Detection of Active Mycobacterium Tuberculosis Infection and Multidrug Resistance by Multiplex Suspension Arrays

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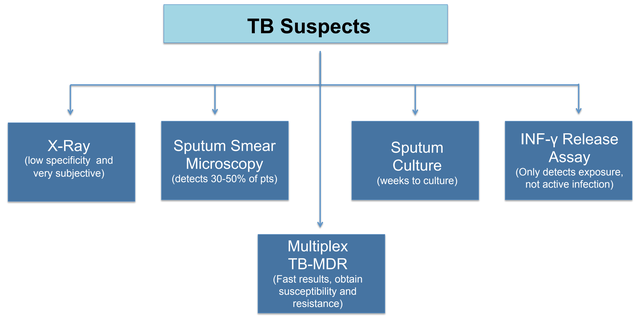
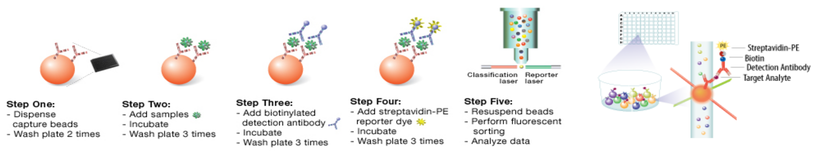
Introduction

Mycobacterium tuberculosis (*M.tb.*) is an aerobic, gram positive, and acid-fast bacillus that leads to the complex disease manifestation of TB. Multidrug resistance (MDR) is a growing concern in endemic countries as a result of poor compliance, length of treatment, and adverse medication effects. Current diagnostic methods for detecting active *M.tb* infection are slow, unreliable and are not cost effective for high-risk populations. The Khan laboratory is currently developing novel strategies for studying blood based immune-biomarkers as well as intracellular signaling proteins and pathways in cell lysates using high-throughput multiplex microbead immunoassays. The goal of my summer project was aimed at optimizing the detection of active Mycobacterium tuberculosis infection and multidrug resistance by multiplex suspension arrays.



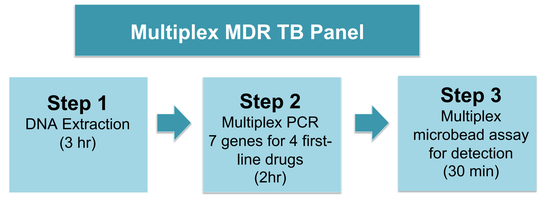
Materials

- **Multiplex kits for measuring cytokines, chemokines and growth factors, for use on the Luminex platform (Luminex Corp, Austin, Tx), were obtained from BioRad, Hercules, CA. Assays were performed per manufacturer's instructions.**
- **Plasma samples from healthy individuals in the same TB endemic areas were used to compare confirmed TB patient samples.**
- **Primers targeting variable genomic targets observed in multi-drug resistant *M.tb.* strains were designed from genomic biomarkers for TB-MDR.**



Methods

- **DNA extracted from sputum samples of TB patients was used to identify TB-MDR through multiplex PCR.**
- **Multiplex-PCR was used to amplify seven *M.tb.* genes in DNA isolated from purified TB patient sputum samples to identify multidrug resistance.**



Results

Sample	P38-PN F 100ug	P38-PN F 25ug	Rv0934-P38 4ug	P38-PN 25ug	P38-PN 12.5ug	Antihuman IgG 20ug	188 BSA 100ug pool
HBP 1/12	55	17	21	23	14	**6965**	25
	63	17	21	24	14	**6692**	25
HBP 3/12	114	21	30	36	20	**6838**	33
	104	22	34	33	18	**6745**	30
TB-159/11	**3305**	97	**2384**	**468**	35	**6006**	18
	3342	91	**2333**	**413**	36	**6306**	19
TB-138/11	**313**	34	**619**	95	24	**6778**	11
	327	33	**609**	102	28	**6778**	11

- **Figure 1: Optimization of the plasma antibody multiplex method using microbead suspension arrays to study blood-based biomarkers associated with active TB. P38 and Rv0934 are *M.tb* specific antigens used in the serodection of TB in healthy blood plasma (HBP) against plasma from culture confirmed *M.tb* infected patients.**

Antibiotic	Genes	Multiplex-PCR Probes
Rifampin	rpoB	18
Isoniazid	katG, inhA	9
Pyrazinamide	none	0
Ethambutol	embB	5
Streptomycin	rpsL, rrs	8

- **Figure 2: First line anti-*M.tb.* drugs with their corresponding genes identified in MDR-*M.tb.* strains. Multiplex-PCR probes corresponds to the available targets used in the identification of MDR-TB.**

Results

Sample	katG Ser WT	katG 315 Thr	rpoB 531 Ser WT	rpoB 531 Leu	rpsL 43 Lys WT	rpsL 43 Arg	EMB 306 Met WT	EMB 306 Val	IS6110
Blank	104	86	101	99	177	96	148	133	56
	96	71	54	96	205	147	156	91	73
H37RV	**2911**	104	**959**	205	**2596**	95	**1495**	664	**2776**
	3283	96	**920**	211	**2826**	140	**1535**	688	**2806**
MDR isolate	325	**4002**	190	**2322**	181	**2883**	225	**900**	**2339**
	349	**4103**	109	**2362**	156	**2458**	187	**871**	**2190**

- **Figure 3: Multiplex-PCR assay detection of genomic targets observed in multi-drug resistant *M.tb.* in comparison to corresponding wild-type genomic targets. H37RV is a wild-type *M.tb.* strain used to differentiate between the culture confirmed, isolated MDR-*M.tb.***

Future Directions

- **Optimize TB-MDR assay for fresh patient sputum samples.**
- **Continue to validate and identify more biomarkers for multidrug resistance *M.tb.***
- **Verify results via a field validation study for sensitivy and specificy.**
- **Ongoing developments for commercialization of blood-based diagnostic assay.**

References

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