing):ti,ab,kw OR (Sequencing, High-Throughput DNA):ti,ab,kw	
#3	#1 OR #2	1643
#4	MeSH descriptor: [Sepsis] explode all trees	4960
#5	(Bloodstream Infection*):ti,ab,kw OR (Infection, Bloodstream):ti,ab,kw OR (Pyemia*):ti,ab,kw OR (Pyohemia*):ti,ab,kw OR (Pyaemia*):ti,ab,kw OR(Septicemia*):ti,ab,kw OR (Poisoning, Blood):ti,ab,kw OR (Blood Poisoning*):ti,ab,kw OR (Poisonings, Blood):ti,ab,kw OR (Severe Sepsis):ti,ab,kw OR (Sepsis, Severe):ti,ab,kw	8836
#6	#4 OR #5	9468
#7	#3 AND #6	17

Search Strategy Used in Web of Science 2022/08/22

No.	Search items	Items found
#1	High-Throughput Nucleotide Sequencing (Topic) or High Throughput Nucleotide Sequencing (Topic) or Nucleotide Sequencing, High-Throughput (Topic) or Sequencing, High-Throughput Nucleotide (Topic) or Next-Generation Sequencing (Topic) or Next Generation Sequencing (Topic) or Sequencing, Next-Generation (Topic) or Illumina Sequencing (Topic) or Sequencing, Illumina (Topic) or Ion Torrent Sequencing (Topic) or Sequencing, Ion Torrent (Topic) or Ion Proton Sequencing (Topic) or Sequencing, Ion Proton (Topic) or Deep Sequencing (Topic) or Sequencing, Deep (Topic) or High-Throughput RNA Sequencing (Topic) or High Throughput RNA Sequencing (Topic) or RNA Sequencing, High-Throughput (Topic) or Sequencing, High-Throughput RNA (Topic) or Massively-Parallel Sequencing (Topic) or Massively Parallel Sequencing (Topic) or Sequencing, Massively-Parallel (Topic) or Pyrosequencing (Topic) or High-Throughput Sequencing (Topic) or High Throughput Sequencing (Topic) or Sequencing, High-Throughput (Topic) or High-Throughput DNA Sequencing (Topic) or DNA Sequencing, High-Throughput (Topic) or High Throughput DNA Sequencing (Topic) or Sequencing, High-Throughput DNA (Topic)	314756
#2	Sepsis (Topic) or Bloodstream Infection* (Topic) or Infection, Bloodstream (Topic) or Pyemia* (Topic) or Pyohemia* (Topic) or Pyaemia* (Topic) or Septicemia* (Topic) or Poisoning, Blood (Topic) or Blood Poisoning* (Topic) or Poisonings, Blood (Topic) or Severe Sepsis (Topic) or Sepsis, Severe (Topic)	384143
#3	#1and#2	1180

Supplementary Table 2. Detailed data of the subgroup analysis.

Subgroup analysis				
Subgroup	**Number of studys**	**Pooled Sensitivity (95% CI)**	**Pooled Specificity (95% CI)**	**AUC (95% CI)**
Country				
China	7	0.92 (95% CI: 0.85-0.96)	0.40 (95% CI: 0.23-0.57)	0.84 (95% CI: 0.58-0.93)
Not China	2	0.73 (95% CI: 0.5-0.9)	0.42 (95% CI: 0.17-0.73)	0.74 (95% CI: 0.21-0.95)
Sequencing platform				
BGISEQ-100	2	1 (95% CI: 1-1)	0.55 (95% CI: 0.11-0.91)	0.95 (95% CI: 0.12-0.99)
BGISEQ-50	2	0.88 (95% CI: 0.62-0.99)	0.39 (95% CI: 0.13-0.72)	0.79 (95% CI: 0.32-0.99)
Illumina	2	0.73 (95% CI: 0.50-0.90)	0.42 (95% CI: 0.17-0.73)	0.74 (95% CI: 0.20-0.92)
Whether the samples were submitted at the same time				
Yes	6	0.89 (95% CI: 0.78-0.96)	0.39 (95% CI: 0.25-0.53)	0.82 (95% CI: 0.68-0.97)
No	2	0.93 (95% CI: 0.85-0.98)	0.25 (95% CI: 0.10-0.48)	0.93 (95% CI: 0.28-0.98)
Sample type				
Blood	8	0.81 (95% CI: 0.74-0.89)	0.50 (95% CI: 0.32-0.67)	0.81 (95% CI: 0.42-0.89)
BALF	2	0.97 (95% CI: 0.89-1.00)	0.18 (95% CI: 0.05-0.39)	0.98 (95% CI: 0.21-0.99)

Supplementary Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart of included studies.

Supplementary Figure 2. Quality Assessment of Diagnostic Accuracy Studies-2 tool assessment for bias.