# Mycobacterium tuberculosis.....can we beat it?

Thursday, 21 March 2013
The Royal College of Pathologists, London, UK

*Mycobacterium tuberculosis* is one of the most successful human pathogens. Treatment of tuberculosis requires a long duration time with the use of multiple drugs. There is also an alarming emergence of multidrug resistant M. tuberculosis. As a result a need have arisen to develop novel anti-tubercular agents. This EuroSciCon meeting will present cutting-edge research on developments in the detection and treatment of tuberculosis.

This event has CPD accreditation and will have a discussion panel session.

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

Meeting Chair: ***Dr. Sanjib Bhakta***, Head of Mycobacteria Research Laboratory, Institute of Structural and Molecular Biology, Birkbeck, University of London & UCL Research Department of Infection**.**

9:00 – 9:45 **Registration**

9:40 –10:00 **Introduction by the Chair**: *Dr. Sanjib Bhakta*
Birkbeck, University of London, London , United Kingdom

**An integrative approach in targeting different physiological stages of *Mycobacterium tuberculosis*.** Dr Sanjib Bhakta, Head of Mycobacteria Research Laboratory, Institute of Structural and Molecular Biology, Birkbeck, University of London & UCL Research Department of Infection

10:00 – 10:20 M***ycobacterium tuberculosis* in the Moonlight: The Unusual Preponderance of Moonlighting Proteins Used by *Mycobacterium tuberculosis* as Virulence Factors**
*Professor Brian Henderson*, Eastman Dental Institute, London, UK
Protein moonlighting defines a recently recognised property of some proteins to have more than one unique biological function. Evidence is emerging that moonlighting proteins can play a role as bacterial virulence factors with a growing number of human pathogens. Most pathogens identified as having moonlighting proteins generally have only one, or at most two, such molecules. In contrast, at the time of writing, *M. tuberculosis* has 12 of its proteins functioning as moonlighting molecules each playing a potential, or identified, role in the virulence phenotype of this organism. Examples include the antigen 85 complex proteins which function both as mycosyltransferases and as ligands for the major host component fibronectin. Binding to fibronectin is a well-recognised method for bacteria both to adhere to the host and also to invade host cells. The cell stress protein, chaperonin 60.2 is found on the cell surface and functions as an adhesin and invasin by binding to the host cell receptor CD43 on macrophages. The paralogous protein, chaperonin 60.1, plays a major role in the generation of the multinucleate giant cells found in the tuberculoid granuloma, but appears not to be an adhesin. These chaperonins also have major effects on macrophage activation. The role of the various mycobacterial moonlighting proteins in tubercular disease will be described, and their potential as therapeutic targets will be explored

10:20 – 10:30 **POLYTB: A genome-browser web tool to investigate *m. tuberculosis* genetic polymorphisms derived from next generation sequencing data**
Francesc Coll, Mark Preston, Kim Mallard, Ruth McNerney, Nigel Martin, Taane Clark
London School of Hygiene and Tropical Medicine, London, UK; BirkBeck College, London, UK

10:30 – 10:40 **Investigation of the structural contributions to the antimicrobial peptides activity against Mycobacterium tuberculosis**
Y Lan, WC Yam, AJ Mason and JKW Lam*
*Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 2/F, Laboratory Block, 21 Sassoon Road. Hong Kong*

10:40 – 11:00 **Speakers' photo then mid-morning break/networking , trade show and POSTER SESSION 1**
*Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you*

11:00 – 11:20 **New approaches to sputum analysis; implications for treatment and transmission**
*Professor Mike Barer*, Professor of Clinical Microbiology, University of Leicester, UK

11:20 – 11:40 **Simple diagnosis of TB infection**

*Christopher Granger*, Director, Global Professional Relations, Oxford Immunotec Ltd, Abingdon,United Kingdom
The T-SPOT.TB test identifies TB infection. Guidelines in many countries indicate its use in preference to the tuberculin skin test in many of the following patient groups:

- Contact tracing
- Healthcare workers
- HIV patients
- Immuno-suppressed patients, including pre-TNF screening
- New Entrants
- Hard-to- reach groups, including prisoners

The design of the test ensures it has excellent sensitivity and specificity. Additionally the simple phlebotomy and robust assay methodology allows the test to be carried out easily and simply in all these patient groups.

11:40- 12:00 **How we can use drugs to treat tuberculosis better**

*Professor Stephen Henry Gillespie, University of St Andrews**, Scotland***
There is a growing body of research at preclinical and clinical stages investigating new drugs and regimens for the treatment of tuberculosis. We have also learned much about the differing cell states of tuberculosis and how they pose a challenge to treatment. This talk will summmarise some of thie key new data in the area and attempt to indicate what the challenges are to much shorter treatment regimens and how these might be achieved.

12:00 - 12:10 **A new approach for fast diagnosis of tuberculosis using gas chromatography-mass spectrometry and chemometrics**

NA Dang[a,*], S Kuijper[a], E Walters[b], M Claassens[b], D Soolingen[c], G Vivo-Truyols[a], HG Janssen[a,d], AHJ Kolk[a]
Analytical Chemistry & Forensic Science, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

12:10- 12:20 **Development of aminocoumarins as a new class of potential drug candidates against multi-drug resistant tuberculosis (MDR-TB)**

R. Tandon, P. Ponnan, K. Garima, N. Aggarwal, M. V. Basil, M. Nath, V.S. Parmar, H. G. Raj, A. K. Prasad, M. Bose
Department of Microbiology, V. P. Chest Institute, University of Delhi, India

12:20- 12:30 **Genes mutations of Drug-Resistant, (Rifampicin, Isoniazidr and Fluoroquinoloner), *Mycobacterium tuberculosis* from TB patients in Thailand**

Pannamthip Pitaksajjakul, Dhruba Kumar Khadka, Pongrama Ramasoota
Faculty of Tropical Medicine, Mahidol University 420/6 Rajwithii Road, Bangkok, Thailand

12:30 – 13:30 **Lunch/networking and trade show**

*This is also a good time to fill out your feedback forms and any questionnaires*

13:30 – 14:20 **Question and Answer Session**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:20 – 14:40 **Development of immune-based diagnostics for TB in a TB-endemic setting**

Jayne Sutherland, Acting Head of TB Immunology, MRC Unit, The Gambia
One of the major roadblocks in reducing TB transmission is the lack of fast and accurate diagnostic tests for use in resource-poor settings. We analysed Mtb-antigens stimulated blood samples from TB suspects using a 13-plex cytokine assay. Our results indicate increased sensitivity when analytes are combined with sCD40L, IL10 and TGFα resulting in 89% correct classification of TB. Following verification in genetically diverse populations (including stratification based on strain of infection, bacterial load, HIV status and location) these will subsequently be used to develop a rapid, lateral-flow based test for screening of TB in resource-poor, TB-endemic settings.

14:40- 14:50 **Monitoring early host responses associated with starting effective tuberculosis treatment**

AL. den Hertog, AF. de Vos, PR. Klatser, RM. Anthony
Royal Tropical Institute, KIT Biomedical Research, Meibergdreef 39 1105 AZ Amsterdam, The Netherlands

14:50 – 15:00 **Mangosteen extract coated pre-filter could inhibit aerosol *M. tuberculosis***

Natthakarn Tipkrua, Sunit Suksamrarn,[b] Pannamthip Pitaksajjakul, and Pongrama Ramasoota.
Faculty of Tropical Medicine, Mahidol University 420/6 Rajwithii Road, Bangkok, Thailand

15:00 – 15:30 **Afternoon Tea/Coffee, networking, trade show and POSTER SESSION 3**

15:30 – 15:50 **Mycobacterium tuberculosis – new ideas, new drugs and new vaccination strategies**
*Professor Graham Bothamley,* Homerton University Hospital, London, UK
Effective short-course (6 month) treatment for tuberculosis has been available for almost 30 years. The failure to contain this problem is largely one of health care delivery. To short-circuit this problem, we need drugs which reduce the time required for effective treatment and vaccination strategies which prevent latent TB developing into infectious smear-positive disease. An understanding of latent tuberculosis is essential for both targets. M/XDR-TB offers an opportunity to test bactericidal drugs to replace isoniazid and drugs which act on slowly dividing populations of bacilli to replace rifampicin. Genomic and proteomic studies permit an examination of latency in Mycobacterium tuberculosis. New immunological tests offer the opportunity to examine markers of latency and reactivation.

15:50 – 16:10 **Targeting the cell wall of Mycobacterium tuberculosis**
*Dr Luke Alderwick*, Director of the Birmingham Drug Discovery and Screening Facility, University of Birmingham, UK
The D-arabinan-containing polymers arabinogalactan (AG) and lipoarabinomannan (LAM) are essential components of the unique cell envelope of the pathogen Mycobacterium tuberculosis. Biosynthesis of AG and LAM involves a series of membrane-embedded arabinofuranosyl (Araf) transferases whose structures are largely uncharacterised, despite the fact that several of them are pharmacological targets of ethambutol, a frontline drug in tuberculosis therapy. Recently, benzothiazinone (BTZ) inhibitors have shown nanomolar potency against both drug-susceptible and multidrug-resistant strains of the tubercle bacillus. However, their proposed mode of action is lacking structural evidence. This talk will cover some recent advances regarding the biosynthetic pathways leading to cell wall assembly including a report on the crystal structure of the BTZ target, a FAD-containing oxidoreductase M. tuberculosis DprE1, which is essential for viability. These results mark a significant step forward in the characterisation of a key TB drug target.

16:10 – 16:30 **Novel targets for tackling M. tuberculosis inside macrophage**
*Professor Edith Sim*, Dean of the Faculty of Science Engineering and Computing at Kingston University, Kingston University, Surrey, UK

16:30 – 16:50 **Biomarkers for monitoring TB treatment**
*Professor Timothy McHugh,* Centre for Clinical Microbiology, UCL, UK

16:50 - 17:00 **Chairman's summing up**

Dont forget to sign up to Euroscicons' e-newsletter at www.euroscicon.com/signup.htm to keep up to date with European Life Science news and events and to be notified of the follow up to this event

About the chair

**Sanjib Bhakta's** continued research interest (currently funded by Medical Research Council, UK and Austrian Research Fund, EU) is focused on developing novel therapeutics to tackle persistence and drug resistance in Tuberculosis (XDR-TB, a global health emergency). He has published more than 25 original research articles in last 10 years for a number of internationally acclaimed journals including J. Exp. Med., JBC, Tuberculosis, Biochem. J., J. Antimicrob. Chemother., FEBS J. and Mol. Microbiology.
Following a BSc (Hons), an MSc and a PhD in Molecular Biology & Biochemistry from world class Universities & Research Institutions in India, Dr Bhakta joined the Oxford University Department of Pharmacology in 2001 as an ISIS innovation Senior Research Scholar and shortly after he was awarded with a Wellcome Trust International Travelling Fellowship. He graduated from The Queen's College, University of Oxford in 2005 completing a second doctoral degree (DPhil) and received a "Sir William Paton Prize" for the best PhD presentation in Pharmacology. In 2006 he attained his first academic appointment at Birkbeck as a University Lecturer to lead his research and teaching. To date, he has supervised five PhD students, all of them completing their degree within four years, and he is supervising a number of UG/PG project students, three PhD students, two post-doctoral scientists and a UNESCO-L'Oreal International Fellow in his Laboratory. He became a Fellow of the Higher Education Academy, UK after achieving a post graduate certificate in Teaching and Life Long Learning in Higher Education (PGCHE) from the University of London in 2008.
He is a core member of Tuberculosis Drug Discovery-UK (http://www.tbd-uk.org.uk), the Institute of Structural and Molecular Biology, NIMR/Birkbeck/UCL and an affiliated academic Fellow of the Centre for Infection, Immunity and Disease mechanism, Brunel University. He is a member of a number of international societies and a review Editor for the Frontiers in Infectious Diseases. He was elected as a Fellow of the Royal Society of Medicine in 2008 and recognised as a Chartered Biologist in 2011.

About the speakers

**Brian Henderson** is Professor of Biochemistry at UCL's Eastman Dental Institute and is one of the early discoverers that bacteria secrete molecular chaperones which signal to host cells. This has led on to the thesis that bacteria use their molecular chaperones as secreted moonlighting proteins which aid in the process of bacterial virulence.

**Stephen Gillespie** has worked in Kenya researching the relationship between malaria and lower respiratory tract infection and the prevalence of parasitic infection in children in Kilifi, Kenya. He has investigated a cholera epidemic and vaccine failures in Guerrero, Mexico. He has been working in collaboration with colleagues at Kilimanjaro Christian Medical Centre since 1988 where he has, variously, studied respiratory and parasite diagnostics and novel antimalarials, anti-helminthics and anti tuberculosis agents. His main research activity is in the area of tuberculosis drug development. For the last twenty years Stephen Gillespie has been involved in various aspects of tuberculosis drug development. This has included the evaluation of new candidate antituberculosis agents in vitro. This work has expanded into studies of the molecular mechanisms of resistance and the development of model systems to measure the fitness deficits found in resistant strains. He has been involved in the development of fluoroquinolones for tuberculosis having performed early bacterial activity studies and clinical trials of ciprofloxacin. More recently he has led the group working on the clinical development of moxifloxacin in collaboration with the Global Alliance for TB Drug Development as Chief Investigator of the REMox TB study. He is also one of the three Chief Investigators of the PanACEA consortium that is developing Europe and Africa's clinical trials capacity.

**Graham Bothamley** is a clinician experienced in the management of tuberculosis with a keen interest in translational research. He is Chair-elect of the Tuberculosis Network European Trialsgroup (TBNET, http://www.tb-net.org/). He is a member of the Stop TB Proposal Review Committee. He has published >75 papers in tuberculosis, recently addressing the clinical problems of M/XDRTB in Europe, the role of immunodiagnostic tests, TB control programs in the UK and the detection of latent TB in people living with HIV, a randomized controlled trial of vitamin D in TB and the potential diagnostic value of exhaled breath. He graduated from Pembroke College, University of Oxford in 1980 and gained a PhD in the immunology of TB from work undertaken at the MRC Tuberculosis and Related Infections Unit from 1985-88.

**Edith Sim** studied Biochemistry in Edinburgh University graduating in 1973. She then carried out her D. Phil. using the then new technique of 31PNMR of membrane phospholipids under the supervision of Charles Pasternak in the Biochemistry Department in Oxford. After 2 years as a Royal Society Exchange Fellow at the Centre d'Etudes Nucleaires in Grenoble studying hydrogen production in Pseudomonas aeruginosa she returned to Oxford, firstly as a Demonstrator in the Biochemistry Department working in collaboration with Bob Sim on immune system proteins of the complement system. A seminal paper on the study of the mechanism of activation of complement components C3 and C4 was the basis for a study on Hydralazine induced autoimmune disease, as a Wellcome Trust senior lecturer in the Pharmacology Department. The work in turn led to investigation of the pharmacogenetics of arylamine N-acetyltransferases (NATs) and development of understanding of these enzymes in animals and also in bacteria, identifying their mechanism of action. One NAT isoenzyme in humans (NAT1) is a breast cancer marker and she has developed specific ligands along with colleagues Steve Davies and Angie Russell which change colour on binding to human NAT1.She investigated NAT in mycobacteria and showed that the NAT protein along with other gene products of the same operon are good targets for anti-tubercular therapy. Research Interests: Currently working on a series of enzymes known as azoreductases.

**Luke Alderwick** is a Lecturer in Molecular Microbiology in the Institute of Microbiology and Infection (IMI) at the University of Birmingham. His many research interests revolve around understanding the biochemistry and molecular genetics of cell wall assembly in Mycobacterium tuberculosis. In close collaboration with Prof Gurdyal Besra and Dr Apoorva Bhatt, Dr Alderwick forms a trio of Principle Investigators heading one of the worlds leading academic research groups studying mycobacterial biochemistry, genetics and molecular microbiology. He is also the Director of the Birmingham Drug Discovery and Screening Facility (BDDSF), which is a new £700k high-throughput screening facility within the IMI designed specifically to allow academic-led translational drug discovery research, particularly in the area of discovering new anti-infectives.

**Chris Granger** has a graduate degree in pharmacology and a masters degree in business administration. He has spent the last 10 years developing the use and application of the T-SPOT.TB test throughout the world. He has been responsible for planning and running the clinical studies that were used to obtain regulatory approvals for the test and for providing data to many Guideline Development Groups.

**Jayne Sutherland** completed her PhD in cancer immunology at Monash University, Melbourne, Australia. She spent 2 years on further cancer research at UCL, London before moving to the Medical Research Council Unit in The Gambia in 2006. She is currently acting Head of TB research involved in the TB case-contact platform for vaccine research, diagnostic development, childhood tuberculosis and protective biomarkers for TB. Other projects include HIV-TB co-infection, Mtb antigen-diversity, Mtb strain differences (m. africanum), pleural TB diagnostics and BCG vaccine immune profiles.

**Stephen Gillespie** is the foundation Sir James Black Chair of Medicine at the University of St Andrews. He is currently the Chief Investigator of the REMoxTB project that is investigating two treatment-shortening regimens in a regulatory pivotal study. This study is recruiting patients throughout the world and, if successful, will support a four month treatment for tuberculosis. Professor Gillespie is also one of the three Chief Investigators for the PanACEA consortium which is funded by EDCTP and is the main European-African clinical trials network. He is also the Chair of TB-Drug development UK which brings together researchers from throughout the UK from

medicinal chemists to clinicians. His current work in St Andrews is to develop new and more comprehensive mathematical models of tuberculosis treatment and investigating novel approaches to identify differing tuberculosis cell cycle by non-invasive methodology.

This meeting was organised by Euroscicon (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry.

Keywords: TB, latent, infection, diagnosis, IGRA,Latent TB; M/XDRTB; genome; proteome; immunome, cell wall, arabinogalactan, drug discovery, benzothiazinone, tuberculosis; treatment; biomarkers; antibiotics, mutation, *rpoB*, *gyrA*, *gyrB*, *katG*, *inhA*, Rifampicin resistance, Fluoroquinolone resistance, Isoniazid resistance, *Mycobacterium tuberculosis*, Thailand, Mangosteen extract, Antimycobacterial activity, air filter, pre-filter

Registration Web Site: **www.regonline.co.uk/tb2012**

Media partners

**NOTES ABOUT THIS EUROSCICON EVENT**

For your convenience we would like to bring your attention to the following

- You will be issued with a FULL delegate list within 14 days of the event, which will include the email addresses of the delegates (we are sorry that there is this delay in emailing the list, but we need to make sure that it takes into account any late arrivals). You will not be included in this list if you have opted out and you can do this by logging into your registration details. This list will not be sold or ever give out to third parties. Only people attending or sponsoring the event have access to the list
- There may be an independent meeting report published within a few months of this event. If this is published we will send you an email to let you know the reference details
- Notepads and pens are available from the Euroscicon reception desk
- We cannot give out the slides from our speaker's presentations as they are deleted immediately after each event. If you require a particular set of slides please approach the speaker
- Please remember that EuroSciCon is a small independent company with no subsidies from society memberships or academic rates for venues. We try to be as reasonably priced as possible and our delegate rates are substantially lower than comparable commercial meeting organisations
- To keep updated on our events and other Life Science News, please sign up for our newsletter at www.eurosciconnews.com
- We may take pictures during the meeting. These pictures will be used to promote our events and placed on our various websites and the closed Euroscicon group on Facebook. If you do not want your photograph distributed please let one of the Euroscicon staff know.

## DEVELOPMENT OF AMINOCOUMARINS AS A NEW CLASS OF POTENTIAL DRUG CANDIDATES AGAINST MULTI-DRUG RESISTANT TUBERCULOSIS (MDR-TB)

R. Tandon, P. Ponnan, K. Garima, N. Aggarwal, M. V. Basil, M. Nath, V.S. Parmar, H. G. Raj, A. K. Prasad, M. Bose*
*Department of Chemistry, University of Delhi, Delhi-110007, India*
* Corresponding author: Emeritus Scientist, Department of Microbiology, V. P. Chest Institute, University of Delhi, Delhi-110007, India.
E-mail: mridulabose@hotmail.com

The emergence of increasingly drug-resistant strains of *Mycobacterium tuberculosis* across the globe constitutes one of the major bottlenecks in the path of effective control and management of TB. Till recently, humanity was faced with the grave challenge of combating various MDR and XDR strains when cases of totally drug-resistant tuberculosis (TDR-TB) came into light further increasing the gravity of the problem necessitating strong measures to curb the menace. The current situation clearly manifests the need to develop new inhibitory molecules / novel approaches that could help tackle drug resistance and contain the spread of the disease. As part of our initiative, we also set forth to develop new potential drug candidates against *M. tuberculosis* especially MDR-TB.

A series of amino and acyl amino coumarins were synthesized and evaluated for their *in-vitro* activity against *M. tuberculosis* H37Rv strain as well as drug-susceptible and drug-resistant clinical isolates. The compounds were found to be effective with MICs in the range of 1-3.5 mg/L. They were also reported to be bactericidal in nature and act in synergy with isoniazid (INH) and rifampicin (RMP)[1]. The most effective compound of the series, 7-amino-4-methylcoumarin (NA5) was shown to bring down the MIC of INH by 1/30$^{th}$ and that of RMP by 1/20$^{th}$ when used in combination – a fact that may enhance their prospects of being incorporated in standard regimen, if found to be effective even under *in-vivo* conditions and further along the drug development procedure. *In-silico* prediction of pharmacokinetic properties of the test compounds using combination of ADMET prediction programs revealed the 'drug-likeness' of these molecules, making them worthy enough to be pursued further. In addition, NA5, was also tested against intracellular *M. tuberculosis* H37Rv as well as an MDR strain using THP-1 macrophage cell line. After 96 hrs of drug treatment, the colony counts were found to decrease by approximately 92% and 84% for H37Rv and MDR strain, respectively.

Another key finding was the 'cell-wall attacking' characteristic of these molecules that was established by means of electron microscopy (EM). When mycobacterial cultures were grown in the presence of sub-lethal doses of the test molecules, pronounced changes in the cell wall morphology were noticed[1]. These observations were further extended to probe the mechanism of action and identify the target of the test compounds. Since, mycolic acid constitutes the major portion of the mycobacterial cell wall, it was thought to be one of the probable targets. A panel of genes involved in the FAS-II pathway of mycolic acid biosynthesis were thus, selected and fold-change in their expression as a result of drug exposure was analysed through qRT-PCR. Significant changes in the expression of certain genes were observed indicating towards the possible interaction of the test compounds with the enzymes involved in the pathway. Moreover, EM studies had suggested that the test molecules may have an INH-like effect on cell morphology. To further investigate this, molecular docking calculations were employed to predict the potential binding modes of these molecules in the InhA binding pocket. The compounds were found to interact with the active site residues forming H-bonds and hydrophobic interactions. Similar studies were carried out using DNA gyrase- the known target of aminocoumarins. The compounds interacted with *E. coli* DNA gyrase B subunit (having structural similarity with *M. tuberculosis* DNA gyrase B) in the novobiocin-binding active site.

These compounds were thus shown to not only affect the cell wall of *M. tuberculosis* via their effect on mycolic acid biosynthetic pathway but were also found to interact with DNA gyrase. Thus, the molecules exhibit a 'multi-targeted approach' towards *M. tuberculosis* which indeed is the need of the hour. In order to combat drug-resistant TB, it's required that the drugs should have a multi-pronged approach that will help reduce the probability of development of resistance. The experiments thus carried out provided sufficient evidence for the fact that aminocoumarins could be potential drug candidates.

## A NEW APPROACH FOR FAST DIAGNOSIS OF TUBERCULOSIS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND CHEMOMETRICS

NA Dang[a,*], S Kuijper[a], E Walters[b], M Claassens[b], D Soolingen[c], G Vivo-Truyols[a], HG Janssen[a,d], AHJ Kolk[a]
[a]Analytical Chemistry & Forensic Science, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands
[b] Desmond Tutu TB Centre, Stellenbosch University, P.O. Box 19063, Tygerberg 7505, South Africa
[c] National Institute of Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[d] Unilever Research and Development, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands
**Presenting author: NgocA Dang, Analytical Chemistry & Forensic Science, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands. Phone +31 65 75 30 203 and fax number +31 20 52 55 604, Email n.dang@uva.nl.*

Tuberculosis (TB) is a global epidemic. One third of the world population is infected. Finding biomarker(s) for rapid diagnosis of TB becomes an essential requirement for TB control. Recently we have developed a fully automated thermally-assisted hydrolysis and methylation gas chromatography - mass spectrometry (THM-GC-MS) method that uses a combination of two biomarkers, tuberculostearic acid (TBSA) and hexacosanoic acid (C26), for detection of *Mycobacterium tuberculosis* (MTB) in culture and sputum (Kaal et al. 2009). Unfortunately this combination of a few biomarkers fails in identification of MTB because these markers are also present in some non tuberculous mycobacteria (NTM). Rapid differentiation of MTB complex from NTM is critical for TB control because many positive cultures in South Africa grow NTM instead of MTB. Via THM-GC-MS and advanced chemometrics, we have built a model that uses information on the levels of 20 potential biomarkers and gives more than 95% accuracy for the fast

differentiation of MTB from NTM (Dang et al. 2013 submitted). The 20-compound model has been validated with two independent sample sets including 40 mycobacterial strains representing MTB and NTM strains cultivated in mycobacteria growth indicator tubes (MGIT) from the Netherlands and 86 primary isolates from sputum grown in MGIT cultures from Stellenbosch, South Africa. The model gave a sensitivity of 100% and specificity of 93% with the 40 samples from the Netherlands and a sensitivity of 93% and specificity of 100% with the samples from South Africa. In the future we would like to develop a test for the direct identification and differentiation of mycobacteria directly in sputum using these biomarkers and finally develop a portable device, a micro GC with a simple detector that uses these biomarkers for the diagnosis of TB in sputum.

Literature

[1]Kaal E, Kolk AHJ, Kuijper S, Janssen HG. 2009. J Chromatogr A. 2009 1216(35): 6319–25

[2]Dang NA, Kolk AHJ, Kuijper S, Janssen HG, Vivo-Truyols G. Metabolomics 2013 (submitted for publication)

## GENES MUTATIONS OF DRUG-RESISTANT, (RIFAMPICIN, ISONIAZIDR AND FLUOROQUINOLONER), *MYCOBACTERIUM TUBERCULOSIS* FROM TB PATIENTS IN THAILAND

Pannamthip Pitaksajjakul [a], Dhruba Kumar Khadka[a], Pongrama Ramasoota [a]*

[a] *Faculty of Tropical Medicine, Mahidol University 420/6 Rajwithii Road, Bangkok 10400*

**Abstract—** The main mechanism of drug resistant *Mycobacterium tuberculosis* that falling in to mutations in specific genes, altering in drug target enzyme, have been frequently reported. By this mechanism, several molecular techniques have been used to verify these mutations. In present study, the mutations in *rpoB* (drug target for RIF$^r$-MTB), *gyrA*, *gyrB*, (drug target of FQr-MTB), *katG*, and *inhA* genes (drug target for INHr-MTB) of MDR-TB isolates have been studied by PCR-DNA sequencing. From 425 bps region (cover RRDR) of *rpoB* gene from 70 isolates of RIF$^r$-MTB, twenty-eight isolates (40%) had single-point mutations, and 26 of those isolates had mutations at positions never reported before, of which, just one had a substitution at Val-432 (Asp), and the remaining 25, has a silent mutation at Gln-517. All other isolates had multiple mutations, of which 24(34%) had mutations at two positions; 9(13%), at three positions; 2(3%), at five positions; and 1(1%) at six positions. Five isolates (7%), reported to have the RIF$^r$ phenotype, contained no mutation in the examined region of the *rpoB* gene. Surprisingly, one RIF$^r$ strain had silent mutations at 29 positions. By far the dominant mutation was the silent mutations at Gln-517 (86%). For FQ$^r$, 92 isolates of FQ$^r$-MTB were studied and the result shown that 70 of 92 (76.08%) exhibited single-point mutations in different positions, at Asp94 (Gly/Ala/His/Asn) (42.39%), Ala90Val (20.65%), Ser91Pro (9.78%), Gly88 (Cys/Ala) (2.17%), in *gyrA* QRDR and one isolates (1.09%) with Asp495Asn mutation in the *gyrB* QRDR. The other 22 FQr-MTB isolates (23.91%) had no mutation. For INHr, 29 isolates of INH$^r$-MTB were studied. Single point mutations at one or two positions of *katG* gene were found (both Ser315Thr and Arg463Leu, 58.6%, only Arg463Leu, 34.5%, only Ser315Thr, 3.4%, Thr308Pro, 3.4%). Accordingly, 9 of 29 isolates were identified as mutants in inhA promoter and inhA-ORF, of which 5 isolates found Cytosine/Thymine mutation at position –15 (17.2%), 1 isolate found Thymine/Cytosine mutation at upstream position –8 (3.4%), 1 isolate found Cytosine/Thymine mutation at position -15 plus mutation at Ile21Thr (3.4%), 1 isolate found Cytosine/Thymine mutation at position -15 plus Ser94Ala (3.4%), and 1 isolate with Ile21Val (3.4%), whereas 20 isolates (69%) had no mutation at any position. Furthermore, it was found that mutation position at Trp308Pro in *katG* gene and Thymine/Cytosine at position –8 of *inhA* gene is firstly reported here.

**Keywords—**mutation, *rpoB*, *gyrA*, *gyrB*, *katG*, *inhA*, Rifampicin resistance, Fluoroquinolone resistance, Isoniazid resistance, *Mycobacterium tuberculosis*, Thailand

* Corresponding author. Pongrama Ramasoota, Faculty of Tropical Medicine, Mahidol University 420/6 Ratchawithi Road, Bangkok 10400 Tel.: 66 02 3549100-19 ext 1562-4; Fax: 66 02 3549167; E-mail: pongrama.ram@mahidol.ac.th

## MANGOSTEEN EXTRACT COATED PRE-FILTER COULD INHIBIT AEROSOL *M. TUBERCULOSIS*

*Natthakarn Tipkrua,[a] Sunit Suksamrarn,[b] Pannamthip Pitaksajjakul,[a] and* ***Pongrama Ramasoota.*** [a]

[a] *Department of Social and Environmental Medicine, and Center of Excellence for Antibody Research (CEAR), Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawittee Road, Ratchadhewee, Bangkok 10400, Thailand.*

[b] *Department of Chemistry, Faculty of Science, Srinakharinwirot University; Sukhumvit 23, Bangkok 10110, Thailand.*

**Background:** Tuberculosis (TB) is an airborne transmission disease that caused by Mycobacterium tuberculosis (MTB). It is a global public health problem which major cause of death. Pre-filter and HEPA filter have been widely used in air filtration system for long time. But they face with the problem of dust and microbes accumulation on the filter. These microbes on filter can release back to indoor air and cause health effect especially by pathogenic bacteria like MTB. Since Mangosteen exstract (MSE) from Mangosteen rind was found to be effective anti-mycobacterial agent. So this study has objective to add anti-mycobacterial activity to air-filter by using MSE coated on pre-filter. Then its mycobactericidal activity was tested against MTB H37Ra strain.

**Methods:** MSE was tested for antimicrobial activity against MTB and determined Minimal inhibition concentration (MIC) using MTT assay. All MSE coated pre-filters were tested for antimycobacterial activity using disc diffusion assay and observed characteristic of

MSE coated pre-filter under scanning electron microscope. For filtration efficacy of coated pre-filter, it was tested in the experimental chamber model, using 5 ml of M. smegmatis $10^7$ cells / ml sprayed passed through pre-filter and collected the air and examined bacterial number by culture technique. After filtration testing, each pre-filter was cut and cultured.MTB suspensions were dropped on the pre-filter, then bacterial viability at difference time was observed. The filtration efficacy of each pre-filter was compared using the calculated as follow; {total number of bacterial in impinger (without pre-filter) – total number of bacteria in impinger (when used pre-filter)} $\times$ 100 and divided by the total number of bacterial in impinger (without pre-filter). Antimycobacterial activity at difference exposure time using bacterial viability testing by culture technique was also performed.

**Results and conclusion:** MSE was shown antimycobacterial activity against MTB at the MIC of 31.25 µg / ml. The 100 µl of MSE coated on 2 cm diameter of pre-filter disc showed inhibition zone began at 625µg/ml. There was no difference between un-coated and MSE coated pre-filter, only the color was changed, the pore size and fibers were the same as uncoated pre-filter, when observed under electron microscope. There was also no different in air flow (filtration efficiency) between MSE coated and uncoated pre-filter. After bacterial filtration, the viability of collected MTB in the pre-filters was decreased when the exposure time was increased. MSE coated pre-filter showed 98 % inhibition of Mycobacterial growth in pre-filter. The results showed that MSE could be use as effective alternative anti-mycobacterial substance activity of air filtration system especially in TB and HIV hospitals where many cases of TB infected medically personnel were reported.

**Key words:** Mangosteen extract, Antimycobacterial activity, air filter, pre-filter

.

**Corresponding author;** P. Ramasoota E-mail pongrama.ram@mahidol.ac.th

## MONITORING EARLY HOST RESPONSES ASSOCIATED WITH STARTING EFFECTIVE TUBERCULOSIS TREATMENT

AL. den Hertog, AF. de Vos, PR. Klatser, RM. Anthony

***Presenting author*** *R.M. Anthony, Royal Tropical Institute, KIT Biomedical Research, Meibergdreef 39 1105 AZ Amsterdam, The Netherlands, r.anthony@kit.nl*

There is no truly cheap and simple diagnostic test for active tuberculosis, easily detectable and specific biomarkers for active TB are elusive and may unfortunately remain so. In parallel to the essential extensive efforts in diagnostic biomarker discovery and platform development being performed in this field, we would like to suggest established and new biomarkers of TB disease be considered for use in a "treat-to-test" strategy for confirmation of infection and treatment monitoring. Biomarkers non-specifically associated with TB infection that have already been identified or detected in ongoing systematic studies that are not useful for simple near-patient tests may nonetheless be informative when measured serially in patients starting treatment, potentially confirming clinical response as well as infection.

We have explored this concept in a placebo controlled infection controlled mouse model using a multiplex magnetic bead cytokine array to look for shifts in immune response related to the initiation of treatment. Our study identified a number of immunological markers that change measurably after only 1 week's anti tuberculosis therapy in the mouse model for TB only in infected mice. This supports the potential of a Treat-to-Test approach for TB diagnosis. Clinical studies are necessary to validate these results in human TB suspects and to determine whether this approach will have utility in TB control. Treatment monitoring and detection of drug resistance could also be facilitated by knowledge of the kinetics of markers for bacteriological response. Collection and testing of samples in the early stages of chemotherapy suitable to study the kinetics of easily measurable and potentially highly informative markers is a priority.

## POLYTB: A GENOME-BROWSER WEB TOOL TO INVESTIGATE *M. TUBERCULOSIS* GENETIC POLYMORPHISMS DERIVED FROM NEXT GENERATION SEQUENCING DATA

Francesc Coll[1], Mark Preston[1], Kim Mallard[1], Ruth McNerney[1], Nigel Martin[2], Taane Clark[1]

[1]London School of Hygiene and Tropical Medicine, London, UK; [2]BirkBeck College, London, UK

*Room 341, Bonham Carter House, 52-54 Gower Street, London, WC6E 6ET*

*Mycobacterium tuberculosis* (*Mtb*) is the causal agent of tuberculosis disease (TB), the second leading cause of death from an infectious disease worldwide. Despite the availability of highly efficacious treatments for decades, TB remains a major global health threat due to multidrug resistance emergence and spread. Next generation sequencing (NGS) platforms allow the survey of genetic polymorphisms across large numbers of samples at an unprecedented cost and resolution. Two important applications of this technology include large-scale epidemiological studies and association studies to determine the genotypes that explain phenotypes such as virulence or drug susceptibility.

Here we describe the development of a web-based tool to display genetic variation from all publicly available *Mtb* isolates. Whole genome data produced by Illumina paired-end technology have been processed to identify variation including Single Nucleotide Polymorphisms (SNPs), small insertion and deletions (indels) and large structural variants. We have developed a web-based genome browser to display the resulting allele calls for the global dataset and make them accessible for the TB research community.

PolyTB browser page displays colour-coded genetic polymorphism for the chromosome region and samples selected by the user along with annotation information. The web-page allows the investigation of allele frequencies at the geographical regions from where sequenced samples were collected in a Google maps view. We incorporated *in silico* inferred strain genotypes, in particular spoligotypes (1), to allow the visual detection of relationships between certain alleles and strain types. The construction of phylogenetic trees based on whole genome polymorphisms has been implemented as an additional tool to investigate population structure.

Future extensions will also incorporate meta data and phenotypes (e.g. drug resistance), association analysis tools, links to other publicly available TB databases and epidemiology-related functionality.

(1) Coll F. et al. (2012). *Bioinformatics*. SpolPred: rapid and accurate prediction of Mycobacterium tuberculosis spoligotypes from short genomic sequences.

## INVESTIGATION OF THE STRUCTURAL CONTRIBUTIONS TO THE ANTIMICROBIAL PEPTIDES ACTIVITY AGAINST MYCOBACTERIUM TUBERCULOSIS

Y Lan[1], WC Yam[2], AJ Mason[3] and JKW Lam*[1]

[1] *Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 2/F, Laboratory Block, 21 Sassoon Road. Hong Kong*

* Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 2/F, Laboratory Block, 21 Sassoon Road. Hong Kong, jkwlam@hku.hk

[2] Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong

[3] King's College London, Institute of Pharmaceutical Science

Tuberculosis is a severe infectious disease caused by *Mycobacterium tuberculosis* (Mtb), and poses a serious threat to the public. A novel class of antimicrobial peptides (AMPs), the D-formed cationic amphipathic a-helical LAK peptides, is being investigated as potential anti-tuberculosis agent. These peptides contain 24-25 D-amino acid residues and have the ability to penetrate and destabilize the bacterial membrane. The antimicrobial potency and selectivity between host membrane and pathogenic membrane are determined by their structure characteristics, including charge angle (angle subtended by positively charged amino acids), hydrophobicity, and the incorporation of proline residues. To investigate the structure-activity relationships, *in vitro* anti-tuberculosis assays of a series of AMPs were carried out on eight different strains of Mtb in order to identify the most potent peptide candidates. Selected AMPs were then tested for their activity against intracellular Mtb. The cytotoxicity of the AMPs was also evaluated by MTT assay.
It was found that D-AMPs could greatly inhibit the extracellular growth of Mtb, including the multi-drug resistant and the extensive drug resistant strains. Peptides with a charge angle of 120° had the most potent antibacterial activity against both Mtb, but they were also more cytotoxic to the host human macrophage-like cells (THP-1). Increasing hydrophobicity decreased the antimicrobial potency, and due to the reduction of self-association of the peptides in solution, a significant decrease of cytotoxicity was also observed. Our finding suggested that hydrophilic D-AMPs with charge angle 120° conferred the most potent activity against extracellular Mtb, and remain active against intracellular Mtb. Overall, this study showed the potential of using antimicrobial peptides as a novel therapeutic agent for the treatment of tuberculosis.

## ATP-DEPENDENT MUR LIGASES IN THE BIOGENESIS OF CELL WALL PEPTIDOGLYCAN IN *MYCOBACTERIUM TUBERCULOSIS*: NOVEL TARGETS FOR ANTI-TB DRUG DISCOVERY

T. Munshi, A. Gupta, D. Evangelopolous, J. D. Guzman, N. H. Keep, S. Gibbons and S. Bhakta*

*Department of Biological Sciences, Institute of Structural & Molecular Biology, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK*

*s.bhakta@bbk.ac.uk

Cell-wall peptidoglycan is a well-established target for antibacterial chemotherapy; however our knowledge on structure, function and regulation of enzymes involved in the early stages of peptidoglycan biosynthesis in *Mycobacterium tuberculosis* is still limited. ATP-dependent Mur ligases - MurC, MurD, MurE and MurF play crucial role in peptidoglycan biosynthesis as they catalyse the sequential addition of key amino acid residues to the stem peptide; thus representing new targets for anti-tubercular drug discovery. To this end, we have characterised the *mur*-operon and extended our investigations [1, 2] to express, purify, biochemically characterise all the Mtb-Mur ligases, and developed novel assays to determine enzyme mechanisms and inhibitions.

Sassetti *et al* [3] in their transposon site hybridisation analysis reported that seven out of nine enzymes are essential for the viability of *M. tuberculosis.* Our evaluation of over-expression of these *M tuberculosis* Mur synthetases in *M. bovis* BCG resulted in slow growth and altered morphology indicating their role in cell division. Furthermore, in line with the previous findings on the regulation of mycobacterial MurD and corynebacterial MurC via phosphorylation [4, 5], we found that all the Mur synthetases were interacting with Ser/Thr protein kinases, PknA and PknB. In addition, we have critically analysed the interaction network of all the Mur synthetases with proteins involved in cell division and PG biosynthesis to re-evaluate the importance of these key enzymes in cell wall PG-biogenesis pathway as novel therapeutic targets for antitubercular drug discovery.

1. Basavannacharya *et al* (2010) *Tuberculosis (Edinb)* 90: 16-24.
2. Basavannacharya *et al* (2010) *Protein & Cell* 1: 1011–1022.
3. Sassetti et al (2003) *Molecular Microbiology* 48: 77–84
4. Thakur et al (2008) *Biochem J* 415: 27-33.
5. Fiuza et al (2008) *J Biol Chem* 283: 36553-36563

**. GENES TARGETED PEPTIDE NUCLEIC ACIDS ACT AS ANTIMICROBIALS AND KILL *MYCOBACTERIUM SMEGMATIS***

Md. Ariful Islam[a,b], Nammalwar Sriranganathan[a], Stephen M Boyle[a*]

[a]*Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061-0342, USA*

[b]*Department of Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*

*Corresponding author
Dr. Stephen M Boyle
Professor of Microbiology
e-mail: smboyle@vt.edu

Antisense peptide nucleic acids (PNAs) targeted to essential genes have been shown to be novel therapeutic compounds to inhibit bacterial growth. The PNAs are very stable, short synthetic nucleic acid analogues (10-12 bp) in which the sugar-phosphate backbone of natural nucleic acid has been replaced by a synthetic peptide backbone usually formed from N-(2-aminoethyl) glycine units. PNAs are very long-lived antisense constructs that inhibit expression of targeted genes at either the transcriptional or translational level. An investigation was undertaken to evaluate the ability of several antisense PNAs to inhibit extracellular *M. smegmatis* in broth culture and intracellular *M. smegmatis* in J774 murine macrophage cell line. Six PNAs obtained from a commercial supplier were designed to inhibit the expression of the following essential genes: *inhA* (a fatty acid elongase), *rpsL* (ribosomal S12 protein), *gyrA*(DNA gyrase), *pncA* ( pyrazinamidase), *polA* ( DNA polymerase I) and *rpoC* ( RNA polymerase β subunit) of *M. smegmatis.* Each PNA was used at 20µM, 10µM, 5µM and 2.5µM concentrations to test whether they induced a dose dependent inhibition of *M. smegmatis* cultured in Middlebrook medium at 37 °C. PNAs targeting *inhA* and *rpsL* exhibited strong growth inhibition at all four concentrations, whereas only 20µM concentration of PNAs targeting *pncA*, *polA* and *rpoC* genes strongly inhibited the growth of *M. smegmatis*. PNAs targeting *gryA* and a mismatch PNA targeting *dnaG* ( DNA primase) did not inhibit *M. smegmatis* in pure culture. Four PNAs at 20µM, 2µM and 0.2µM concentrations targeting *inhA, rpoC*, *polA* and *gryA* were also tested in murine macrophages infected with *M. smegmatis*. All four PNAs exhibited statistically significant growth inhibition *($p<0.05$)* of *M. smegmatis in* murine macrophages. Data from this study extends the sole report of *M. smegmatis* susceptibility to a PNA against *InhA* in pure culture. Furthermore, the data suggests that PNAs could be a novel therapeutic approach against *Mycobacterium* infections. Experiments are in progress to examine the effects of PNAs administered as targeted nanoparticles to mice infected with *M. smegmatis*.

**MYCOBACTERIUM TUBERCULOSIS INFECTION IN CATTLE IN CROATIA - CASE REPORT**

S.Spicic, V. Katalinic-Jankovic, I. Racic, M. Zdelar-Tuk, S. Duvnjak, Z. Cvetnic
E-mail: spicic@veinst.hr
*Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia*

Among the diseases that have threatened the health and the lives of people and animals in the past century, tuberculosis (TB) played a significant role. In Croatia, incidence of human TB was 15/100.000 in 2011. Bovine TB occurrence has decreased as a result of a planned fight against the disease in the past decades. Despite the effort, it has not been eradicated yet. In Croatia, the control of bovine tuberculosis is based on annual testing of all bovine older than six weeks by tuberculin skin test. Bovine positive on tuberculin skin test are slauthered and their organs taken for bacteriological testing on TB. Methods for molecular epidemiology are also used in order to enhance the control of TB.

Described here are the only two cases of *M. tuberculosis* infection found in cattle in Croatia. In 2008, one heifer on a small cattle farm was slaughtered due to positive reaction on bovine tuberculin skin test. No gross pathological changes were visible on lymph node and tissue specimens inspected at slaughter. *M. tuberculosis* was isolated from bronchial lymph nodes. Epidemiological investigation based on MIRU-VNTR typing database enbled us to find the man – the source of the infection and connect him to the infection in heifer.

In the second case, in 2010 we found 3 cows positive on bovine tuberculin in the same flock. Only changes detected at slaughter were enormously increased bronchial and mediastinal lymph nodes without visible tubercles. *M. tuberculosis* was isolated from bronchial lymph nodes in one cow. Despite extensive epidemiological investigations and molecular MIRU-VNTR genotyping of the cattle strain we were not able to find identical strain in human database in Croatia or possible pathways for introduction of the infection to the heard. This second case of *M. tuberculosis* infection in cattle with unknown strain opens up questions about the sources of infection, ways of dissemination, but also points to the need to change the system of detection of tuberculosis in humans.

Although rare, *M. tuberculosis* infection may occur in cattle and other animal species. *M. tuberculosis* does not appear to have an indigenous animal host or reservoir and the animals that become infected represent most probably accidental hosts. Humans suffering from active TB are strongly believed to represent the main source of *M. tuberculosis* infection in animals, including cattle. Improvement of diagnosis of tuberculosis in humans and rapid detection of carriers ensures limiting of spreading of *M. tuberculosis* in humans and in animals.

## ADHERENCE TO TUBERCULOSIS ISONIAZID TREATMENT REGIMENS CAN BE RELIABLY ASSESSED USING A POINT-OF-CARE TEST BASED ON THE ARKANSAS METHOD: A PROSPECTIVE COHORT STUDY.

Soobratty R,[1] Whitfield R,[1] Subramaniam K,[2] Grove G,[3] Carver A,[3] O'Donovan GV,[4] Wu HHT,[5] Lee O Y-C,[5] Swaminathan R,[3] Cope GF,[5] Milburn HJ.[4]

[1]Dept of Respiratory Medicine, Mayday University Hospital, Croydon, [2]Dept of Clinical Biochemistry, University Hospital of North Staffordshire, [3] Dept of Chemical Pathology, King's College London School of Medicine, King's College, University of London, [4]Dept of Respiratory Medicine, Guy's and St Thomas' NHS Foundation Trust, London, [5]University of Birmingham, UK.

Grahamcope@btconnect.com

***Background:*** Anti-tuberculosis chemotherapy is highly effective, but a minimum of six months' treatment with a regimen containing isoniazid is difficult for some patients to adhere to. Failure to do so increases morbidity and mortality, prolongs treatment, expands costs and enhances the chance of drug resistance. Detection of drug metabolites in urine can demonstrate adherence to treatment.
***Aim:*** To assess the performance of a rapid, simple point-of-care colorimetric test for isoniazid metabolites over 72 hours following ingestion of isoniazid.
***Method:*** Urine specimens from 105 patients treated for active or latent tuberculosis were tested at 12, 24, 48 and 72 hours following the final dose of isoniazid.
***Results:*** At 12 hours, all samples were positive (purple or blue); at 72 hours, 87% were negative (yellow) and 13% intermediate (green). There was a significant probability of a purple result at 12, blue at 24, green at 48 and yellow at 72 hours (OR 0.84; 95%CI 0.81-0.88; p=0.0001). Test sensitivity, specificity, positive and negative predictive values were all 100% at 12 hours. Acetylation status had no effect on the test result.
***Conclusion:*** The rapid, point-of-care colorimetric test for isoniazid metabolites is reliable, rapid and safe, and is a useful tool as a spot check for adherence to anti-tuberculosis medication.

## DETERMINANTS OF DELAY IN TUBERCULOSIS DIAGNOSIS AND TREATMENT IN MIDDLE–INCIDENCE COUNTRY

A.Jurcev-Savicevic, R. Mulic, B. Ban, K.Kozul, Lj. Bacun-Ivcek, J. Valic, G. Popijac-Cesar,
S. Marinovic-Dunatov, D. Puntaric, A. Simunovic
*Teaching Public Health Institute of Split and Dalmatia County, Vukovarska 46, Split, Croatia*

Background: Early detection and treatment of tuberculosis patients have been key principles of tuberculosis control. Delayed diagnosis and treatment increase the severity of disease level as well as extend the period of infectivity. A number of studies have addressed this issue but mostly in high- or low- incidence countries. Our understanding of delay has been quite limited in settings with intermediate tuberculosis burden, especially in circumstances of free health care and sufficient network of health services providing tuberculosis diagnosis and care.
Objectives: Exploring both health seeking behaviour for tuberculosis symptoms and the time needed to establish the diagnosis and start the therapy from physicians under these particular circumstances in Croatia.
Methods: A total of 241 consecutive adult cases with culture-confirmed pulmonary tuberculosis were interviewed and their medical records were evaluated. Patient delay was defined as the period from the appearance of any symptoms to the first visit to a medical provider. Health system delay was defined as the number of days from the first consultation with physicians to the initiation of treatment. Long delay was defined as a period exceeding the median of delay.
Results: The median patient and health system delays were 38 and 15 days. Long patient delay was associated with the lowest level of education (p=0.016), current smoking (p=0.029), and coughing (p=0.022) in multivariate logistic regression.
The most common reasons for delay were supposed influenza or symptoms improving over time (34.5%) and underestimated symptoms (32.9%).
Almost 30 % of patients remained undiagnosed more than 30 days after the initial health care visit. Female patients (p=0.008), negative sputum smear (p=0.003) and having symptoms other than usual (0.037) were found to be significantly associated with long health system delay in multivariate analysis.
Conclusions: People with the lowest level of education, smoking habits and health seeking behaviour which may favour advanced disease and prolonged infectiousness contributed to delay. Some groups of tuberculosis patients experienced health system delay, mainly due to the lack of suspicion. It is imperative that physicians are able to recognize and promptly treat tuberculosis. Therefore, efforts should be made to improve their diagnostic skills and awareness. There is a need not only to maintain but to raise awareness of tuberculosis symptoms with emphasis on developing general health awareness as well. These measures may be useful to reduce the number of missed opportunities for tuberculosis diagnosis as one of the approaches toward tuberculosis elimination in middle–incidence countries.
Coressponding author: anamarijajs@gmail.com
Meeting: Mycobacterium tuberculosis.....can we beat it?

## INSERTION SITE MAPPING: A RAPID PROTOCOL TO DETERMINE GENOMIC LOCATION OF REPEATED ELEMENTS IN *MYCOBACTERIUM TUBERCULOSIS*

Kartyk Moganeradj[1], Ibrahim Abubakar[1], Timothy McHugh[2], Pamela Sonnenberg[3], and Catherine Arnold[1]

[1] *Microbiology Services, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK.*

[2] *Centre for Clinical Microbiology, Department of Infection, Royal Free Campus, University College London, Rowland Hill Street, London NW3 2PF, UK.*

[3] *Centre for Sexual Health & HIV Research, Department of Infection and Population Health, University College London, Mortimer Market, off Capper Street, London WC1E 6JB, UK.*

Insertion elements in the genome can be viewed as evolution genes and play a role in changing the physical and biochemical traits of the organism they belong to. They have also been used extensively as genetic markers for differentiation of bacteria by mapping their movement within the bacterial genome. Determination of the precise genetic location of the transposable elements' insertion site could shed light on the putative altered function of adjacent genes. A rapid and simple method of insertion site mapping using Insertion site *6110* (IS*6110*) fluorescent amplified fragment length polymorphism (FAFLP) PCR was developed and applied to *Mycobacterium tuberculosis* H37Rv strain to compare the experimental data with the *in silico* results.

## HIGH THROUGHPUT WHOLE-CELL INTRACELLULAR SCREENING MODEL FOR ANTI-TUBERCULARS

Antima Gupta and Sanjib Bhakta*

*UNESCO-L'Oréal International Fellow*

*Department of Biological Science, ISMB*

*Birkbeck, University of London,*

*London, WC1E 7HX, UK*

**Theory:** Tuberculosis (TB) is one of the highly infectious diseases responsible for millions of death and billions of infected population around the world. The continuous increase of drug-resistance and prolonged latent infection are two major concerns in the current TB-control strategies. It has massive health and economic impact on global development. There is large number of novel chemical libraries available for a comprehensive phenotypic evaluation at the preclinical stage of TB drug development, the search for novel chemotherapeutics against drug-resistant and dormant TB bacilli is severely impeded by the slow growth of pathogenic organism and the need to work in highly stringent and expensive containment level-3 laboratories. This poses considerable obstacles, such as complex handling, expensive set up and special training requirements that are major bottlenecks towards extensive drug screening research using different physiological state bacilli. In order to alleviate these critical issues, surrogates have been introduced in the drug discovery process, of which non-pathogenic, fast-growing *Mycobacterium aurum* is one of the most promotable strains because of its similar drug susceptibility profile and drug-resistance mechanism to *M. tuberculosis*.

**Aim and Objectives:** In order to explore new anti-TB molecules in quick and more efficient assay method, the aim of this study is to develop and characterise the rapid and convenient semi-automated high-throughput intracellular screening model for *M. aurum* and RAW 264.7 macrophage cell line as a gold standard to substitute highly virulent, extremely slow-growing *M. tuberculosis* in the early stage of anti-TB screening.

**Results:** Exploiting the characteristics of *M. aurum* and the solid agar-based spot culture growth inhibition (SPOTi) assay, we have developed an integrated surrogate drug screening model for intracellular screening of inhibitor. The sensitivity to an acidic pH environment and the ability to multiply inside RAW 264.7 macrophages provided additional advantages for implementing surrogate *M. aurum* in intracellular drug screening methods. Using the developed model we will be achieving a number of potential inhibitors against active tubercular bacteria. Moreover, the established model will be suitable as an anti-infective drug screening system for wide spectrum use. Importantly, the model does have not only academic but industrial applications.