BCIL Biotech Consortium India Limited

Molecular Probes for Detection of Mycobacteria

TTO@BCIL
Accelerating Technology Translation

TECHNOLOGY AVAILABLE FOR TRANSFER

UNMET NEED

- Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and is a leading cause of deaths worldwide. Late diagnosis of TB is one of the major reasons for its prevalence and resulting deaths.
- Microscopic, culture, and nucleic acid amplification tests are commonly used to screen and diagnose TB, but poor sensitivity, selectivity, specificity, and cross-reactivity are the major issues faced by these tests.
- Extra-pulmonary TB (EPTB) alone represented nearly 15% of reported TB cases in 2018. formalin-fixed paraffin-embedded (FFPE) tissue sections are the only tissues available for examination in case of EPTB. But the presence of a very low number of mycobacteria and inability of current enzyme activity-dependent diagnostics methods are the key hurdles in developing EPTB diagnostics. Consequently, current EPTB diagnostic methods like microscopy/PCR/ antibody/ aptamer suffers from poor efficacy and therefore there is a demand for better diagnostics, especially for extra-pulmonary TB.
- Mycobacterial membrane composition provides a unique target that can be exploited to engineer mycobacterial membrane-specific molecular probes for its precise diagnosis.
- Such uniquely developed probes are believed to detect TB early with high specificity and sensitivity without cross-reacting with other bacteria. Such early diagnosis will help to start TB treatment timely and can help to manage TB spread effectively.
- So in the current scenario, there is an urgent need for diagnostics technologies that can facilitate early detection of mycobacteria with high specificity and sensitivity without cross-reactivity with other bacteria.

TECHNOLOGY

- The technology is based on novel synthetic modified Cholic acid or deoxycholic acid-based amphiphile lipid small molecules with the ability to bind specifically to mycobacterial lipids, trehalose monomycolate, and phosphatidylinositol mannoside 6.
- These small molecules are further developed into probes by conjugating them with standard Fluorophore/ imaging agents or with Biotin or Alkyne / Azide for selective binding and easy detection of *mycobacteria* from the samples, which can be detected by corresponding detection systems
  - flow cytometry and fluorescence microscopy.
  - Alkyne Streptavidin-Fluorophore conjugates/Streptavidin tagged quantum dots/ Streptavidin conjugated enzymatic assays
  - Copper catalysed/Copper-free click chemistry.

Validation

- In-house lab validation completed for probe selectivity for Mycobacteria, in presence of different Gram-negative, Gram-positive bacterial strains along with microbial and poly- microbial biofilms. Probes are also validated for the detection of extra-pulmonary TB in 30 human tissue samples.
- Selectivity of developed probes were tested against different Gram-negative, Gram-positive, and mycobacterial strains using flow cytometry studies at different concentrations of the probe. which proved that these probes can stain >90% of the mycobacterial cells and are unable to stain Gram-negative strains

BCIL Biotech Consortium India Limited

Molecular Probes for Detection of Mycobacteria

TECHNOLOGY AVAILABLE FOR TRANSFER

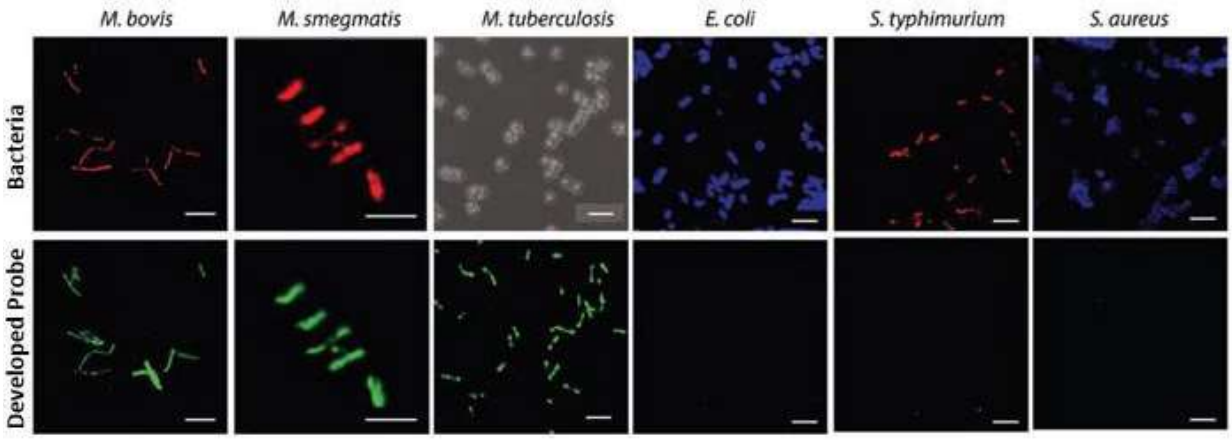

Fig. Probes specificity and sensitivity when compared with bacterial stains

- Detection of mycobacteria in polymicrobial cultures is validated by using fluorescence- the experiment has confirmed the specific co-localization of developed probes only with mycobacteria and not with Gram-negative or Gram-positive bacteria.

- Detection of mycobacteria in biofilms and polymicrobial biofilms by using developed probes has also been performed. It has confirmed the co-localization of developed probes only with mycobacteria and not with other bacterial species under confocal microscopy.

- Detection of mycobacteria in human tissue sections- The ability of the developed probe to detect the mycobacteria in infected human tissues was tested. the results show that the probes can detect the presence of mycobacteria in human tissue sections (FITC Channel). Co-localization of antibody staining confirm the presence of mycobacteria in the sections.

- Differentiation between Gastrointestinal TB and Crohn's Disease human tissue sections-

  The ability of developed probes to differentiate between the human Gastrointestinal and Crohn's Disease tissues was tested. The presence of mycobacteria was confirmed only in the TB positive sections by the colocalization of antibody staining with the developed probe, but it did not show the presence of mycobacteria in Crohn's disease sections.

  Next, the research team has performed a blind screening of mycobacteria-infected tissue sections representing gastrointestinal tuberculosis from patients and noninfected tissue sections from patients with developed probe and mycobacteria-specific antibodies.

  The developed probes could detect mycobacteria in all the mycobacteria-infected FFPE tissue sections confirmed by antibody staining and clinical diagnosis. In contrast, the research team did not find any staining in the FFPE tissue sections of noninfected patients, which was validated with the help of antibody staining, histopathological, and clinical diagnosis.

BCIL Biotech Consortium India Limited

Molecular Probes for Detection of Mycobacteria

TECHNOLOGY AVAILABLE FOR TRANSFER

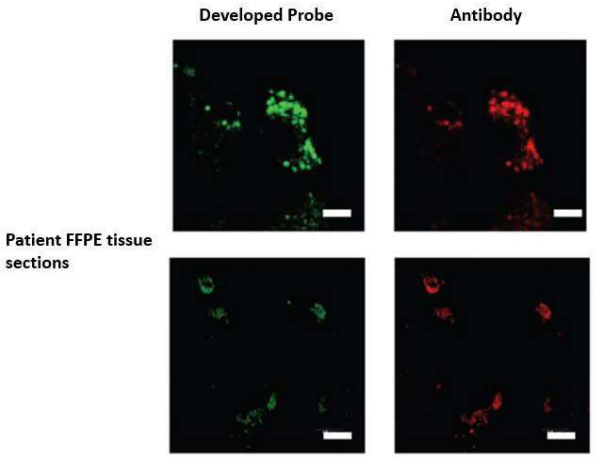

Fig. Fluorescence micrographs of FFPE tissue sections of suspected mycobacteria infected patients after staining with developed probe (green fluorescence) and mycobacteria specific antibody (Red).

APPLICATIONS

- Development of precise point of care diagnostic systems (probes/assays/kits/sensors) for TB.
- Effective extrapulmonary TB detection.

UNIQUE SELLING PROPOSITION

- Molecular probes for pulmonary as well as extra-pulmonary TB detection.
- Probes detects pathogenic, non-pathogenic, drug-sensitive, drug-resistant, persistent, live/dead, metabolically active, and inactive mycobacteria among other microorganisms, mammalian cells, polymicrobial planktonic cultures and biofilms or other biological and nonbiological materials.
- Detection of single mycobacterium in the presence of 10, 000 other bacilli.
- Rapid (in 5 min.) and accurate under in-vitro and in-vivo settings with specificity and sensitivity of 99%.
- Unlike antibody-based TB detection systems the synthetic amphiphile-based TB detection systems are free from batch to batch variation.
- Differentiation of clinical mycobacterial infections from other diseases e.g. Crohn's disease.

Biotech Consortium India Limited

Molecular Probes for Detection of Mycobacteria

TECHNOLOGY AVAILABLE FOR TRANSFER

OPPORTUNITY

- The global Tuberculosis Diagnostics market was estimated at USD 2.58 billion in 2019 and expected to grow at a CAGR of 4 % from 2020 – 2027
- The increasing prevalence of multi-drug resistant (MDR) tuberculosis, patient awareness, improved health care infrastructure and increased expenditure, rising geriatric population, government initiatives, and investments are the key factors driving the growth of the market.
- Advanced diagnostic methods like PoC's is the fastest-growing market in this sector.
- As there is a longstanding need for precise diagnostics technologies for extrapulmonary TB detection. These probes provide a promising prospectus to develop such diagnostic systems by using the same.

STAGE OF TECHNOLOGY

- Lab Scale technology and ready for industrial scale-up for production of probes.
  Further, these probes can be modified for their easy application and convenience as per the need of the diagnostic system/Kit/Assay to be developed.

INTELLECTUAL PROPERTY

- Indian patent application published- Nov 2019
- PCT patent application published- Nov 2019

LICENSING OPPORTUNITY

BCIL is looking for a suitable industrial partner for the development and commercialization of rapid and precise diagnostics for *mycobacterial* infections.

CONTACT:
Dr. Purnima Sharma, Managing Director
BIOTECH CONSORTIUM INDIA LIMITED
V Floor, Anuvrat Bhawan, 210, Deen Dayal Upadhyaya Marg,
New Delhi:110002 Phone: +91-11-2321 9064-67
Fax: +91-11-23219063
Email: tto.bcil@biotech.co.in & info.bcil@biotech.co.in
Website: www.biotech.co.in