Indian Council of Medical Research (ICMR)

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(Ministry of Health and Family Welfare)

Investigator-Initiated Research Proposals for Small Extramural Grants - 2025

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Proposal Id: IIRPSG-2025-01-00715, **Version Id:** F1,
Proposal Title: Development of a novel CRISPR-Cas12a-based diagnostic platform for ESKAPE pathogens and associated antimicrobial resistance in sepsis patients (CASPR)

Personal details of Principle Investigator (PI)

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Nature of Employment	Permanent
Institute	Postgraduate Institute of Medical Education & Research, Govt , Chandigarh, Chandigarh

Proposal Details PART-A

Nature of employment in Medical Institutes, Research Institutes, Universities, Colleges, recognized Research & Development laboratories, Government and semi-government organizations, and NGOs.	Permanent
Advertisement	Call for Investigator-Initiated Research Proposals for small extramural grants - 2025

Summary (up to 250 words): A structured summary should contain the following subheadings: Rationale/ gaps in existing knowledge, Novelty, Objectives, Methods, and Expected outcome.
Rationale/ gaps in existing knowledge. Antimicrobial resistance (AMR) is a major global threat leading to human health and socio-economic concerns. Bacterial AMR contributed to an estimated 1.27 million deaths in 2019, making AMR a leading cause of death above HIV/AIDS and malaria, globally. The 6 leading bacterial pathogens associated with AMR are Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species (ESKAPE). A rapid and cost-effective method for early diagnosis of ESKAPE pathogens and AMR is still a challenge in treating critical conditions such as sepsis. Therefore, empirical treatment is given to patients based on the provisional diagnosis. Novelty. We aim to use Clustered Regularly interspaced short palindromic repeats (CRISPR)-based nucleic acid detection approach for the diagnosis of ESKAPE pathogens and AMR. No such test currently exists for diagnosing sepsis patients. Objectives. The present study proposes the development of a novel CRISPR-based diagnostic platform for ESKAPE pathogens and AMR for early diagnosis of sepsis patients directly from blood samples. Methods. A novel CRISPR-based platform for the diagnosis of ESKAPE pathogens and AMR will be developed and standardized using a multi-target approach. The assay will be further validated on the blood samples from presumptive sepsis patients. The diagnostic accuracy of the developed assay will be assessed against the gold standards. Expected outcome. This novel CRISPR-based diagnostic platform could open new avenues for rapidly diagnosing ESKAPE pathogens and AMR and can guide the clinician about the therapeutic aspect of the disease overcoming the need for empirical therapy.

Priority Area/Priority Area diseases	Communicable Diseases (bacterial,viral, fungal, parasitic) / Antimicrobial resistance
Keywords Six keywords separated by comma which best describe your project may be provided.	AMR, ESKAPE, CRISPR, Sepsis, Diagnostics, AST
Abbreviations Only standard abbreviations should be used in the text. List of abbreviations maximum of ten may be given as a list.	AMR: Antimicrobial resistance, ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species, ICU: Intensive Care Unit, TPP: Target Product Profile, BCID2: Blood Culture Identification 2, CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats, Cas: CRISPR-associated proteins, SHERLOCK: Specific High Sensitivity Enzymatic Reporter UnLOCKing, RPA: Recombinase Polymerase Amplification, gRNA: Guide RNAs.

Skill and experience of the research team (Highlight only salient points (along with 5 relevant publications) that provides confidence to reviewers that team can implement the project with quality.) **:** Dr. Sagarika Haldar (PI): She has more than twelve years of experience working in TB research (both basic and clinical) and has been awarded the Ranbaxy Science Foundation Young Scientists Scholarship Award, the Innovation award by THSTI and the DST INSPIRE Faculty Award for her research on TB diagnosis. Her research focus has been directed toward developing effective diagnostic tests for pulmonary TB, drug-resistant TB, and extra-pulmonary TB. Her research work includes industry-academia initiatives to translate laboratory innovations into kits for improved TB diagnosis. She has been successful in the development of 3 kits namely the 'TB Detect' kit for improved smear microscopy for TB TB Concentration Transport' kit for dry and bio-safe sputum transport and the 'TB DNA Extraction kit' for M. tuberculosis DNA isolation for improved TB and drug-resistant TB diagnosis. These patient-friendly kits address several of the existing challenges in TB control and are designed to provide 'Universal Access' for rapid TB diagnosis, including drug-resistant disease, and have been evaluated by ICMR and recommended for integration into the National TB programme. She has done pioneering work in the field of cell-free nucleic acid-based diagnosis in India and has published work for the diagnosis of various forms of extra-pulmonary TB. Her translational research laboratory is focused on developing molecular/protein detection platforms for the diagnosis of various forms of extra-pulmonary tuberculosis. Presently, an ICMR funded project on CRISPR-based diagnostic platform for Intrathoracic tuberculosis in children is also ongoing. Publications (relevant to the project) • Sharma P, Gupta RK, Anthwal D, Dass M, Yadav R, Behera A, Sethi S, Singhal R, Dhooria S, Aggarwal AN, Haldar S, 2023. Evaluation of Mycobacterium tuberculosis derived cell-free DNA using pleural fluid and paired plasma samples for the diagnosis of pleural tuberculosis in Tuberculosis 142 (2023) 102369. • Dass M, Kaur M, Aittan S, Sharma P, Punia S, Muthumohan R, Anthwal D, Gupta RK, Mahajan G, Kumari P, Neera Sharma N, Taneja RS, Sharma LK, Shree R, Tyagi JS, Lal V, Haldar S. MPT51 and MPT64-based antigen detection assay for the diagnosis of extrapulmonary tuberculosis from urine samples, Diagnostic Microbiology and Infectious Disease, 107:1 (2023) 115973. • Kumari P, Dhiman A, Lavania S, Sharma P, Rath D, Anthwal D, Gupta RK, Kochar A, Sharma Nc, Gadpayle AK, Taneja RS, Sharma L, Haldar S, Sharma TK, Tyagi JS, 2022. Assessment of DNA aptamers targeting GlcB and HspX antigens for application in the diagnosis of abdominal tuberculosis in Tuberculosis, 134 (2022) 102206. • Dass M, Aittan S, Muthumohan R, Anthwal D, Gupta RK, Mahajan G, Kumari P, Neera Sharma N, Taneja RS, Sharma LK, Shree R, Lal V, Tyagi JS, Haldar S. Utility of cell-free transrenal DNA for the diagnosis of Tuberculous Meningitis: A proof-of-concept study, Tuberculosis 135 (2022) 102213. • Sharma P, Anthwal D, Kumari P, Gupta RK, Lavania S, Sharma N, Sharma LK, Rath D, Soraganvi PK, Sharma A, Gadpayle AK, Taneja RS, Tyagi JS, Haldar S. Utility of circulating cell-free Mycobacterium tuberculosis DNA for the improved diagnosis of abdominal tuberculosis. PLoS One. 202015(8): e0238119. • Anthwal D, Lavania L, Gupta R K, Verma A, Myneedu VP, Sharma PP, Verma H, Malhotra V, Gupta A, Gupta NK, Sarin R, Haldar S, Tyagi JS Development and evaluation of novel bio-safe filter paper-based kits for sputum microscopy and transport to directly detect Mycobacterium tuberculosis and associated drug resistance. PLOS ONE (2019) 14(8): e0220967. • Kumari P, Lavania S, Tyagi S, Dhiman A, Rath D, Anthwal D, Gupta RK, Sharma N, Gadpayle AK, Taneja RS, Sharma L, Ahmad Y, Sharma TK, Haldar S, Tyagi JS. A novel aptamer-based test for the rapid and accurate diagnosis of pleural tuberculosis. Anal Biochem. (2019)1 564-565:80-87. • Anthwal, D, Jamwal, S, Gupta RK, Singhal R, Verma A, Bhalla M, Myneedu VP, Sarin R, Choudhury S, Tyagi JS, Haldar S. Direct Molecular Detection of Drug-Resistant Tuberculosis from Transported Bio-Safe Dried Sputum on Filter-Paper. Current Microbiology (2022) 79:110. • Anthwal D, Gupta RK, Singhal R, Bhalla M, Verma A, Myneedu VP, Sarin R, Gupta A, Gupta NK, Singh M, Tyagi JS, Haldar S. Compatibility of a novel filter paper-based bio-safe sputum transport kit with Line Probe Assay for diagnosing drug-resistant tuberculosis: a single-site evaluation study. European Respiratory Journal Open Research (2021) 7: 00137-2021. • Anthwal D, Gupta RK, Gomathi NS, Tripathy SP, Das D, Pati S, Panwalkar N, Desikan P, Bala K, Singh UB, Bhalla M, Singhal R, Verma AK, Khayyam KU, Myneedu VP, Sarin R, Sharma S, Bansal AK, Gupta UD, Patil SA, Goyal A, Gupta A, Singh M, Gupta NK, Haldar S, Tyagi JS. Evaluation of 'TBDetect' sputum microscopy kit for improved detection of Mycobacterium tuberculosis: a multi-centric validation study. Clin Microbiol Infect (2021) 27:911.e1e911.e7. Dr. Barnali Kakati (Co-PI): Dr. Barnali is a clinical microbiologist, serves as the Professor and Head of the Department of Microbiology at Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, a leading tertiary care health center of Uttarakhand. With 16 years of extensive experience in teaching, diagnostics, and research, Dr. Barnali has authored 44 research papers in prestigious national and international journals. Her expertise lies in antimicrobial resistance and bacteriology. Publications (relevant to the project): • Comparative performance of biofire pneumonia panel and standard culture-based methods for diagnosing pneumonia in critically ill patients: Impact on antibiotic stewardship. B Kakati, R Singh, G Mittal, N Koul. Indian J Med Microbiol. 202449:100564. doi:10.1016/j.ijmmb.2024.100564. • Emergence of carbapenem-resistant non-fermenting gram-negative bacilli isolated in an ICU of a tertiary care hospital. S Agarwal, B Kakati, S Khanduri, S Gupta. Journal of clinical and diagnostic research: JCDR 11 (1), DC04. • ESBL and MBL in cefepime resistant Pseudomonas aeruginosa: an update from a rural area in Northern India.A Kotwal, D Biswas, B Kakati, M Singh.Journal of clinical and iagnostic research: JCDR 10 (4), DC09. • Detection of Klebsiella pneumoniae carbapenemases (KPCs) among ESBL/MBL producing clinical isolates of Klebsiella pneumoniae. M Singh, B Kakati, RK Agarwal, A Kotwal. Int J Curr Microbiol App Sci 4 (4), 726-31. • Antibiotic resistance pattern in intensive care unit of a tertiary care teaching hospital. A Bhatia, J Kalra, S Kohli, B Kakati, R Kaushik.Int J Basic Clin Pharmacol 7 (5), 906-911. • Severe sepsis due to Chryseobacterium indologenes, a possible emergent multidrug-resistant organism in intensive care unit-acquired infections. S Agarwal, B Kakati, S Khanduri.Indian Journal of Critical Care Medicine: Peer-reviewed. • Emerging issues regarding management of MDR non-fermenting gram-negative ventilator associated pneumonia in a rural catering tertiary care hospital. B Kakati, S Agarwal, S Gupta. J Med Sci Clin Res 4 (10), 13232-13238. • Urinary tract infection in critically ill patients with diabetes mellitus: Spectrum of uropathogens and antimicrobial susceptibility pattern. N Kishore, S Modi, S Khanduri, B Kakati. Bali Journal of Anesthesiology 4 (Suppl 2), S55-S60. • Universal Presence of blaNDM-1 Gene in Carbapenem-Resistant Gram-Negative Bacilli in an Indian Hospital in 2015.B Chatterjee, N Khanduri, B Kakati, A Kotwal.Journal of Clinical and Diagnostic Research: JCDR 11 (9), DL01. • Colistin resistance in organisms causing ventilator-associated pneumonia-Are we going into pre-antibiotic era S Agarwal, B Kakati, N Kishore, S Khanduri, M Singh. Critical Care Shock 21 (2) 2017. • Use of the Combined Modified Carbapenem Inactivation Method and EDTA-modified Carbapenem Inactivation Method for Detection of Carbapenemase-Producing Enterobacteriaceae Causing. N Koul, B Kakati, S Agarwal. J Pure Appl Microbiol 16 (2), 1239-1244. • Ventilator-Associated Tracheobronchitis–Etiology and Outcome at an Intensive Care Unit of a Tertiary Care Center in North India. B Kakati, N Koul, S Agarwal.Iranian Journal of Medical Microbiology 17 (1), 103-106. • Alarming Rise in Secondary Infections and Antimicrobial Resistance in COVID-19 Patients Admitted at a Tertiary Care Centre in Dehradun, Northern India. R Singh, N Koul, M Mittal, B Kakati, G Mittal. Journal of Clinical Diagnostic Research 16 (7). • Study of colistin and tigecycline sensitivity for carbapenem-resistant isolates of Klebsiella pneumoniae (CRKP) patients in the intensive care unit (ICU) of HIMS, Dehradun. N Kishore, B Kakati, S Khanduri, Int J Acad Med Pharm 4 (4), 71-75. • Correlation of VITEK-2 and conventional tests in identification of Non-Fermenting Gram Negative Bacilli in a Tertiary Care Hospital. S Sharma, RK Agarwal, B Kakati. DOI: 10.18231/2394-5478.2018.0042. Dr. Dr. Sonika Aggarwal (Co-I) : Dr. Sonika Agarwal is a Clinician, Professor, and Head of the Department of Critical Care Medicine at the Himalayan Institute of Medical Sciences, Swami Rama Himalayan University. She has more than 12 years of experience in managing critically ill patients and was associated with renowned institutes like GSVM Medical College Kanpur and SGPGIMS Lucknow. With over 15 national and international publications to her credit, her research has been associated with advancing our understanding of critical areas such as hospital-acquired infections and acute respiratory distress syndrome. Publications (relevant to the project): • Kakati B, Agarwal S, Gupta S. Emerging Issues Regarding Management of MDR Non-Fermenting Gram Negative Ventilator-Associated Pneumonia in a Rural Catering Tertiary Care Hospital. 2016 Journal of Medical Science And Clinical Research, 04:10. • Agarwal S, Kakati B, Kishore N, Khanduri S, Singh M. Resistance in organisms causing ventilator-associated pneumonia - Are we going into a pre-antibiotic era Crit Care Shock (2018) 21:78-87. • Agarwal S, Kakati B, Mahalingam, V, Rana P. Effectiveness of Staff Education on Prevention of Ventilator-Associated Pneumonia and Recent Trends of Antimicrobial Susceptibility of Organism Causing VAP in ICU. EAS J Anesthesiol Crit Care. Volume-1 | Issue- 4| July-Aug-2019. • Sonika Agarwal, Barnali Kakati1, Sushant Khanduri. Severe Sepsis Due to Chryseobacterium indologenes, a Possible Emergent Multidrug Resistant Organism in Intensive Care Unit Acquired Infections. Indian Journal of Critical Care Medicine, Volume 22 (11) 2018. • Nupur Koul, Barnali Kakati, Sonika Agarwal, Garima Mittal. Multidrug-resistant Acinetobacter baumannii causing Ventilator-associated respiratory infections at a tertiary care center of India. International Journal of Medical Microbiology and Tropical Diseases 20228(1):15–18. • Sonika Agarwal, Barnali Kakati, Sushant Khanduri, Shalini Gupta. Emergence of Carbapenem Resistant Non-Fermenting Gram-Negative Bacilli Isolated in an ICU of a Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. 2017, 11(1). • Nupur Koul, Barnali Kakati and Sonika Agarwal. Use of the Combined Modified Carbapenem Inactivation Method and EDTA-modified Carbapenem Inactivation Method for Detection of Carbapenemase Producing Enterobacteriaceae Causing Ventilator-associated Respiratory Infections. J Pure Appl Microbiol.2022. • Barnali Kakati, Nupur Koul, Sonika Agarwal. Ventilator-Associated Tracheobronchitis – Etiology and Outcome at an Intensive Care Unit of a Tertiary Care Center in North India. J Med Microbiol. 2023 17(1): 103-106. • Singhal, Sanjay, Gurjar, Mohan, Sahoo, Jyoti Narayan, Saran, Sai, Dua, Ruchi, Sahoo, Alok Kumar, Sharma, Ankur, Agarwal, Sonika. Aerosol drug therapy in critically ill patients (Aero-in-ICU study): A multicentre prospective observational cohort study. Lung India 41(3):p 200-208, May–Jun 2024. Dr. Divya Anthwal (Co-I):

Dr. Divya Anthwal is a keen researcher with training in Infectious disease diagnostics with more than 9 years of proven experience in the field of Tuberculosis. Her research focus has been directed toward developing effective molecular diagnostic tests for pulmonary TB, drug-resistant TB, and extra-pulmonary TB. She was an integral part of the industry-academia collaborative initiative (in collaboration with Advanced Microdevices Pvt. Ltd., Ambala, and AIIMS, New Delhi) to develop 3 kits namely the 'TB Detect' kit for improved smear microscopy for TB TB Concentration Transport' kit for dry and bio-safe sputum transport and the 'TB DNA Extraction kit' for M. tuberculosis DNA isolation. She has been involved in several ICMR projects for the development of molecular diagnostics tests for TB and associated drug resistance including independent ICMR-SRFship. Publications (relevant to the project): • Anthwal D, Gupta RK, Bhalla M, Bhatnagar S, Tyagi JS, Haldar S, 2017. Direct detection of Rifampicin, Isoniazid and multi-drug resistance in sputum samples from tuberculosis patients by High-Resolution Melt curve analysis in Journal of Clinical Microbiology 55:1755–1766. • Anthwal D, Lavania S, Gupta RK, Verma AK, Myneedu VP, Sharma PP, Verma H, Malhotra V, Gupta A, Gupta NK, Sarin R, Haldar S, Tyagi JS, 2019. Development and evaluation of novel bio-safe filter paper-based kits for sputum microscopy and transport to directly detect Mycobacterium tuberculosis and associated drug resistance in PLoS ONE 14(8): e0220967. • Sharma P, Anthwal D, Kumari P, Gupta RK, Lavania S, Sharma N, Sharma LK, Rath D, Soraganvi PK, Sharma A, Gadpayle AK, Taneja RS, Tyagi JS, Haldar S, 2020. Utility of circulating cell-free Mycobacterium tuberculosis DNA for the improved diagnosis of abdominal tuberculosis in PLoS ONE 15(8): e0238119. • Anthwal D, Gupta RK, Gomathi NS, Tripathy SP, Das D, Pati S, Panwalkar N, Desikan P, Bala K, Singh UB, Singhal R, Bhalla M, Verma AK, Myneedu VP, Sarin R, Bansal AK, Gupta UD, Patil SA, Goyal A, Gupta A, Gupta NK, Singh M, Haldar S, Tyagi JS, 2021. Evaluation of 'TBDetect' sputum microscopy kit for improved detection of Mycobacterium tuberculosis: a multi-centric validation study in Clinical Microbiology and Infection 2021 27:911.e1e911.e7. • Anthwal D, Gupta RK, Singhal R, Bhalla M, Verma A, Myneedu VP, Sarin R, Gupta A, GuptaNK, Singh M, Tyagi JS, Haldar S, 2021. Compatibility of a novel filter paper-based bio-safe sputum transport kit with Line Probe Assay for diagnosing drug-resistant tuberculosis: a single-site evaluation study in ERJ Open Research 2021,7:00137-2021. • Anthwal D, Jamwal S, Gupta RK, Singhal R, Verma AK, Bhalla M, Myneedu VP, Sarin R, Choudhary S, Tyagi JS, Haldar S, 2022. Direct Molecular Detection of Drug-Resistant Tuberculosis from Transported Bio-Safe Dried Sputum on Filter-Paper in Current Microbiology (2022) 79:110. equal first author.

Institutional Support/ Facilities: The proposed study will be implemented through an inter-institutional collaboration between PGIMER, Chandigarh, and the Himalayan Institute of Medical Sciences at Swami Rama Himalayan University, Dehradun. Both institutes were involved in conceptualizing and formulating the study. Role and Responsibility of all Participating Investigators Dr. Sagarika Haldar and Dr. Ashok Kumar Yadav (PGIMER): (i) Development of the CRISPR-based diagnostic platform for ESKAPE pathogens and associated drug resistance (Objective 1), (ii) Validation of the developed assay on collected samples and evaluation of its diagnostic accuracy against standard blood culture diagnosis and BIOFIRE Filmarray Blood Culture Identification panel (Objective 3) Dr. Barnali Kakati, Dr. Sonika Aggarwal, and Dr. Divya Anthwal (SRHU): (i) Enrollment of suspected sepsis patient (n100) admitted to the ICU of Himalayan Hospital and collection of their blood samples, (ii) Execution of the gold standard testing (standard blood culture diagnosis AST and BIOFIRE Filmarray Blood Culture Identification panel- Objective 2).

Laboratory facilities (in-vitro/ in-silico) Institutional resources such as instruments/ equipment and other physical resources available for use in the project proposed animal house etc.
PI Lab (PGIMER): 1. Real-time PCR, 2. Bio-safety Cabinets, 3. High-speed refrigerated Centrifuge, 4. -20 degree fridge, 5. -80 degree fridge Co-PIs Lab (SRHU): 1. BIOFIRE System, 2. VITEK-2 system, 3. BACT/ALERT system, 3. Incubator, 4. Microscope

Conflict of Interest declaration (if any) We declare no Conflict of Interest.

Duration (in Months) 36 Months

Investigator Details

#	Name	Institute	Designation	Email	Contact No.	Nature of Employment	Role in Proposal
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4	Dr SONIKA AGARWAL	Swami Rama Himalayan University	Professor	sonikakatiyar@srhu.edu.in	9935060858	Permanent	Co-PI
5	Dr Divya Anthwal	Swami Rama Himalayan University	Scientist C	anthwal.divya14@gmail.com	8527155938	Permanent	Co-PI

Documents consideration

#	Document Name	Is Applicable?	Uploaded Document	Remarks
1	Declaration & Attestation Form(duly signed by Head of Department/ Director)	Yes	View	Declaration Attestation form with Declaration of Interest forms, CV of investigators, and a copy of the Statutory Audit Authority of the collaborating Institute (SRHU, Dehradun)
2	Additional supplementary information including figures tables flow diagrams etc can be shared as PDF	Yes	View	Research Proposal with figures and tables