Research Protocol

(Version No.: 1.0; Version Date: 2026.01.31)

Project Title： A Multicenter Prospective Study Evaluating the Diagnostic Performance and Impact on Clinical Outcomes of the NuRapid-CRISPR Pathogen Profile Assay in ICU Patients With Sepsis

Sponsor：

Responsible Department：

Principal Investigator：

Lead Unit：

Participating Units：

Investigator's Statement and Signature Page

As the Principal Investigator of this study, I hereby affirm that I will adhere to the ethical principles outlined in the Ministry of Health's "Measures for Ethical Review of Biomedical Research Involving Humans" (2016), the WMA "Declaration of Helsinki" (2013), CIOMS "International Ethical Guidelines for Biomedical Research Involving Human Subjects" (2002), and GCP. I will conduct the research according to the protocol approved by the Ethics Committee, under the guidance of Good Clinical Practice for pharmaceutical products, to ensure the scientific integrity of the study and protect the health and rights of the subjects.

Name: _____

Signature: _____

Date: _____

Protocol Summary

Protocol Title	Multicenter Prospective Study Evaluating the Diagnostic Performance and Impact on Clinical Outcomes of the NuRapid-CRISPR Pathogen Profile Detection Technology in ICU Patients with Sepsis
Version Number/Date	1.0/January 31, 2026
Sponsor and Participating Institutions	Shanghai Tongji Hospital; Shanghai Liangrun Biotechnology Co., Ltd., Dongfang Hospital Affiliated to Tongji University, Yangpu Hospital Affiliated to Tongji University
Principal Investigator	
Study Design	Multicenter Prospective Study
Study Objective	To evaluate whether a diagnostic and treatment strategy based on the NuRapid-CRISPR rapid pathogen detection technology can reduce the 28-day all-cause mortality rate in patients with sepsis/septic shock in the ICU compared to traditional pathogen culture.
Sample Size	The calculated sample size per group is 172 cases. Considering a 15% dropout rate, the final total sample size was determined to be 396 cases (198 cases per group). Based on the multicenter study design involving three hospitals, each center will enroll at least 132 cases (approximately 66 cases per group).
Study Population	This study is a cluster-randomized controlled trial. All patients meeting the following criteria will be enrolled and randomly assigned to a group (rapid diagnostic guidance group or standard care group) based on their ICU ward/time unit to receive the corresponding treatment strategy.
Study Methods	This study employs a practical cluster-randomized controlled design to evaluate the impact of the NuRapid-CRISPR rapid pathogen detection technology on diagnostic performance and clinical outcomes in ICU patients with sepsis. **1. Study Design and Grouping** A multicenter, prospective, cluster-randomized controlled design was adopted. ICU units or consecutive time periods participating in the study served as the units of randomization, and participants were cluster-randomized into the following two groups: Intervention Group (Rapid Diagnosis Strategy Group): Clinicians received NuRapid-CRISPR test results within

	2–4 hours and adjusted early antimicrobial therapy based on these results (in conjunction with clinical judgment). Control Group (Conventional Strategy Group): Followed standard clinical pathways; treatment was based on clinical experience prior to the availability of traditional culture results (typically 24–72 hours); NuRapid-CRISPR results were not used for clinical decision-making (blinded). **2. Study Population** Population: Adult patients with sepsis or septic shock admitted to the ICU and meeting the Sepsis-3 diagnostic criteria. Sample Size: Determined through statistical estimation based on a superiority hypothesis for the primary endpoint (28-day all-cause mortality). **3. Study Protocol and Data Collection** Concurrent Testing: Qualified specimens were collected from all enrolled patients and simultaneously submitted for NuRapid-CRISPR (study endpoint) and conventional microbiological culture (reference standard). Process and Outcome Measure Collection: Data on diagnostic turnaround time, time to adjust antimicrobial therapy, pathogen coverage, duration of organ support, ICU/total length of stay, and 28-day survival status will be prospectively collected. **4. Statistical Analysis** Primary Analysis: Using the intention-to-treat principle, the difference in the primary endpoint (28-day mortality rate) between the two groups will be compared. Secondary Analysis: Comparison of diagnostic accuracy metrics (sensitivity, specificity, etc.) and secondary clinical endpoints. **5. Quality Control and Ethics** An independent Data and Safety Monitoring Board (DSMB) will be established to conduct interim monitoring of safety and efficacy. The study protocol was approved by the ethics committees of each center; informed consent was strictly enforced to safeguard the rights of participants and ensure data security.
Inclusion Criteria	Age ≥ 18 years; length of stay in the ICU ≤ 24 hours; meets the Sepsis-3.0 diagnostic criteria (SOFA score increased by ≥2 points from baseline, with evidence of

	infection); clinically suspected sepsis or septic shock with unknown pathogen; plans to collect sterile or suitable specimens (e.g., blood, respiratory specimens, cerebrospinal fluid, ascites) for microbiological testing;Expected ICU stay of ≥48 hours, with the ability to complete at least 28 days of clinical follow-up; written informed consent signed by the patient or their legally authorized representative.
Exclusion Criteria	A definitive microbiological diagnosis (based on microbial culture, reliable molecular testing, or serological evidence) has already been established at admission, and targeted antimicrobial therapy against the identified pathogen has been initiated for more than 48 hours; Vital signs are extremely unstable, with death expected within 24 hours;Severe underlying immunodeficiency (e.g., AIDS, active hematologic malignancy, post-solid organ or hematopoietic stem cell transplantation, long-term use of high-dose glucocorticoids [prednisone ≥20 mg/day or equivalent dose for more than 4 weeks] or other potent immunosuppressants); Pregnant or lactating women; The patient or their authorized representative explicitly refuses to undergo any pathogen testing; Inability to obtain a suitable specimen for testing due to anatomical, physiological, or technical reasons; concurrent participation in another interventional clinical trial that may interfere with the assessment of the primary outcome of this study; refusal by the patient or their authorized representative to participate in this study.
Endpoint for Study Completion	**1. Completion as planned** Primary objective met: Successful recruitment and completion of the protocol-specified total sample size for subject enrollment, intervention, and follow-up of the primary endpoint (survival status at Day 28). **2. Early termination based on the recommendation of the Independent Monitoring Committee** The Independent Data and Safety Monitoring Board (DASMB) recommends early termination of the study based on predefined interim analysis results, citing clear reasons related to efficacy, futility, or safety, and such recommendation is approved by the Study Steering Committee

	3. Termination Based on External Requirements Termination of the study is required by the Institutional Review Board (IRB) or relevant regulatory authorities based on sufficient grounds. The study cannot continue due to force majeure (e.g., major public health events, policy changes). **4. Termination Based on Study Feasibility** The study is assessed as unable to yield scientifically valid conclusions due to severely insufficient enrollment rates, protocol violations, or the unavailability of essential or critical study resources.
Withdrawal/Exclusion Criteria	The patient, legal representative, or guardian voluntarily withdraws informed consent; following enrollment, verification reveals that the subject does not meet the inclusion criteria or meets any of the exclusion criteria; a serious adverse event directly related to the testing procedure occurs during the study, and the Safety Review Committee determines that the subject's participation must be terminated; results from NuRapid-CRISPR or conventional culture are invalid due to failure in specimen handling, transport, storage, or testing, and a qualified specimen cannot be reobtained;Key data cannot be obtained due to errors in the execution of the study protocol; testing fails due to malfunctions of reagents, consumables, or equipment, and the failure cannot be remedied; key follow-up data required to assess primary study endpoints (e.g., 28-day survival status) cannot be obtained.
Early Withdrawal Criteria	**1. Voluntary withdrawal by the subject** The patient or their legal representative voluntarily requests withdrawal from the study at any time, without providing a reason. **2. Withdrawal at the Investigator's discretion (for safety or scientific reasons)** A serious adverse event clearly related to study procedures or the intervention strategy occurs, and continued participation in the study is deemed too risky. A significant change in the subject's condition (e.g., transition to hospice care, discontinuation of active treatment) renders the subject unable to continue adhering to the study protocol. It is discovered that the subject does not actually meet the inclusion criteria or meets the exclusion criteria (enrollment error).

	The subject commits a serious protocol violation, resulting in the inability to obtain valid study data. **3. Withdrawals related to the study process** Missing key data: Necessary data for the primary outcome analysis cannot be obtained (e.g., 28-day survival status is unknown due to loss to follow-up). Early study termination: The entire study is terminated prematurely due to safety, efficacy, or other reasons.
Dosage Regimen	This study does not involve drug research; therefore, there is no dosing regimen.
Primary Efficacy Endpoints	Pathogen Diagnostic Turnaround Time (TAT): The duration (in hours) from the completion of specimen collection to the issuance of a definitive diagnostic report; 28-day all-cause mortality in patients with sepsis: The proportion of deaths occurring within 28 days of enrollment relative to the total number of enrolled patients.
Secondary Efficacy Endpoints	Diagnostic performance metrics: positive rate, sensitivity, specificity, positive predictive value, negative predictive value, and AUC of the microbiological test; Treatment-related measures: time to initiation of targeted antimicrobial therapy, duration of broad-spectrum antibiotic use, number of antibiotic adjustments, and cost of antimicrobial therapy; Clinical outcome measures: ICU length of stay, total length of hospital stay, duration of mechanical ventilation, duration and dosage of vasoactive drug use, incidence and severity of multiple organ dysfunction syndrome (MODS), and medical costs during hospitalization; Safety indicators: Incidence of adverse events related to microbiological testing (e.g., bleeding after specimen collection, infection, etc.), and incidence of antibiotic-related adverse reactions.
Safety indicators	**1. Adverse Events and Serious Adverse Events** Incidence, type, and severity: Record and compare the occurrence of all adverse events and serious adverse events in both groups. Relevance to the study: The investigator will assess the causal relationship between each AE/SAE and the study intervention (rapid diagnostic information support) or study procedure (specimen collection). **2. Safety Events of Particular Interest** New or worsening organ dysfunction: Assessed based

	on changes in the daily SOFA score. Antimicrobial-associated adverse events: Including Clostridioides difficile infection, hepatic and renal toxicity, allergic reactions, etc. Signs of clinical deterioration related to diagnostic delay (with a focus on the conventional strategy group): such as escalation of antimicrobial therapy due to suspected treatment failure, or progression to septic shock requiring urgent intervention. **3. All-cause mortality** The 28-day all-cause mortality rate is the primary efficacy endpoint of this study and also the most critical overall safety indicator.
Study Timeline	Phase 1 (Months 1–6) is the preparation and initiation phase, primarily involving finalizing the protocol, obtaining ethical approval, staff training, and system testing, with the enrollment of the first subject serving as a milestone. Phase 2 (Months 7–30) is the core implementation phase, during which patient recruitment, randomization, intervention implementation, and data collection will continue under independent monitoring, with the goal of completing enrollment of the full sample size.Phase III (Months 25–33) is the follow-up and data lock-in period. After completing the 28-day follow-up for the last patient, final data cleaning will be performed and the database will be locked. Phase IV (Months 34–36) is the analysis and summary period, during which statistical analyses will be completed, summary reports and research papers will be drafted, and the final study report will be submitted.
Statistical Analysis Methods	Continuous variables will first undergo a Kolmogorov-Smirnov test to assess normality. Normally distributed data will be presented as mean ± standard deviation (X ± S), and comparisons between groups will be performed using an independent samples t-test; non-normally distributed data will be presented as median (interquartile range) [M (Q1, Q3)], and comparisons between groups will be performed using the Mann-Whitney U test.Categorical data are presented as frequency (percentage) [n (%)], and comparisons between groups are performed using the chi-square (χ^2) test or Fisher's exact test (when the expected frequency is < 5).
Publication Format	Publication of 1–2 SCI papers; Patent: Rapid detection

	of common ICU pathogens based on NuRapid-CRISPR; New device: NuRapid-CRISPR all-in-one point-of-care pathogen detection system
Study Duration and Follow-up Arrangements	Patients will be followed up via telephone or outpatient follow-up visits 7, 14, and 28 days after discharge to record their survival status, readmission status, continuation of antimicrobial therapy, and recovery of organ function.For patients who die during the follow-up period, the time of death and cause of death (sepsis-related or other causes) will be documented. Dedicated follow-up personnel will be assigned to maintain a follow-up registry; reasons for loss to follow-up will be analyzed and recorded to ensure a follow-up success rate of ≥90%. Patient privacy will be strictly protected throughout the follow-up process, and all follow-up data will be promptly entered into the study database and verified.

I. Study Objectives

1. Primary Objectives

Diagnostic Accuracy Study

Using traditional pathogen culture techniques as the reference standard, this study evaluates the diagnostic performance of the NuRapid-CRISPR technology in pathogen detection through concurrent testing of various qualified patient body fluid samples (blood, sputum, urine, and peritoneal fluid, etc.). Key comparative metrics include sensitivity, specificity, and positive/negative predictive values, with a direct comparison of the diagnostic turnaround time between the two methods.

Clinical Utility (Practicality) Cohort Study

Following the receipt of rapid molecular diagnostic results, patients will be naturally divided into a “rapid diagnostic guidance group” and a “conventional care group” based on whether clinicians adopt these results to guide early, targeted antimicrobial therapy.Through prospective follow-up, we will evaluate whether NuRapid-CRISPR-based rapid diagnosis can shorten the time required for optimizing antimicrobial therapy, improve the coverage of initial treatment targets, and ultimately improve key clinical outcomes for patients, such as 28-day all-cause mortality,

duration of infection-related organ dysfunction, and ICU length of stay.

II. Study Background

Sepsis is a syndrome characterized by a dysregulated systemic response to infection, leading to life-threatening organ dysfunction [1].The WHO's latest Global Burden of Disease study indicates that there are approximately 50 million sepsis cases worldwide each year, with over 10 million ultimately dying from the condition. Furthermore, 85% of sepsis cases originate in low- and middle-income countries, including China; thus, high incidence, high mortality, and a heavy disease burden characterize the condition [2].A 2020 epidemiological study of sepsis in Chinese ICUs led by Professor Qiu Haibo's team also found that sepsis patients accounted for approximately 20% of the total ICU population, with a mortality rate as high as 35.5% [3]. Consequently, sepsis is a common disease that poses a serious threat to human health today, and improving patient outcomes remains a critical challenge that critical care medicine urgently needs to address.

In clinical practice, sepsis often presents with subtle symptoms, making early definitive diagnosis a significant challenge; a substantial proportion of patients miss the optimal window for treatment due to delayed diagnosis [4].Although previous studies have demonstrated that early antibiotic therapy can significantly reduce mortality in patients with sepsis [5–7], it has not been clearly established that empirical broad-spectrum antibiotic therapy without a definitive pathogen identification can lead to a series of adverse events, such as allergic or hypersensitivity reactions, renal injury, thrombocytopenia, and antibiotic resistance, which similarly impact patient prognosis [8, 9].Therefore, **early definitive pathogen diagnosis not only reduces mortality in patients with sepsis but also avoids the adverse consequences of empirical broad-spectrum antibiotic therapy, making it of great significance for improving patient outcomes**.

Microbial culture is the most established method for bacterial detection, relying on the direct cultivation and inoculation of microorganisms, and currently serves as the gold standard for diagnosing pathogenic agents. However, many microorganisms are difficult to grow in culture media (e.g., Treponema pallidum, Bartonella spp.) or cannot be cultured at all (e.g., certain viruses), while others (e.g., mycobacteria and

fungi) may take several weeks to grow and form colonies [10–12]. Standard culture techniques also require substantial laboratory equipment, consumables, and time to detect pathogens. Only rigorously trained laboratory personnel can accurately interpret culture results, making it difficult for many non-specialists to perform these tests [13].

Advances in nucleic acid detection and genomic sequencing technologies have significantly transformed the study of pathogenic microorganisms. Compared to traditional methods, molecular biological detection techniques can improve detection efficiency for an increasing number of infectious pathogens, and such methods offer advantages such as greater speed, higher sensitivity, and greater specificity [14,15].High-throughput nucleic acid amplification methods have now been developed, generating vast amounts of data on various types of pathogens—such as bacteria, fungi, parasites, and viruses—that possess specific disease markers (e.g., virulence, antibiotic resistance, and susceptibility factors) present in various types of specimens, including blood, stool, swabs, urine, cerebrospinal fluid (CSF), and respiratory secretions [16,17]. Nucleic acid-based methods include polymerase chain reaction (PCR), high-throughput sequencing (mNGS, metagenomic Next-Generation Sequencing), and pathogen-targeted sequencing (tNGS, targeted Next-Generation Sequencing), among others [18,19].Molecular biological detection technologies provide sensitive and rapid detection for many pathogens; for example, PCR is widely used for the detection of pathogenic microorganisms due to its accuracy, speed, and sensitivity.Among these new PCR technologies, extreme PCR requires less time to detect microorganisms than traditional PCR due to its rapid heat conduction. When the concentrations of primers and polymerase are increased, the reaction cycle rate increases by 10–20 times, allowing results to be obtained in a short period [20].However, PCR technology also has significant shortcomings. One of the most notable limitations is the difficulty in distinguishing between live and dead pathogens [21]; due to PCR’s high sensitivity, sample contamination may yield misleading DNA results; primer design requires prior sequence data, limiting the technology to detecting only known pathogens or the presence or absence of specific genes.Furthermore, the primers used may anneal to similar DNA sequences and

anneal to the target DNA, thereby yielding incorrect results; incorrect nucleotides may be erroneously incorporated during PCR, leading to misleading results [22].

High-throughput sequencing technologies compatible across Nanopore and Illumina platforms are suitable for analyzing cell-free DNA (cfDNA) from various body fluids, ranging from low-cell-density cerebrospinal fluid (CSF) to purulent fluids with high human host DNA content, enabling the detection of different body fluid samples without the need for culture to obtain purified DNA [23].Due to the high background of human host DNA, purulent fluids typically indicate an infectious etiology, making them difficult to analyze via mNGS and thereby reducing detection sensitivity [24]. Furthermore, the coverage depth of sequencing samples may lead to false-negative results and complicate the interpretation of sequencing reports, which imposes certain limitations on the application of this detection technology [25].

Targeted sequencing technologies allow for the selection or enrichment of a specific organism or a group of organisms of interest, either before or after library preparation. PCR amplification and probe hybridization can be used to target specific organisms or communities of interest [26,27].All these methods feature minimal human DNA interference and higher detection sensitivity, making them suitable for sample types containing a large number of human cells (such as tissue or sputum) [19]. However, this targeted sequencing method can detect only a limited number of pathogens, and its lack of flexibility makes it unsuitable for on-site testing within healthcare facilities. Therefore, the development of new detection technologies to address the current limitations of pathogen detection techniques is crucial for shortening diagnosis time and improving diagnostic accuracy.

Point-of-Care Testing (POCT) technologies for nucleic acid detection of pathogens causing ICU infections have been a research focus both domestically and internationally in recent years.POCT applies technologies such as PCR (polymerase chain reaction), LAMP (loop-mediated isothermal amplification), and CRISPR (gene editing system) to rapid bedside detection of infectious pathogens. By identifying common sources of infection—including bacteria, viruses, and fungi—it can significantly shorten diagnostic time and holds significant clinical value for high-risk patients, such as those with sepsis [28, 29].Internationally, several relatively mature

nucleic acid detection technologies have been applied to POCT. The FilmArray system, launched by the U.S.-based company BioFire and based on multiplex PCR technology, can simultaneously detect multiple infectious pathogens (such as viruses, bacteria, and fungi) within approximately one hour [30]. Another U.S. company, Cepheid, offers the GeneXpert system, a leading POCT product that has been deployed globally for the rapid detection of pathogens such as Mycobacterium tuberculosis and MRSA [31]. With advancements in microfluidic technology and portable devices, miniaturized PCR devices are continuously being introduced, such as the MinION device from Oxford Nanopore Technologies in the UK, which uses nanopore sequencing technology for pathogen DNA/RNA detection and has been deployed in multiple emergency medical settings. In China, POCT research is aligning with international trends, particularly in technologies such as PCR and LAMP, with several products already developed.Companies such as Maccura and BGI Genomics have successively launched portable PCR-based testing devices [32,33], suitable for scenarios requiring rapid diagnosis, such as ICUs. Maccura's nucleic acid testing platform enables simultaneous detection of multiple pathogens and reduces testing time to less than one hour.Companies such as Aikon Biotech use LAMP technology to rapidly detect various infectious pathogens and read results on handheld devices, making them suitable for hospital POCT settings [34]. Many domestic research institutions are also dedicated to developing multiplex nucleic acid detection technologies. For example, research teams from the Chinese Academy of Sciences and Peking University are exploring the combination of CRISPR and LAMP technologies for the rapid identification of pathogens in ICUs [35], though these have not yet entered clinical trials.

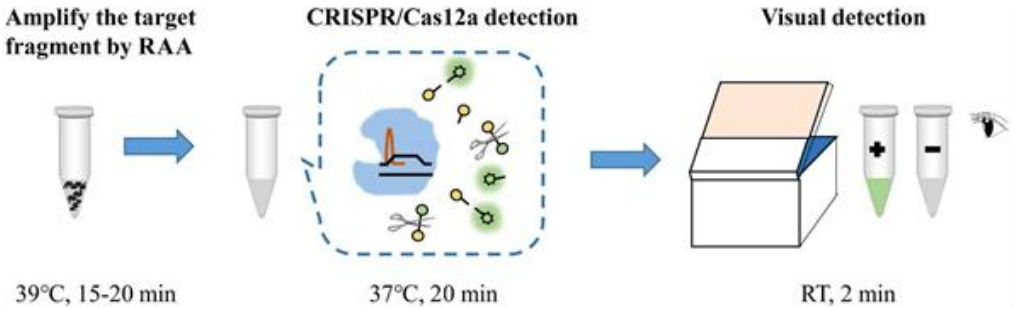

Figure 1: Schematic diagram of RCA/CRISPR-based detection technology

The NuRapid-CRISPR pathogen profiling technology, based on the CRISPR/Cas system, represents a "next-generation molecular diagnostic technology." The detection principle of this technology is as follows (see Figure 1 for details): When an exogenous gene invades a bacterium, the immune defense enters an adaptation phase, and the bacterium acquires the exogenous gene, which is inserted into its genome.Upon subsequent invasion by the exogenous gene, the CRISPR sequence, under the regulation of the leader region, transcribes CRISPR RNA (crRNA) and trans-acting crRNA (tracrRNA). TracrRNA pairs with crRNA to form guide RNA (gRNA), which forms a complex with Cas proteins to specifically recognize and cleave the exogenous gene for defense [36].By designing gRNA sequences, the gRNA/Cas protein complex can specifically recognize foreign fragments, activate the nuclease activity of Cas proteins, and act on fragments unfamiliar to the cleavage system [37].When the system contains a specifically modified "reporter probe," the activated Cas protein cleaves it; the integrity of the "reporter probe" is then used to detect the presence of "target DNA" in the system [38–40].RAC is an emerging isothermal nucleic acid amplification technique that utilizes three enzymes: recombinase (UvsX), single-strand binding protein (SSB), and DNA polymerase (Klenow). At an optimal temperature of 37°C, DNA fragments can be amplified within 5–20 minutes, enabling highly efficient DNA amplification [41].The combination of the CRISPR/Cas system with Recombinase-Assisted Amplification (RAC) enables rapid detection of pathogens in various clinical samples.

The NuRapid-CRISPR pathogen profiling technology first uses RAC-preloaded lyophilized beads to rapidly amplify nucleic acids in the sample, then employs CRISPR-preloaded lyophilized beads to rapidly detect the nucleic acid sample, and finally analyzes the collected fluorescent signals to generate a pathogen detection report for the sample.This technology utilizes the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, combined with Recombinase-mediated Isothermal Amplification (RAC), to establish an MTB-specific detection method, thereby enhancing the sensitivity of the detection system and achieving "two-stage" amplification of the detection signal.**The NuRapid-CRISPR POCT technology for**

pathogen profiling and antibiotic resistance gene profiling has been developed into 1-hour in-hospital rapid testing protocols for respiratory, urinary, bloodstream, reproductive, and central nervous system pathogens, as well as bacterial antibiotic resistance genes, providing clinicians with timely identification of pathogens causing refractory or critical infections. Furthermore, this testing eliminates the need for specialized technical expertise and sophisticated equipment, making it equally suitable for the vast majority of primary care hospitals.

Supported by the clinical study “Clinical Research on Rapid Pathogen Detection Based on pCIS” (Shanghai Tenth People’s Hospital Ethics Approval No.: 23KT82), which was initiated by the hospital in 2023, this method has undergone clinical validation for 10–30 cases across select targets, demonstrating a clinical concordance rate exceeding 90%. Sample types included swabs, sputum, and bronchoalveolar lavage fluid.However, **there remains a significant lack of relevant clinical research data on the NuRapid-CRISPR pathogen profiling technology across different body fluid samples, and the efficacy of this technology for rapid pathogen detection requires further evaluation**.

Therefore, this study, led by Tongji Hospital Affiliated to Tongji University in collaboration with Dongfang Hospital Affiliated to Tongji University, Yangpu Hospital Affiliated to Tongji University, and Shanghai Liangrun Biomedical Technology Co., Ltd., will enroll ICU patients with sepsis as study subjects.based on the choice of microbiological diagnostic methods, an intervention group (NuRapid-CRISPR detection group) and a control group (conventional pathogen culture group) will be established. A prospective cohort study will be conducted to evaluate whether the NuRapid-CRISPR technology can shorten the turnaround time for microbiological diagnosis in patients with sepsis and, through the early implementation of targeted antimicrobial therapy, significantly reduce the 28-day all-cause mortality rate in these patients.

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III. Experimental Basis

1. Animal experiments and literature review from previous studies

Animal Experimentation Background

To validate the analytical performance of the NuRapid-CRISPR detection system in complex biological samples, we have conducted preliminary animal studies. By establishing mouse models of bacterial (Klebsiella pneumoniae, Staphylococcus aureus) and fungal (Candida albicans) lung infections, we collected bronchoalveolar lavage fluid and blood samples at various time points post-infection. Using the preliminary sample processing workflow and reagent system, the results showed:

Sensitivity and Limit of Detection: In simulated samples with bacterial loads as low as 10^2 CFU/mL, the technology could still reliably detect the target pathogens. Its sensitivity was significantly higher than that of traditional culture methods and showed good agreement with qPCR results.

Specificity Validation: In a mixed infection model, the system accurately distinguished the target pathogen from the mouse's native commensal flora (e.g., enterococci, lactobacilli), with no non-specific cross-reactions observed.

Interference resistance: In alveolar lavage fluid samples containing high concentrations of host cell debris and inflammatory mediators, the optimized nucleic acid extraction protocol effectively removes inhibitors, ensuring the efficiency and stability of subsequent RCA/CRISPR reactions.

The above experimental results provide direct proof of concept for the feasibility of this technology in detecting pathogens in complex human body fluids (such as sputum and pus).

Literature and Technical Support

The implementation of this study is based on thorough literature review and a mature technical platform:

CRISPR diagnostic basis: Numerous published studies (e.g., J. S. Chen et al., Science, 2018; M. J. Kellner et al., Nat. Protoc., 2019) have demonstrated that CRISPR-Cas12a/Cas13a-based systems offer high specificity, high sensitivity, and

rapid response in pathogen nucleic acid detection, providing a solid theoretical foundation for the selection of this technical approach.

Multiplex Detection and Signal Amplification Strategies: Preliminary experiments have successfully combined rolling circular amplification (RCA) with CRISPR detection. RCA technology enables efficient isothermal amplification of low-abundance target nucleic acids, effectively enhancing detection sensitivity; the reliability of this methodology has been validated in publications such as Nucleic Acids Res.

gRNA Design Experience: The team has established a gRNA design workflow based on bioinformatics prediction and in vitro screening. For the target pathogens in this study (such as common Gram-negative/Gram-positive bacteria and fungi), we have preliminarily completed the computational design and specificity evaluation of candidate gRNA libraries, laying the foundation for subsequent experimental validation.

Preliminary Exploration of Freeze-Drying Process: Drawing on established experience in the freeze-dried preservation of vaccines and molecular diagnostic reagents (see relevant formulation studies), we have conducted preliminary screening of preservative formulations. Laboratory pilot tests indicate that the activity retention rate of freeze-dried RCA/CRISPR core reagents remains above 90% after one week of storage at room temperature, providing a process foundation for subsequent product development.

In summary, preliminary animal experimental data and systematic literature and technical reviews indicate that the NuRapid-CRISPR detection system is conceptually feasible and technically achievable, and has established a preliminary experimental foundation for transitioning from a laboratory system to clinical application. Building on the current foundation, this study will further address key translational challenges such as clinical sample compatibility, integration of the detection workflow, and standardization.

2. Criteria for Subject Selection

The subjects of this study are patients with sepsis or septic shock admitted to the ICU. This population was selected based on the following core criteria:

- Disease Severity and Clinical Need: Sepsis is a common critical condition in the ICU, and early, definitive pathogen diagnosis is key to improving prognosis. This population has the most urgent clinical need for rapid and accurate pathogen detection technology.
- Time-Sensitivity Challenges in Pathogen Diagnosis: Traditional microbial culture is time-consuming, often leading to delayed or blind initial treatment. If the novel rapid diagnostic technology (NuRapid-CRISPR) being evaluated in this study can shorten the time to diagnosis, it is expected to have the most direct and significant impact on clinical decision-making for this population.
- Suitability of the study design: The condition of sepsis patients changes dynamically, and adjustments to their treatment and clinical outcomes are easily observable and assessable, making this setting suitable for comparing the effects of different diagnostic strategies (rapid diagnosis vs. conventional diagnosis) on the treatment process and final outcomes.
- Feasibility: ICUs feature standardized diagnostic and treatment protocols, centralized patient resources, and comprehensive data recording systems, facilitating strict adherence to the study protocol, the implementation of a cluster-randomized intervention, and the collection of high-quality prospective data and follow-up.

Therefore, using ICU sepsis patients as the study population will most effectively evaluate the diagnostic efficacy of the NuRapid-CRISPR technology and its practical value in improving clinical outcomes, with study results possessing strong generalizability and clinical significance.

3. Basis for Dose Selection/Dosage Regimen/Dose Adjustment

This study is a clinical investigation of the diagnostic efficacy of the NuRapid-CRISPR pathogen profiling technology in ICU patients with sepsis and does not involve aspects such as dose selection, dosing regimens, or dose adjustments.

4. Basis for Endpoint Selection

Primary Endpoint:

- 28-day all-cause mortality: Death from any cause within 28 days from enrollment.

Secondary Endpoint:

- Time to First Targeted Therapy: The time (in hours) from enrollment to the first administration of an antimicrobial agent that covers the final confirmed pathogen (based on conventional culture or clinical diagnosis).
- Initial Treatment Adequacy Rate: The proportion of empirical antimicrobial regimens that cover the final confirmed pathogen within 24 hours of enrollment.

Clinical outcome measures:

- Length of ICU stay (days);
- Total length of hospital stay (days);
- Number of ventilator-free days (within 28 days);
- Number of days without vasopressor support (within 28 days);
- Incidence of new or worsening organ dysfunction (based on changes in SOFA score);

Health economics indicators:

- Total medical costs during hospitalization (RMB);
- Exploratory endpoints/diagnostic accuracy metrics (derived from the first part of the study):
- Diagnostic turnaround time, sensitivity, specificity, etc., of the NuRapid-CRISPR technology compared to conventional culture.

5. Risk and Benefit Rationale

(1) Potential Risks and Mitigation Measures

This study is a controlled trial evaluating the effectiveness of a diagnostic strategy and does not involve invasive therapeutic interventions for participants. Overall risks are manageable. The primary potential risks and corresponding mitigation measures are as follows:

Risk of diagnostic delay: For patients in the conventional strategy group, NuRapid-CRISPR results are blinded, and clinical decisions rely on traditional culture. Theoretically, there is a risk of delayed treatment due to diagnostic delay.

Countermeasures: ① The study strictly adheres to ethical principles; patients in

both groups receive current standard anti-infective diagnosis and treatment. The conventional strategy group follows the “Chinese Guidelines for Emergency Treatment of Sepsis/Septic Shock” for empirical treatment and pathogen testing, with the intensity of care consistent with routine clinical practice.② An independent Data and Safety Monitoring Board (DSMB) was established to monitor the study in real time. If interim analyses indicated that the primary outcome measures (e.g., 28-day mortality) in the conventional strategy group were significantly worse than those in the intervention group, the DSMB had the authority to terminate the study early and unblind the trial.

Data Breach and Privacy Risks: The study involves sensitive patient clinical information and genetic testing data.

Mitigation Measures: ① All data will be de-identified and assigned unique study identifiers. ② Data will be stored on a dedicated hospital server protected by firewalls and password security, accessible only to authorized researchers. ③ When study results are published, they will be ensured to contain no personally identifiable information.

Risks Related to Testing Technology: As NuRapid-CRISPR is a new technology under evaluation, there is a theoretical possibility of false-positive or false-negative results.

Mitigation Measures: ① All test results are provided solely as a reference for clinical decision-making; final diagnosis and treatment adjustments must be based on the physician’s comprehensive assessment of the patient’s full clinical presentation and should not serve as the sole basis for decision-making. ② Document all treatment adjustments made based on rapid test results and evaluate their concordance with final diagnostic outcomes in subsequent analyses.

(2) Expected Benefits

Direct and Potential Benefits to Participants:

Direct benefits: All enrolled patients will receive one free high-sensitivity molecular pathogen test, which will facilitate earlier and more comprehensive understanding of the infectious pathogen.

Potential benefits: For patients in the rapid diagnostic strategy group, earlier access to accurate pathogen-specific diagnoses may lead to faster initiation of targeted treatment, which is expected to improve infection control, shorten intensive care unit (ICU) stays, and ultimately improve prognosis.

Benefits to Science and Society:

Generation of high-level clinical evidence: This study will provide high-quality, multicenter, prospective, randomized controlled evidence from the Chinese population regarding the application of rapid molecular diagnostic technology in the critical setting of ICU sepsis.

Optimizing clinical pathways: The study results are expected to provide a decision-making basis for establishing a new clinical pathway for "rapid pathogen diagnosis and precision treatment of sepsis," thereby promoting the standardization and precision of sepsis diagnosis and treatment.

Promoting public health: Through early identification of pathogens and their antibiotic resistance, this study will aid in the precise prevention and control of nosocomial infections and the rational use of antimicrobial agents, playing a significant role in curbing bacterial resistance.

(3) Risk-Benefit Ratio Assessment

This study aims to evaluate a new clinical strategy designed to improve patient outcomes. The intervention employed (rapid diagnostic information support) is not physically invasive in itself; the primary risk lies in a theoretical potential for diagnostic delay compared to routine care. This risk has been minimized through rigorous ethical review, standard treatment protocols, and an independent monitoring mechanism.In contrast, the study offers participants the potential for improved clinical care and provides more effective diagnostic approaches for future sepsis patients, demonstrating clear scientific value and societal benefits. Therefore, the anticipated benefits of this study far outweigh the potential risks, and the risk-benefit ratio falls within a reasonable and acceptable range.

IV. Study Content

1. Study Population

This study is a whole-cohort randomized controlled trial. All patients meeting the criteria described below will be enrolled and randomly assigned to one of two groups (rapid diagnostic guidance group or standard care group) based on their ICU ward and time unit, where they will receive the corresponding treatment strategy.

2. Sample Size Calculation

Based on previous cohort studies and pilot study results related to the etiological diagnosis of sepsis, we hypothesize that the 28-day all-cause mortality rate in the conventional culture group is 35%, and that the NuRapid-CRISPR technology group can reduce the 28-day all-cause mortality rate by 12% (i.e., lowering the mortality rate to 23%).The significance level α is set at 0.05 (two-sided), the power (1-β) at 0.8, and the sample size ratio between the control and intervention groups is 1:1.The sample size formula for cohort studies is used: $n = (Z\alpha \times [2p(1-p)] + Z\beta \times [p0(1-p0) + p1(1-p1)])^2 / (p0 - p1)^2$, where p0 = 0.35,p1 = 0.23, p = (p0 + p1)/2 = 0.29, Z0.05 = 1.64, Z0.20 = 0.84. Calculations yielded a sample size of 172 participants per group. Considering a 15% dropout rate, the final total sample size was determined to be 396 participants (198 per group).Based on the multicenter study design involving three hospitals, each center must enroll at least 132 cases (approximately 66 cases per group).

3. Specific Research Content

This study will enroll patients who meet the inclusion criteria and will conduct studies on diagnostic accuracy and clinical utility. The specific study content is as follows:

Diagnostic Accuracy Study:

Study Population: All patients with sepsis included in the study.

Study Method: Prospective, blinded, paired design. For all eligible specimens (blood, sputum, urine, and peritoneal fluid, etc.) from enrolled patients, the test under evaluation (NuRapid-CRISPR technology) and the reference standard (conventional pathogen culture + clinical composite diagnosis) will be performed simultaneously. Laboratory personnel and result interpreters will be blinded to the results of the other

method.

Comparison Criteria:

Primary Outcomes: Calculate the sensitivity and specificity of the NuRapid-CRISPR technology for detecting various pathogens (bacteria, fungi, viruses, etc.).

Secondary endpoints: Calculate the positive predictive value and negative predictive value; plot receiver operating characteristic (ROC) curves and calculate the area under the curve (AUC).

Process indicators: Directly compare the diagnostic turnaround time (the duration from specimen submission to issuance of the official report) between the two technologies.

Clinical Utility Study: A Practical, Prospective, Cluster-Randomized Controlled Trial

Study Design: This study will employ a practical, prospective, cluster-randomized controlled design.

Randomization and Grouping: Using ICU wards or specific time units (e.g., 2-week periods) as the randomization units, participants will be cluster-randomized into the following two groups:

Intervention group (rapid diagnosis strategy group): NuRapid-CRISPR test results are provided to clinicians immediately upon reporting (e.g., within 2 – 4 hours), accompanied by expert recommendations for adjusting antimicrobial therapy based on the results.

Control Group (Conventional Strategy Group): Clinicians follow standard clinical pathways, initiating antimicrobial therapy based on clinical experience and routine indicators such as C-reactive protein prior to receiving traditional culture and susceptibility test results. NuRapid-CRISPR results are not used for clinical decision-making at this stage (but are collected as research data).

Comparison Parameters:

Treatment Process Measures: The primary comparison focuses on the time from enrollment to the initiation or adjustment of precision-targeted antimicrobial therapy, as well as the coverage of the pathogen spectrum in the initial treatment.

Clinical outcome measures: Comparison of 28-day all-cause mortality, duration of

infection-related organ dysfunction, duration of mechanical ventilation, ICU length of stay, and total hospital length of stay between the two groups.

Health economic indicators: Comparison of total medical costs during hospitalization.

Supporting Analyses:

Per-protocol analysis and intention-to-treat analysis.

Subgroup Analysis: Stratification by patient age (≥65 years vs. <65 years), severity of sepsis (sepsis vs. septic shock), and site of infection to explore differences in the effects of the rapid diagnostic strategy across different subgroups.

Adjustment analysis: A multilevel model will be used to adjust for potential baseline imbalances between groups.

Through the above design, this study will separately clarify the diagnostic accuracy of the NuRapid-CRISPR technology and its net benefits in altering decision-making and improving outcomes in real-world clinical settings, thereby providing a comprehensive evidence chain from multiple dimensions to support its clinical application.

V. Study Methods

1. Inclusion Criteria (Diagnostic Criteria, Inclusion Criteria, Exclusion Criteria)

Inclusion Criteria

- Age ≥ 18 years, and time since ICU admission ≤ 24 hours;
- Meets the Sepsis-3.0 diagnostic criteria (SOFA score increased by ≥2 points from baseline, and evidence of infection is present);
- Clinically suspected sepsis or septic shock with an unknown pathogen; plans to collect sterile or suitable specimens (e.g., blood, respiratory tract specimens, cerebrospinal fluid, abdominal fluid) for microbiological testing;
- Expected ICU stay of ≥48 hours, and ability to complete at least 28 days of clinical follow-up;
- The patient or their legally authorized representative has signed a written informed consent form.

Exclusion Criteria

- A definitive microbiological diagnosis (based on microbial culture, reliable molecular testing, or serological evidence) has already been established at admission, and targeted antimicrobial therapy against the identified pathogen has been initiated for more than 48 hours;
- Vital signs are extremely unstable, with death expected within 24 hours;
- Severe underlying immunodeficiency (e.g., AIDS, active hematologic malignancy, post-solid organ or hematopoietic stem cell transplantation, long-term use of high-dose glucocorticoids [prednisone ≥ 20 mg/day or equivalent dose for more than 4 weeks] or other potent immunosuppressants);
- Pregnant or lactating women;
- The patient or their authorized representative explicitly refuses any pathogen testing;
- Inability to obtain a suitable specimen for testing due to anatomical, physiological, or technical reasons;
- Concurrent participation in another interventional clinical trial that may interfere with the assessment of the primary outcome of this study;
- The patient or their authorized representative refuses to participate in this study.

Withdrawal/Exclusion Criteria

- The patient, legal representative, or guardian voluntarily withdraws informed consent;
- Verification after enrollment reveals that the patient does not meet the inclusion criteria or meets any of the exclusion criteria;
- A serious adverse event directly related to the testing procedure occurs during the study, and the Safety Review Committee determines that the patient's participation must be terminated;
- Invalid results for NuRapid-CRISPR or conventional culture due to failure in specimen handling, transport, storage, or testing, and an inability to obtain a qualified specimen;
- Key data cannot be obtained due to errors in the execution of the study protocol;
- Test failure due to malfunction of reagents, consumables, or equipment, and the

situation cannot be remedied;

- Key follow-up data required to assess primary study endpoints (e.g., 28-day survival status) cannot be obtained.

For patients who withdraw from the study, all data collected up to the time of withdrawal will be retained and included in the intention-to-treat analysis set for statistical analysis. For patients whose follow-up is terminated due to death, their data will be fully retained and used for the primary outcome analysis.

2. Subject Grouping

Treatment

Dose Selection/Adjustment

This study employs an efficacy-based cluster randomized controlled design, with the intervention being **clinical decision support based on the results of NuRapid-CRISPR rapid pathogen detection**.

Rapid Diagnosis Strategy Group:

Testing and Reporting: Eligible specimens from enrolled patients undergo NuRapid-CRISPR testing concurrently with submission for conventional culture. Test results (including pathogen species and antibiotic resistance gene information) are delivered to the attending physician via the hospital information system and/or telephone notification within 2–4 hours after validation.

Decision Support: The test report was accompanied by an abstract of the "Expert Consensus on Clinical Interpretation of Rapid Molecular Test Results and Treatment Recommendations," developed by experts in infectious diseases and clinical microbiology. Clinicians were encouraged and authorized to adjust antimicrobial treatment regimens as appropriate based on these rapid results and the patient's specific condition, even before receiving traditional susceptibility test results.

Documentation Requirements: The time of the decision to adjust antimicrobial therapy based on the rapid results, the specific regimen, and the rationale for the adjustment must be documented in detail.

Conventional Strategy Group:

Testing and Reporting: Specimens from enrolled patients are submitted for conventional pathogen culture and susceptibility testing only according to standard

clinical procedures. NuRapid-CRISPR testing is conducted concurrently, but its results are blinded to clinicians until the conventional culture report is issued and are not used as a basis for clinical decision-making.

Clinical Decision-Making: The initial selection and adjustment of antimicrobial agents are based entirely on clinical experience, routine inflammatory markers such as C-reactive protein, and subsequent traditional culture and susceptibility test results.

Data Documentation: All treatment decisions and their rationale are documented as usual.

3. Experimental Treatment

The core intervention of this study is the "clinical decision-support strategy based on NuRapid-CRISPR rapid pathogen detection results," rather than an investigational drug or device. Therefore, the relevant guidelines are as follows:

1) Provision and Adoption of Intervention Information

Intervention Group (Rapid Diagnostic Strategy Group): The research team will provide clinicians with clear information on the pathogen and resistance genes within 2–4 hours of the test report being issued, accompanied by treatment recommendations based on expert consensus.

Principles for Information Adoption: Clinicians should carefully consider these rapid results and make independent, comprehensive judgments based on the patient's complete clinical presentation (including signs, other laboratory tests, and imaging findings) to determine whether and how to adjust the antimicrobial treatment regimen. These results are not intended as mandatory treatment directives.

2) Time Window for Intervention Implementation

The provision of rapid diagnostic information and the resulting treatment adjustments primarily occur in the early phase after enrollment (prior to the reporting of conventional culture results), with the aim of evaluating the value of "early intervention."

3) Trial Blinding/Unblinding

Blinding Design: This study is a partially blinded efficacy trial.

Blinding of clinicians in the conventional strategy group: NuRapid-CRISPR results are not disclosed to them prior to the reporting of traditional culture results.

Blinding of statisticians: Statisticians are unaware of group assignments until the database is locked.

Due to the nature of the intervention (providing information), it is not possible to blind clinicians in the intervention group or the researchers administering the intervention.

Emergency unblinding: Emergency unblinding on a case-by-case basis will be permitted only upon approval by the principal investigator if a serious adverse event occurs and clinical management requires knowledge of the patient's group assignment (to determine whether the event is related to treatment decisions influenced by rapid diagnostic information). All instances of unblinding must be documented in detail, including the reason.

4) Standards for Concomitant Medications

Basic Principle: The prescription, dosage, and duration of all concomitant medications (including antimicrobial agents and all other drugs) shall follow standard clinical practice guidelines and protocols, with the primary goal of meeting the patient's therapeutic needs.

Recording Requirements: All concomitant medications (drug name, dosage, start and end dates, and indications) must be recorded in detail from enrollment through the end of the study for safety analysis and assessment of confounding factors.

5) Management of Adverse Events and Supportive Care

General Principle: The medical safety of the subject always takes precedence over the study itself. When any adverse event occurs, especially a serious adverse event, standard clinical management procedures must be initiated immediately and given priority.

Management Measures: These include, but are not limited to, discontinuation of the suspected drug, symptomatic and supportive care, multidisciplinary consultation, and escalation of life support. All management must follow standard hospital protocols, with costs covered through routine medical channels or study-related insurance/compensation mechanisms.

Study-Specific Considerations: If a SAE is suspected to be related to the study intervention (e.g., an antimicrobial regimen adjusted based on rapid results), the

clinician should immediately adjust or discontinue the relevant treatment regimen based on professional judgment and take all necessary medical measures. The event must be reported immediately in accordance with protocol requirements.

4. Criteria for Early Withdrawal or Termination of the Study

To strictly safeguard the rights and safety of participants and ensure the scientific validity of study data, a participant will be withdrawn from the study or the study-related procedures will be terminated early if any of the following situations occur:

- **The subject or their legal representative actively requests withdrawal**

The subject or their legal representative may withdraw informed consent and request withdrawal from the study at any stage of the research, without providing any reason. Such a request for withdrawal will be immediately respected and implemented.

- **Situations where the investigator determines withdrawal is necessary (for medical or scientific reasons)**

Occurrence of a serious adverse event related to the study: If a serious adverse event occurs that the investigator determines is clearly related to the study intervention (rapid diagnostic strategy) or study procedures, and continued participation may pose an unacceptable risk to the subject.

Emergence of other serious medical conditions: The subject develops any new serious illness, experiences a significant worsening of their condition, or enters a terminal state, rendering them unable to continue following the study protocol or where the risks of participation outweigh the benefits.

Discovery of ineligibility: Discovery after enrollment that the subject does not actually meet the inclusion criteria or meets any of the exclusion criteria.

Serious protocol violation: A major protocol violation occurs that may seriously affect the validity of the study data or the safety of the subject.

- **Withdrawal related to the study process**

Loss to follow-up: The subject or their legal representative cannot be contacted, resulting in the inability to obtain key data such as the primary endpoint (e.g., 28-day survival status).

Early termination of the entire study: The entire study is terminated prematurely due

to recommendations from the Independent Monitoring Committee, requirements from the Ethics Committee, or regulatory authorities.

Post-withdrawal procedures:

Documentation and Reporting: The investigator must document the date of withdrawal, the specific reason, and the subject's health status at the time of withdrawal in detail on the case report form. If withdrawal is due to a serious adverse event, it must be reported promptly in accordance with regulations.

Data Handling: Within the scope of the informed consent obtained, all data collected prior to the subject's withdrawal will be retained and included in the "Intent-to-Treat analysis set" to ensure the scientific rigor of the analysis.

Medical Care Arrangements: After withdrawing from the study, the participant will continue to receive all necessary medical treatment in accordance with medical standards at this hospital, and their medical rights will not be affected in any way. The investigator will provide necessary medical assistance as appropriate.

VI. Trial Procedures

1. Subject Management

Subject Recruitment Methods

To ensure the smooth conduct of the study and protect the rights of potential participants, this study will employ the following multi-tiered, standardized recruitment methods:

1) Primary Recruitment Channel: Identification and Enrollment via Clinical Care Pathways

The target population for this study consists of patients in the ICU who meet the diagnostic criteria for sepsis; therefore, recruitment will be deeply integrated into routine clinical workflows.

Identification: Attending physicians and research staff in the ICUs of each center will screen newly admitted patients with suspected or confirmed sepsis in real-time during routine clinical care, based on the study's inclusion and exclusion criteria.

Initial Approach: Upon identifying a potentially eligible subject, the attending physician or designated researcher will, once the patient's condition has stabilized,

provide the patient's legal representative (as patients are often sedated or in critical condition) with an initial overview of the study's basic details, objectives, significance, and the voluntary nature of participation.

2) Recruitment Materials and Informed Consent Process

Material Preparation: Provide an easy-to-understand informed consent form for family members that has been reviewed and approved by the ethics committee. The consent form will clearly explain the nature of the study (comparison of diagnostic strategies), the grouping method (cluster randomization), the rights and obligations of both parties, potential risks and benefits, privacy protection measures, and the right to withdraw.

Informed Consent: A trained principal investigator or designated researcher will engage in thorough communication with the patient's legal representative in an independent, undisturbed setting. The researcher will ensure the representative understands all key information and is given sufficient time to consider the decision. After all questions have been satisfactorily addressed, the legal representative will voluntarily sign the informed consent form. The process will be documented in writing.

3) Quality and Ethical Control of the Recruitment Process

Principle of Non-Coercion: Participants are explicitly informed that participation in the study is entirely voluntary and will in no way affect the patient's access to any routine and necessary medical care in the ICU.

Privacy Protection: During the initial screening and engagement phase, patient medical information is strictly protected and used solely to assess eligibility for the study.

Ongoing Training: Researchers involved in recruitment at each center will receive regular training on the study protocol and communication skills to ensure standardized and consistent recruitment practices.

Process Documentation: The entire process from identification to completion of informed consent is documented in detail, including the time of contact, key points of communication, and the consent outcome.

4) Anticipated Recruitment Progress

Monthly and overall recruitment targets for each sub-center will be established based on the historical number of sepsis patients admitted to the ICUs of participating centers, combined with the inclusion criteria. The research team will review recruitment progress regularly (e.g., weekly). If delays occur, the causes (e.g., insufficient screening efforts, changes in eligibility rates) will be analyzed, and strategies will be adjusted promptly to ensure recruitment targets are met as planned.

This recruitment protocol is grounded in clinical practice, centered on fully informed consent, and guided by ethical standards. It aims to efficiently and compliantly enroll a representative study population, thereby ensuring the scientific rigor and feasibility of the research.

2. Informed Consent Process

Informed Consent Process and Documentation The informed consent process begins before an individual agrees to participate in the study and continues throughout their participation in the study.The informed consent form will be approved by the Institutional Review Board (IRB), and participants will be asked to read and review the document. The investigator will explain the study to the participant and answer any questions the participant may have. The investigator will verbally explain to the participant, in a manner appropriate to the participant’s understanding: the purpose of the study, the procedures, potential risks, and the participant’ s rights.Before signing the written informed consent form, the participant should have sufficient time to read it carefully and ask questions. Before agreeing to participate in the study, the participant should have the opportunity to discuss the study with family members or a representative, or to consider it on their own. The participant will sign the informed consent form before any study-related procedures are performed. The participant must be informed that participation in the study is voluntary and that they may withdraw from the study at any time without any detriment.The investigator will provide the participant with a copy of the informed consent document for their records. The informed consent process should take place before the participant undergoes any procedures related to the study, and the process (including the date) should be documented in the source documents, along with the retention of the signed informed

consent form. The investigator must specifically inform participants that their quality of medical care will not be adversely affected if they refuse to participate in this study, thereby ensuring the participants' rights and welfare.

3. Eligibility Verification

A trained research coordinator will conduct a point-by-point, two-person verification of patients who have passed the initial assessment, based on the inclusion and exclusion criteria clearly specified in the "Study Protocol." The verification will be based on complete medical records, laboratory test results, and imaging findings. Any uncertainties must be resolved by the principal investigator. All verification processes and conclusions must be recorded in the "Eligibility Verification Form" to ensure that enrolled participants fully meet the requirements of the study protocol.

4. Medical History and Medication Review

Systematically collect and document the patient's complete medical history prior to enrollment, including current medical history, past medical history, and allergy history.At the same time, all concomitant medications (including prescription drugs, over-the-counter medications, and herbal medicines) taken before enrollment and during the study must be documented in detail, noting the drug name, dosage, start and end dates of use, and indications. This process aims to accurately assess baseline health status and provide critical background information for subsequent analyses of efficacy and safety, particularly to determine the causal relationship between adverse events and the study intervention.

5. Assignment of Screening ID

Assign a unique screening ID to each patient who has completed informed consent and entered the eligibility verification process. This ID is used to identify all data and documents generated during the screening period, enabling de-identified management of subject identities and data, and facilitating the tracking of reasons for screening failure.

6. Randomization Code Assignment

This study employs a cluster-randomized design. The unit of randomization is not the individual subject, but rather clusters defined as "ICU wards" or "specific consecutive time units."

Randomization Implementation: An independent statistical center generates a random allocation sequence via computer to pre-assign each cluster to either the "Rapid Diagnosis Strategy Group" or the "Conventional Strategy Group."

Group Assignment: Upon enrollment, a patient's group assignment is automatically determined by the pre-assigned results of their cluster. Patients will receive a group code corresponding to their study cluster, which determines the diagnostic information support strategy they will receive. Group information is blinded to participants and clinicians (except for the intervention implementers); however, due to the nature of the intervention, complete blinding of the investigators is not feasible.

7. Trial Compliance Management

The key compliance aspect of this study lies in the implementation of the two diagnostic strategy protocols.

Rapid Diagnostic Strategy Group: Adherence is defined as whether clinicians review the NuRapid-CRISPR test report and document related treatment decisions after the report becomes available (within 2–4 hours). The time of report review will be verified through electronic system logs, and records of treatment adjustments will be verified through case reviews.

Conventional Strategy Group: Adherence is defined as clinicians not being informed of the NuRapid-CRISPR results in advance, prior to the reporting of traditional culture results (maintaining blinding). This will be monitored through information system access controls and operational logs.

Deviation Handling: Any situation inconsistent with the protocol-specified diagnostic strategy must be recorded as a "protocol deviation." The research team will periodically review deviations, analyze causes (e.g., system failures, urgent clinical situations), and implement corrective actions through retraining or process optimization. All deviations must be analyzed and explained in the final study report.

2. Safety Evaluation Procedures (Assessment, Detection, and Reporting of Adverse Events)

Adverse Event Recording and Reporting Process

Recording: All AEs, regardless of severity or relevance, must be recorded in detail and objectively on a dedicated case report form. The content must include: event

description, start and end times, severity, relevance to the study, measures taken, and outcome.

Reporting Timelines and Channels:

Non-serious adverse events: Recorded in the CRF as required by the protocol and reported in summary form during routine study monitoring.

Serious Adverse Events:

Immediate Reporting: Within 24 hours of becoming aware of an SAE, the investigator must submit a written report to the Institutional Review Board (IRB) of this research center and the study's Data and Safety Monitoring Board (DSMB).

Report Content: Includes subject identification number, detailed description of the SAE, time of occurrence, current status, measures taken, and preliminary assessment of causality.

Follow-up Reports: Monitor the SAE until the event is resolved, stabilized, or the subject is lost to follow-up, and submit follow-up reports in a timely manner.

Review of Safety Data

Investigator Review: The principal investigator periodically reviews all safety data from this center.

Independent Monitoring: The Data and Safety Monitoring Board will periodically (e.g., after every 50 patients are enrolled) conduct a blinded review of the pooled safety data from all study centers, focusing on the incidence of SAEs between the two groups, the balance of specific types of AEs (e.g., new-onset organ failure, drug-related adverse reactions), and evaluating the overall risk-benefit ratio of the study; if necessary, recommendations for adjusting or terminating the study will be made.

Risk Management Plan

Once an AE or SAE is determined to be clearly related to the study, the clinical emergency response plan will be immediately activated to prioritize the medical safety of the subjects. All additional medical expenses incurred as a result will be borne by the sponsor (or the responsible party for the study) in accordance with the national "Good Clinical Practice (GCP)" and relevant laws and regulations.

Strict adherence to this procedure will ensure that any potential risks are promptly

identified, scientifically assessed, and properly managed, providing a core safeguard for the rights and interests of the subjects.

Risk Control and Management Procedures

Control Measures: All study data will be de-identified immediately upon collection and assigned a unique study identifier. Electronic data will be stored on encrypted servers at the hospital's information center, while physical documents will be kept in locked filing cabinets. Database access will be subject to strict hierarchical permission controls. When study results are published, it will be ensured that no personally identifiable information is included.

Psychological Distress or Decision-Making Burden:

Control Measures: The informed consent process ensures that it is fully informed and free from coercion. Patients and their families have the right to withdraw from the study at any time without providing a reason, and their subsequent medical care will not be affected in any way. The research team will provide communication channels to promptly address participants' questions regarding the study process.

Anxiety caused by uncertainty regarding test results:

Control Measures: All participants will be clearly informed that NuRapid-CRISPR is an investigational technology under evaluation in this study, and its results are intended solely for the clinician's reference; they are not to be used as the final or sole basis for diagnosis. Clinical decisions are made by synthesizing all clinical information (symptoms, signs, and other test results), thereby reducing excessive anxiety in patients caused by a single test result.

Summary: The risks of this study primarily stem from routine clinical care itself and have been systematically managed through rigorous ethical design (no delay in standard treatment), standardized operating procedures, comprehensive data security measures, and an independent monitoring mechanism. The entire risk control system is designed to maximize participant safety and ensure that study risks remain within ethically acceptable limits.

4. Efficacy Measurement Procedures

The efficacy assessment in this study employs multidimensional indicators, measured through standardized, prospective data collection processes to ensure the

objectivity, accuracy, and comparability of the results.

- **Measurement of Primary Efficacy Endpoint**

Endpoint: 28-day all-cause mortality.

Measurement Method:

Definition: Death from any cause occurring between the date of randomization and Day 28 (±2 days).

Data Source:

In-hospital deaths: Recorded in real time through daily medical record reviews.

Out-of-hospital deaths: Confirmed via a structured telephone follow-up on day 28 after enrollment. The telephone follow-up will use a standardized questionnaire and be conducted by trained study coordinators.

Adjudication: All death events will be independently adjudicated by a blinded Clinical Endpoint Adjudication Committee in accordance with pre-established protocols. The committee will review relevant medical records (e.g., death certificates, end-of-life medical records) to determine the cause of death, ensuring a consistent standard.

- **Measurement of Secondary Efficacy Endpoints**

Treatment Process Measures:

Time to First Targeted Therapy:

Definition: The time interval (in hours) from the enrollment date to the first administration of an antimicrobial agent covering the final confirmed pathogen (based on conventional culture or clinical diagnosis).

Measurement: Calculated precisely by comparing the time of the antimicrobial prescription with the time of the final microbiological report.

Initial Treatment Adequacy Rate:

Definition: The proportion of empirical antimicrobial regimens initiated within 24 hours of enrollment whose antimicrobial spectrum covers the final confirmed pathogen.

Measurement: Blinded assessment by an infectious disease specialist based on the final microbiological diagnosis and antimicrobial susceptibility profile.

Clinical Outcome Measures:

ICU length of stay, total length of stay: Extracted directly from discharge records in the hospital information system, accurate to the day.

Days without ventilator or vasoactive support: Cumulatively calculated from daily organ support records during the 28-day observation period.

Organ dysfunction: SOFA scores are calculated daily, and new or worsening organ failure is recorded.

Health economics indicators:

Total medical costs: Total medical costs from patient enrollment to discharge are extracted from the hospital's financial system.

- **Data Quality Control**

Standardized Training: All investigators involved in data collection and evaluation must undergo standardized training to ensure consistent understanding of definitions and procedures.

Source Data Verification: Study monitors will conduct periodic on-site source data verification of efficacy endpoint data to ensure consistency between CRF entries and original medical records.

Logical Validation: The electronic data capture system incorporates built-in logical validation rules that provide real-time alerts for critical time points and logical inconsistencies.

Blinded Assessment: Any metrics involving subjective judgment (e.g., adequacy of treatment, adjudication of endpoint events) will be performed by assessors who are unaware of the patients' group assignment.

5. Termination/Withdrawal Procedures

To respect the autonomy of participants and safeguard their safety and rights, this study has established clear procedures for discontinuation and withdrawal.

- **Voluntary Withdrawal by the Subject**

Right: The subject or their legal representative may decide to withdraw from this study at any time and for any reason.

Procedure:

The participant or their representative clearly expresses their intention to withdraw to the research team (physician or research coordinator).

The investigator must respect this decision and immediately cease all study-related activities.

The investigator must communicate with the participant or their representative to confirm whether they consent to the continued use of previously collected data for analysis, and document their choice.

Record the date and reason for withdrawal and complete the "Subject Early Withdrawal Record Form."

- **Study Termination Initiated by the Investigator**

The investigator has the authority (and responsibility) to decide to terminate a subject's participation in the study under the following circumstances:

Safety reasons: A serious adverse event clearly related to the study occurs, and the risks of continued participation outweigh the benefits.

Medical condition: A significant change in the patient's condition (e.g., transfer to hospice care, discontinuation of active treatment) that prevents continued adherence to the study protocol.

Protocol Deviation or Ineligibility: A serious protocol deviation has occurred, or it has been determined that the patient did not meet the inclusion or exclusion criteria at the time of enrollment.

Procedural Failure: Inability to obtain eligible specimens or valid test results due to technical reasons, with no possibility of remediation.

Procedure:

The investigator assesses the situation and discusses it with members of the research team.

Once the decision to discontinue is made, immediately communicate with the subject/proxy and explain the reasons.

Record the date of discontinuation, detailed reasons, and supporting rationale, and complete the relevant forms.

Arrange for standard medical follow-up care for the subject as needed.

- **Data and Medical Management Following Withdrawal/Termination**

Data Handling:

To the extent permitted by the informed consent obtained, all study data collected

prior to the subject's withdrawal will, in principle, be retained and included in the "intention-to-treat analysis."

If a subject requests the withdrawal of all data, their request for data deletion must be processed in accordance with regulatory and ethical requirements.

Medical Arrangements:

Following a subject's withdrawal or discontinuation from the study, all routine and necessary medical services received at this institution will remain unaffected, and the subject is entitled to receive the best possible follow-up care.

If withdrawal is due to study-related harm, insurance and compensation mechanisms will be activated to cover the corresponding medical expenses and provide compensation.

Follow-up:

For subjects who withdraw due to safety concerns, the investigator must conduct necessary medical follow-up until the event is properly resolved or the subject's condition is stable.

- **Recording and Reporting**

All discontinuation/withdrawal events must be documented in detail in the case report form and designated records. If the event involves a serious adverse event, it must be reported to the Ethics Committee and the Data Safety Monitoring Board within the specified timeframe.

6. Blinding/Unblinding Procedures

To ensure the objectivity of the study results and minimize bias, this study adopts the following blinding and unblinding protocols:

- **Blinding Design**

This study is a partially blinded, efficacy-focused randomized controlled trial.

Who is blinded:

Statisticians: Remain fully blinded throughout the data analysis phase until the primary analysis is completed.

Clinicians in the conventional strategy group: They had no access to NuRapid-CRISPR test results prior to receiving conventional culture results (blinding was achieved through information system access controls).

Members of the Clinical Endpoint Adjudication Committee: Were unaware of the patients' group assignment when adjudicating primary endpoint events (e.g., cause of death).

Elements that could not be blinded:

Due to the nature of the intervention being the "provision of diagnostic information," clinicians in the intervention group (rapid diagnosis strategy group) and the research coordinators administering the intervention cannot be blinded.

Patients and their families are not informed of their group assignment in principle; however, they may learn this information from their physicians during actual clinical care.

- **Maintenance of Blinded Status**

Data Management: Grouping variables are stored in the database in coded form (e.g., Group A/Group B), without explicit labels such as "intervention group" or "control group."

Communication Guidelines: Predefined group codes are used in all study documents, meeting minutes, and routine communications to prevent accidental unblinding.

System Controls: In electronic medical records and the study data system, access permissions for physicians in the standard-of-care group to view NuRapid-CRISPR results are technically restricted.

- **Emergency Unblinding Procedure**

Emergency unblinding may only be initiated in the event of a serious adverse event (SAE) where immediate knowledge of the patient's group assignment is clinically necessary for targeted management (e.g., to determine whether the SAE is directly related to a specific treatment decision based on rapid results).

Request: A written request must be submitted by the patient's attending physician or the principal investigator, stating the medical necessity for unblinding.

Approval and Execution: Upon approval by the principal investigator, the query is conducted by an independent third party (such as the pharmacy department or the randomization center) according to the predefined unblinding procedure. The unblinding process must be witnessed and documented in detail.

Recording and Reporting: Within 24 hours of completing the emergency unblinding,

the “Emergency Unblinding Record Form” must be completed, detailing the reason, date, person who performed the unblinding, and the results of the unblinding, and submitted to the study’s Data and Safety Monitoring Board and Ethics Committee.

- **Study Unblinding**

After the analysis of all primary endpoint data is completed and the statistical analysis report is finalized, the Principal Investigator, the statistician, and the Chair of the Data and Safety Monitoring Board (DSMB) shall jointly authorize the formal study unblinding to determine the actual interventions corresponding to Group A and Group B, which will be used for the preparation of the final report and the interpretation of results.

7. Site Visit Requirements

The site visit plan for this study is designed to collect data systematically and efficiently while minimizing additional burden, and is closely integrated with patients’ clinical care pathways. Specific arrangements are as follows:

1) Screening Period

Time Window: Within 24 hours of patient admission to the ICU.

Core Tasks:

Eligibility Assessment: Conduct a comprehensive review of patient information based on inclusion and exclusion criteria.

Informed Consent: Engage in thorough communication with the patient’s legal representative to obtain written informed consent.

Baseline Data Collection: Record demographic information, ICU admission diagnosis, SOFA score, vital signs, laboratory test results, and imaging findings.

Baseline Treatment Documentation: Document the use of all antimicrobial agents prior to enrollment.

Study Specimen Collection: Collect microbiological specimens (e.g., blood cultures, qualified sputum samples) for subsequent conventional culture and NuRapid-CRISPR testing.

2) Treatment Period/Hospitalization Observation Period

Time window: From enrollment until discharge from the ICU or death.

Visit Method: Daily review of medical records and data extraction; no additional

patient visits are required.

Core Data Collection:

Daily Clinical Assessment: SOFA score, vital signs, and details of organ support (mechanical ventilation, vasoactive agents).

Microbiological Updates: Real-time recording of all conventional culture and NuRapid-CRISPR test results and reporting times.

Treatment Decision Tracking: Detailed documentation of all antibiotic regimen adjustments, including timing, specific regimens, and rationale (based on rapid results, culture results, or clinical judgment).

Adverse Event Monitoring: Proactively identify, assess, and document all adverse events.

3) Post-treatment follow-up

Safety and Efficacy Follow-up:

Time Window: From enrollment to discharge or Day 28 (whichever occurs first).

Method: Continuous medical record review (data collected via the ward medical record system after the patient is transferred out of the ICU).

Content: Record the patient's condition upon discharge from the ICU, new infections or complications, subsequent antimicrobial therapy, total length of hospital stay, and discharge diagnosis.

Survival Follow-up:

Time Point: 28 days after enrollment (±2 days).

Method: Conducted via structured telephone follow-up for discharged patients.

Key Information: Confirm the patient's survival status (alive/deceased). If deceased, record the date of death and primary cause whenever possible.

VII. Study Start and End Dates

This study will be conducted strictly within the following timeframe to ensure a controlled process and reliable results.

1. Study Initiation

Start-up Criteria: A participating center may initiate the study only after sequentially meeting the following conditions:

Obtaining written approval from the center's institutional review board (IRB).

Finalization and approval of the study protocol and related documents (informed consent forms, CRFs, etc.).

The principal investigator and all study team members have completed training on the protocol and standard operating procedures (SOPs) and passed the assessment.

The data collection system and randomization system have been installed, tested, and are operational.

The laboratories involved in the study (such as the central laboratory conducting NuRapid-CRISPR testing) have completed accreditation.

Enrollment of the first subject: Centers that meet the above initiation criteria may begin screening and enrolling eligible subjects starting from the effective date of the ethics approval. This date will be recorded as the official start date of the study for that center.

2. Study Conduct Phase

This phase constitutes the core implementation period and includes:

Ongoing recruitment, screening, informed consent, and enrollment of subjects.

Strict adherence to the protocol for randomization of the entire cohort and corresponding treatment strategies.

Systematic collection, recording, and reporting of study data and safety information.

Undergoing regular internal quality audits and reviews by an independent Data and Safety Monitoring Board.

3. Study Termination

This study will formally conclude upon fulfillment of any one of the following conditions:

Study Completion: Successful recruitment and randomization of all subjects required by the protocol, along with follow-up at the primary endpoint (28 days).

Early Termination: Early termination of the study upon recommendation by the Independent Data and Safety Monitoring Board (DSMB) or Ethics Committee based on safety or efficacy considerations, and following a decision by the Study Steering Committee.

Other reasons: The study cannot continue due to force majeure.

4. Post-Study Activities

Upon formal completion of the study, the following tasks will be carried out in an orderly manner:

Data Cleaning and Database Lock-in: After the last subject completes the primary endpoint follow-up, final data verification and resolution of any discrepancies will be performed, followed by the locking of the study database.

Statistical Analysis: A blinded statistician will perform statistical analysis on the locked data in accordance with the pre-specified statistical analysis plan.

Final Report: A clinical study summary report will be prepared and submitted to the Institutional Review Board (IRB), relevant regulatory authorities, and the study sponsor.

Publication and Data Archiving: A manuscript will be prepared in accordance with the publication plan, and all study-related documents (source data, records, reports, etc.) will be archived in accordance with regulations and retained for at least 15 years following the conclusion of the study.

VIII. Clinical Criteria for Early Termination of the Trial

To safeguard the rights of participants and ensure research ethics, this study will initiate an evaluation and consider early termination if any of the following conditions arise:

1. Termination Based on Safety Signals

Clear Evidence of Harm: Following review by the independent Data and Safety Monitoring Board (DSMB), there is sufficient evidence indicating that participants in the rapid diagnostic strategy intervention group (exposure group) face a significantly higher risk of serious adverse events clearly related to the study compared to the standard strategy group (non-exposure group), and the risk-benefit ratio is no longer acceptable.

Unexpected serious issues: The emergence of unexpected, serious safety issues related to the study intervention that pose a clear threat to participant health.

2. Termination Based on Efficacy/Ineffectiveness

Clear Evidence of Efficacy: In an interim analysis, if the DSMB review finds that

the rapid diagnosis strategy demonstrates highly significant and clinically substantial superiority in reducing the primary efficacy endpoint (28-day all-cause mortality)—with statistical power far exceeding the pre-specified interim analysis threshold—and it is ethically unacceptable to continue subjecting the standard-of-care group to the current strategy, early termination may be recommended.

Clear evidence of futility: If, during an interim analysis, the DSMB review finds that the difference between the two groups in the primary efficacy endpoint is minimal, and based on current data projections, it is highly unlikely that the pre-specified statistical superiority endpoint will be met even after the full sample size is enrolled, continuing the study would be scientifically meaningless.

3. Termination Based on Feasibility and Quality

Severe enrollment delays or excessively high dropout rates: Actual enrollment rates or protocol completion rates are significantly lower than expected, preventing the study from reaching reliable conclusions within a reasonable timeframe.

Widespread protocol violations: Systematic and widespread protocol violations occur, severely compromising the scientific validity, reliability, and integrity of the study data.

4. Other Reasons

Requests from the ethics committee or regulatory authorities.

The investigational product (NuRapid-CRISPR detection system) is suspended or recalled due to quality issues.

Force majeure.

5. Termination Decision Process

The decision to terminate early shall not be made unilaterally by the investigator. When any of the above potential situations arise:

The DSMB (or the investigator) will urgently report the relevant information to the Study Steering Committee.

The Study Steering Committee will synthesize all information and conduct a careful assessment.

The final decision to terminate must be reviewed and approved by the ethics committee of the lead institution.

Once the decision to terminate is made, all study sites will be notified immediately to cease recruitment of new participants, and appropriate medical follow-up and care will be arranged for enrolled participants in accordance with ethical guidelines.

IX. Data Safety and Monitoring Plan

This plan has been formulated to ensure the authenticity, accuracy, integrity, and security of study data, and to protect the rights and interests of participants.

1. Overview of Data Management Methods

Data Collection: Standardized case report forms based on an electronic data capture system will be used. Data will be entered by trained study coordinators after verification against source documents.

Data Transmission and Storage: All electronic data will be transmitted via the hospital's internal encrypted network and stored on dedicated servers at the hospital's Information Center, which are protected by firewalls. Physical documents (such as signed informed consent forms) will be stored in locked filing cabinets. Data will be de-identified and linked using a unique study identifier.

Data Validation and Cleaning: The system will include logic checks and numerical range validation. The data manager will periodically generate a list of data discrepancies; researchers at each center will verify the source data and provide feedback on corrections. The study monitor will conduct on-site verification of source data.

Database Lockdown: Prior to the commencement of the primary analysis, the principal investigator, statistician, and data manager will jointly review the data. Once all discrepancies have been resolved, the database will be formally locked. Any modifications to the locked data must undergo a strict approval process and be documented.

2. Reporting and Collection of Adverse Events and Serious Adverse Events

Definition and Identification: Adverse events and serious adverse events are defined strictly in accordance with the protocol. All investigators receive relevant training.

Collection and Documentation: Any AE/SAE, regardless of whether it is considered

study-related, must be documented in detail within 24 hours in the dedicated module of the eCRF and on the Adverse Event Report Form. This includes a description of the event, time of occurrence, severity, relationship to the study, actions taken, and outcome.

Assessment and Follow-up: The principal investigator must promptly assess the causal relationship between the AE/SAE and the study intervention. All SAEs and AEs requiring follow-up must be tracked until the event is resolved, stabilized, or the subject is lost to follow-up, and follow-up information must be recorded.

Reporting Procedures and Timelines:

SAE Reporting: Upon becoming aware of an SAE, the investigator must submit a written report to the Institutional Review Board (IRB) of this center, the Data and Safety Monitoring Board (DSMB) of this study, and the study sponsor/lead institution within 24 hours. Follow-up reports must be submitted as required.

Annual Safety Report: Submit a cumulative summary and analysis of SAEs to the Ethics Committee on a regular basis.

3. Data and Safety Monitoring Plan

1) Overview of Data Management Methods

Establishment of an Independent Data and Safety Monitoring Board (DSMB): Composed of experts in critical care medicine, infectious diseases, statistics, and ethics who are not involved in the study.

DSMB Responsibilities:

Regularly review unblinded cumulative study data, particularly comparisons between the two groups regarding primary efficacy endpoints (e.g., 28-day mortality rate) and key safety indicators (e.g., SAE incidence).

Assess the overall risk-benefit ratio of the study.

Review compliance with study protocols and data quality.

Provide independent recommendations to the Study Steering Committee regarding whether the study should continue, be modified, or be terminated early, based on a pre-established monitoring protocol.

Monitoring Timing and Reporting: The DSMB will convene meetings at key

milestones, such as when study enrollment reaches 25% or 50% of the planned total, and may convene at any time if safety concerns arise. Meeting minutes and recommendations will be submitted to the Study Steering Committee and the Ethics Committee.

2) Reporting and Collection of Adverse Events and Serious Adverse Events

Clinical adverse events may occur during the course of subject treatment. Upon the occurrence of any adverse event (including serious adverse events), the time of occurrence, clinical manifestations, management, duration, outcome, and relationship to the drug must be recorded in detail on the case report form. In cases of abnormal laboratory test results, the patient must be followed up until the test results return to normal, or to pre-treatment levels, or until it is determined that the abnormality is unrelated to the investigational drug.In the event of a serious adverse event, a Serious Adverse Event Form must be completed and reported within 24 hours to the sponsor, the Ethics Committee, the CFDA Safety Supervision Department, and the health administration department.

3) Medical Safety Measures

This study will implement comprehensive medical safety measures to protect the rights and interests of participants:

Priority of Standard of Care: All subjects will receive standard diagnosis and treatment in accordance with the *Chinese Guidelines for Emergency Treatment of Sepsis/Septic Shock*. The study intervention (rapid diagnostic information) shall not replace or delay any necessary clinical management.

Active Monitoring and Management of Adverse Events: A systematic process for monitoring, recording, evaluating, and reporting adverse events (AEs) and serious adverse events (SAEs) will be established. Upon the occurrence of an AE or SAE, the clinical team will immediately initiate standard medical procedures for treatment; related costs will be covered by routine medical channels or study insurance.

Independent Safety Monitoring: An independent Data and Safety Monitoring Board (DSMB) will be established to periodically review unblinded cumulative safety data, assess the risk-benefit ratio, and have the authority to recommend study modification or termination.

Emergency Unblinding Mechanism: If a subject experiences a serious adverse event and clinical management requires knowledge of their group assignment, a predefined emergency unblinding procedure may be initiated to ensure the timeliness and accuracy of medical decisions.

4) Communication with Ethics Committees and Regulatory Authorities

This study will maintain transparent and timely communication with ethics committees and relevant regulatory authorities:

Initial Submission and Approval: All initial documents, including the study protocol and informed consent forms, will be submitted to the Ethics Committees of each participating center for review and approval. The study may only commence after written approval has been obtained.

Ongoing Reporting:

Annual/Periodic Reports: Progress reports, including enrollment status, safety summaries, and protocol amendments, will be submitted to the Ethics Committee.

Safety Reporting: Suspected and unexpected serious adverse events, as well as DSMB recommendations, will be reported in a timely manner in accordance with regulatory requirements.

Protocol Amendments: Any protocol amendments must be submitted to the Ethics Committee for review and approval prior to implementation.

Reports of Serious Protocol Deviations or Early Termination: Any serious or persistent protocol or regulatory violations, or early termination of the study, must be reported immediately.

Final Report: A final summary report must be submitted upon completion of the study.

5) Internal Data Analysis Plan

To ensure the scientific rigor and completeness of data analysis, the following internal plan has been established:

Definition of Analysis Datasets: The full analysis set, protocol-compliant set, and other relevant datasets will be clearly defined in advance.

Statistical Analysis Plan: Prior to database lock, a detailed statistical analysis plan will be drafted and finalized by a blinded statistician, specifying the analytical

methods for all endpoints, subgroup analyses, and missing data handling.

Analysis Phases:

Interim Analysis: Conducted by the DSMB to evaluate efficacy/inefficacy and safety based on predefined protocols; results are for DSMB decision-making purposes only.

Final Analysis: After database lock, a blinded statistician will conduct the final analysis in accordance with the SAP and generate a statistical report.

Quality Control: All analysis procedures will be archived to ensure reproducibility of results. The analysis process is subject to quality audits.

6) Frequency of Data Safety and Monitoring Reports Submitted to the Ethics Committee

This study will report data safety and monitoring status to the Ethics Committee at the following frequencies:

Periodic Reports: At least once a year, as part of the study progress report, summarizing SAE occurrences during the reporting period, DSMB meeting summaries, and key safety conclusions.

Immediate Reporting:

All suspected and unexpected serious adverse events will be reported immediately upon awareness (typically within 7 business days).

Important safety recommendations from the DSMB or decisions to terminate the study early will be reported immediately.

Report any major protocol deviations that could seriously affect the safety of the subjects or the conduct of the study in a timely manner.

Reporting as Required: Provide relevant information at any time in accordance with the specific requirements of the Ethics Committee.

X. Compliance with Ethical Principles and Relevant Regulations

This study will strictly adhere to internationally recognized ethical guidelines, relevant Chinese laws and regulations, and the technical specifications of the National Health Commission, ensuring that the entire research process is scientific and compliant, and prioritizing the rights, safety, and well-being of the subjects.

1. Core Ethical and Regulatory Documents to Be Followed

The Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects)

Good Clinical Practice (GCP)

"Measures for Ethical Review of Biomedical Research Involving Human Subjects"

The Basic Law on Medical and Health Care and Health Promotion of the People's Republic of China

The Personal Information Protection Law of the People's Republic of China

Relevant regulations of the National Health Commission regarding clinical research, the management of human genetic resources, and biosafety.

2. Ethical Review

All relevant documents, including the study protocol, informed consent forms, and recruitment materials, must be submitted to the ethics committee of each participating center for independent and comprehensive review prior to the study's commencement, and written approval must be obtained.

During the course of the study, any substantive amendments to the protocol must be resubmitted to the ethics committee for review and approval before implementation.

The study will submit regular progress reports (annual reports or more frequently) and safety reports in accordance with the requirements of the ethics committee, and a final report will be submitted upon completion of the study.

3. Informed Consent

Obtaining voluntary, written, and fully informed consent is an absolute prerequisite for each subject's participation in the study. Given that the target population of this study (ICU patients with sepsis) often lacks full legal capacity, the consent process will primarily be conducted with their legal representatives.

The informed consent process will take place in a quiet, private setting. The principal investigator or a designated researcher will use the consent form approved by the Ethics Committee to clearly and comprehensively explain, in plain language, the study's objectives, procedures, potential risks and benefits, alternative options, privacy protection measures, compensation and insurance arrangements, and the right to withdraw at any time without conditions.

The original signed informed consent form will be properly preserved as a critical

study document.

4. Protection of Participants' Rights and Safety

Risk Minimization: The study design (cluster randomization) will not delay or compromise any standard medical care that participants should receive. The intervention (rapid diagnostic information support) itself does not pose an additional risk of physical harm.

Conflict of Interest Management: All researchers must declare potential conflicts of interest to ensure the objectivity of the study. Participants' medical decisions will always be based primarily on clinical benefit.

Privacy and Confidentiality: Participants' personal information and medical data will be strictly protected. All research data will be de-identified upon collection and identified using unique codes. Security measures, including encryption, will be implemented for data storage, transmission, and analysis, and access will be subject to strict permission controls.

Compensation and Insurance: The study will purchase liability insurance for all participants to cover trial-related harm. In the event of harm directly related to the study, participants will receive timely, free medical treatment and appropriate financial compensation in accordance with the law.

5. Regulatory Compliance

Management of Human Genetic Resources: As this study involves the testing of genetic information such as pathogen nucleic acids, it will strictly comply with the "Regulations of the People's Republic of China on the Management of Human Genetic Resources." Any required approvals or filings will be completed prior to the start of the study.

Data Security: Any cross-border transfer of research data (if applicable) will strictly comply with the requirements of the Cybersecurity Law, the Data Security Law, and the Personal Information Protection Law.

XI. Statistical Analysis Plan

Data analysis will be performed using SPSS 26.0 or R 4.2.0 statistical software. *A p-value* < 0.05 indicates a statistically significant difference.

1. Comparison of Baseline Data

Continuous variables will first undergo a Kolmogorov-Smirnov test to assess normality. Normally distributed data will be presented as mean ± standard deviation (X ± S), and comparisons between groups will be performed using an independent samples t-test; non-normally distributed data will be presented as median (interquartile range) [M (Q1, Q3)], and comparisons between groups will be performed using the Mann-Whitney U test.Categorical data are presented as frequency (percentage) [n (%)], and intergroup comparisons were performed using the chi-square (χ^2) test or Fisher's exact test (when the expected frequency was <5).

2. Diagnostic Performance Analysis

Using traditional culture results combined with clinical diagnosis as the reference standard, ROC curves were constructed to calculate the AUC, sensitivity, specificity, positive predictive value, and negative predictive value of the NuRapid-CRISPR technology for detecting various pathogens. The McNemar test was used to compare differences in positive detection rates between the two technologies.

3. Survival Analysis

The Kaplan-Meier method was used to plot 28-day survival curves for the two patient groups, and the Log-rank test was used to compare survival differences between groups. The Cox proportional hazards regression model was used to analyze the association between the application of the NuRapid-CRISPR technology and the risk of death at 28 days, adjusting for confounding factors such as age, APACHE II score, and underlying diseases.

4. Comparison of Clinical Outcome Measures

Survival-time indicators, such as ICU length of stay and total hospital stay, were analyzed using the Kaplan-Meier method, with the Log-rank test used for intergroup comparisons; other continuous outcome indicators (e.g., duration of antibiotic use, hospital costs) were analyzed using appropriate parametric or non-parametric tests based on their distribution; categorical outcome indicators (e.g., incidence of MODS) were analyzed using the χ^2 test or Fisher's exact test.

5. Subgroup Analysis

Stratified analysis was performed according to predefined subgroups (e.g., age,

severity of sepsis), and interaction tests were used to determine whether the effect of the NuRapid-CRISPR technology differed across subgroups.

6. Handling of Missing Data

For data with a missing rate <5%, single-variable imputation methods (e.g., mean or median imputation) will be used; for data with a missing rate of 5% – 20%, multiple imputation methods will be employed; for data with a missing rate >20%, the causes of missing data will be analyzed, and sensitivity analyses will be conducted to assess the impact of missing data on the study results.

XII. Format and Timing of Publication of Research Findings

1. Publication Format

The scientific findings generated by this study will primarily be disseminated and shared through the following formats:

Articles in International Peer-Reviewed Journals: We plan to compile the main research findings into 1–2 high-quality academic papers and submit them to internationally authoritative SCI-indexed journals in the fields of infectious diseases, critical care medicine, or clinical laboratory medicine (e.g., Lancet Respiratory Medicine, Intensive Care Medicine, Clinical Infectious Diseases, Clinical Microbiology and Infection, etc.).The papers will be written in accordance with the "Guidelines for the Conduct and Reporting of Research and for the Editorial and Publication Practices of Medical Journals" issued by the International Committee of Medical Journal Editors.

Conference Presentations: Significant interim findings obtained during the research process will be actively presented at high-level academic conferences in the fields of critical care medicine, infectious diseases, and clinical laboratory medicine, both domestically and internationally (e.g., the Annual Meeting of the Critical Care Medicine Branch of the Chinese Medical Association, the European Society of Intensive Care Medicine Annual Congress, and the Annual Meeting of the American Society for Microbiology). These findings will be shared through oral presentations or poster sessions.

Data Sharing: Following the publication of major research findings, and upon approval by the Ethics Committee and completion of de-identification, the anonymized dataset collected in this study will be made available for sharing in public data repositories, as appropriate, in accordance with international practices and subject to compliance with ethical and data security regulations, to facilitate further research and validation within the scientific community.

2. Publication Timeline

6–9 months after study completion (database lock-in): Complete the analysis of primary outcomes, draft, and submit the first main manuscript containing results for primary endpoints (e.g., 28-day mortality rate, diagnostic turnaround time).