Joint Research Project
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Big Impact In Global Health.

Grand Challenges Canada Grant Number S4 0253-01_
Stars in Global Health Round 4 Phase I:
**Development of diagnostic kit for early and rapid detection of pulmonary and extrapulmonary tuberculosis and its evaluation in health centres**

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**Center for Innovative Technology**

**Finacial Support Grand Challenges Canada and Vikrant Group of Institutions, Gwalior**

Project under Investigation
Grand Challenges Canada Grant Number S4 0253-01_
Stars in Global Health Round 4 Phase I: Development of diagnostic kit for early and rapid detection of pulmonary and extrapulmonary tuberculosis and its evaluation in health centres

**Investigators:**

Professor Prakash S Bisen
Mentor

Dr. Ms Ruchika Raghuvanshi
Principal Investigator

Dr. Anish Zacharia Joseph
Research Associate

**List of Patents Applied:**

Indian Patent “A diagnostic kit for detection of tuberculosis”
Indian Patent “A method for the preparation of working reagent for detection of tuberculosis”
Indian Patent “Identification of circulatory markers of Mycobacterium tuberculosis by Gas Chromatography Mass Spectroscopy”

**External Collaborators in the research Programme**

Professor GBKS Prasad
School of studies in Biochemistry, Jiwaji University, Gwalior-474011

Dr. Anubhav Jain
SDiagnostic Division RFCL Limited (Formerly Ranbaxy Fine Chemicals Limited), Avantor Ranbaxy Fine Chemicals Limited Avantor performance materials, New Delhi, India

Dr. Pankaj Krishna
SDiagnostic Division RFCL Limited (Formerly Ranbaxy Fine Chemicals Limited), Avantor Ranbaxy Fine Chemicals Limited Avantor performance materials, New Delhi, India

Dr. R.P.Tiwari
Rapid Diagnostic Group of Companies, New Delhi, India
Objective

To understand the microbial community structure in active TB patients by next gen sequencing

Methodology

Sample collection

All of the procedures for the collection and handling of patient samples and data will be as per local ethics committee and in compliance with the Helsinki Declaration of the World Medical Association. All study subjects will be provided with written informed consent to participate in the study. Total of 30 samples will be collected in which 15 samples will be from healthy people which will serve as control. Rest 15 samples will be collected from TB infected persons which has confirmed with microbiological and clinical finding.

Next-gen sequencing

Metagenomic sequencing technique offers a powerful lens for viewing the microbial world that has the potential to revolutionize understanding of the microbiome, bypassing the need for isolation and lab cultivation of individual species. The workflow for Metagenomic sequencing on Ion torrent platform is detailed below.

16s RDNA amplification and DNA Library preparation

1. 16s DNA amplification – Amplify particular variable region of 16S in the given samples or the entire 16s will be amplified and sheared to form a library.
2. The yield and size of the amplified fragment will be assessed and checked
3. Template Dilution Factor will be determined

DNA Library enrichment

1. QC & QA before enrichment will be done
2. Emulsion on diluted construct will be performed and enrichment of the template will be done
3. QC & QA after enrichment will be done

Sequencing on ION TORRENT by 314/316/318 chip or ION PROTON by PI chip

1. There is possibility to create 1 million 80 million features in 200-400 bp sequencing chemistry
2. This will give 150-350 bp mean read length (0.2 – 80 million reads passing filter)
3. The total data will be roughly 20 to 7500 MB and possible to get 10 GB
4. Possible to do multiplex up to 16 samples in single run

Bioinformatics

▶ Sequencing summary with read length histogram with primary data analysis will be done
▶ Sequence data file in the form of FASQ and BAM
▶ Assembly, Alignment and species identification against 16S database will be done
Research Patent Under Publication

- Indian Patent "A diagnostic kit for detection of tuberculosis"
- Indian Patent "A method for the preparation of working reagent for detection of tuberculosis"
- Indian Patent "Identification of circulatory markers of Mycobacterium tuberculosis by Gas Chromatography Mass Spectroscopy"

Collaborators

Professor Prakash S Bison
Dr. Ms Ruchika Raghuvanshi
Dr. Anish Zacharia Joseph

Book Published

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Research Collaboration with

1. Vellore Institute of Technology

Work is underway on monoclonal antibodies for various antigens of medical importance for diagnostic and research purposes in collaboration with Advanced Centre for Bioseparation Technology, Vellore Institute of Technology, VIT University, Vellore 632014.

Prof. M.A. Vijayakalshmi
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Director. CBST
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2. Biochemistry Department, Jiwaji University Gwalior

Research Paper Under Submission for Publication:


Blood Collection
Blood collection was made from nearby villages from Tuberculosis infected patients of following category:

1. Fresh cases without antibiotic treatment
2. Antibiotic treated patients
3. Positive control
4. Negative control

Visit was made to Chattarpur, Nawgaon, Panna, Shicvpuri, Guna and Bhopal depending of the information available about TB patients.