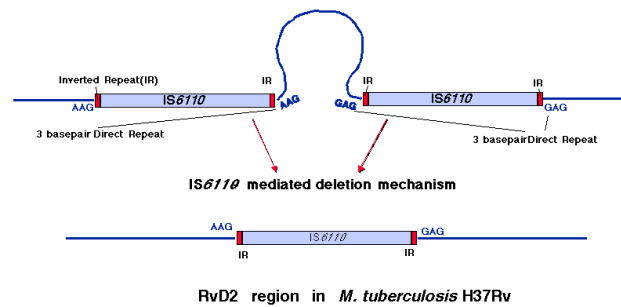
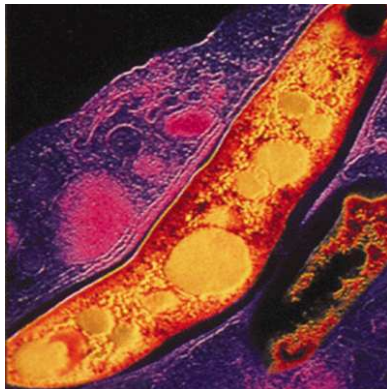
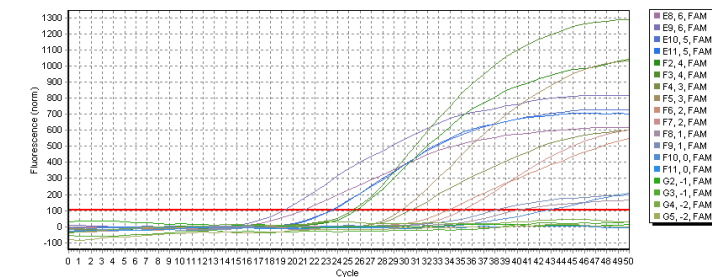


## M.tuberculosis detection Using Real Time PCR- IS6110



*Mycobacterium tuberculosis* is a gram positive bacteria-causative agent for most cases of the tuberculosis in humans. Intrepid Nepal Pvt.Ltd. has worked extensively to develop Real Time PCR based detection of M.tuberculosis. In its recent study, sputum samples from the National TB Centre in Thimi were taken to be tested for presence of the bacteria. AFB and culture were done at NTB Centre. Real Time PCR method detected 6% more cases of TB positive samples than regular AFB method, while concurring 100% with AFB positives.

### INTREPID NEPAL PVT.LTD M.TUBERCULOSIS DETECTION WITH REAL TIME PCR IN ITS IS6110 AND SIMILAR AREA OF THE GENE.



Threshold: 103 (Noiseband)  
 Baseline settings: automatic, Drift correction OFF



### Comparison of Intrepid's Result with NTB Centre's AFB Result

Sample	Q PCR result (DNA copies/ml)	AFB result	Culture result
M001	negative	negative	negative
M002	negative	negative	N/A
M003	negative	negative	N/A
M004	negative	negative	N/A
M005	1.5x10 <sup>5</sup>	negative	N/A
M006	negative	negative	N/A
M007	negative	negative	N/A
M008	negative	negative	N/A
M009	2.0x10 <sup>6</sup>	positive	N/A
M010	negative	negative	N/A
M011	Sample inappropriate	negative	N/A
M012	Sample inappropriate	negative	N/A
M013	3.3x10 <sup>6</sup>	positive	N/A
M014	negative	negative	N/A
M015	negative	negative	N/A
M016	negative	negative	N/A
M017	negative	negative	N/A
M018	negative	negative	N/A
M019	negative e	negative	N/A
M020	Sample inappropriate	negative	N/A
M021	negative	negative	N/A
M022	negative	negative	N/A
M023	negative	negative	N/A
M024	Sample inappropriate	negative	N/A
M025	negative	negative	N/A
M026	negative	negative	N/A
M027	Sample inappropriate	negative	N/A
M028	2.4x10 <sup>6</sup>	negative	N/A
M029	negative	negative	N/A
M030	negative	negative	N/A
M031	negative	negative	N/A
M032	negative	negative	N/A
M033	10.3x10 <sup>6</sup>	negative	N/A
M034	negative	negative	N/A
M035	negative	negative	N/A
M036	10.3x10 <sup>7</sup>	positive	N/A
M037	8.1x10 <sup>7</sup>	positive	N/A
M038	negative	negative	N/A
M039	Sample inappropriate	positive	N/A
M040	negative	negative	N/A
M041	negative	negative	N/A
M042	negative	negative	N/A
M043	negative	N/A	N/A
M044	negative	negative	N/A
M045	negative	negative	N/A
M046	4.1x10 <sup>7</sup>	positive	N/A
M047	negative	negative	N/A
M048	negative	negative	N/A
M049	1.0x10 <sup>9</sup>	positive	N/A
M050	negative	negative	N/A
M051	Sample inappropriate	negative	N/A
M052	negative	negative	N/A
M053	negative	negative	N/A



Sputum Samples of suspected cases were collected from the National TB Centre Clinic (Thimi, Nepal), the samples were divided into two portions- one for AFB and Culture testing done at NTB laboratory and another one for Real Time PCR analysis done at Intrepid Nepal Pvt.Ltd. Laboratory located at Thapathali, Kathmandu.

DNA were extracted using standard protocol. And Real Time PCR was done in IS6110 like target, with 12.5Kb region that is very specific to M.tuberculosis and not M.bovis. To insure and eliminate false positive and false negative scenarios, two internal controls were also simulatneously amplified along with the target.

<b>Sample Received</b>	<b>55</b>			
<b>Real Time PCR Samples</b>	<b>48</b>			
<b>AFB Positive</b>	<b>8</b>			
<b>QPCR Postive</b>	<b>10</b>			
<b>Unprocessable Samples</b>	<b>7</b>			
<b>AFB Positive from Unprocessable Samples</b>	<b>1</b>			
<b>Concurrency AFB QPCR</b>	<b>100%</b>			
<b>Detection (Prevalence) AFB (n=55)</b>	<b>14.50%</b>			
<b>Detection (Prevalence) QPCR (n=48)</b>	<b>20.80%</b>			
<b>Detection (Prevalence) AFB (n=48)</b>	<b>14.50%</b>			
* Unprocessable Samples were sputum samples that were low in quantity when received from NTB				

#### **Observations on the comparative study-**

- **Intrepid Nepal Pvt.Ltd. MTB Real Time PCR (Genomic Detection) is very sensitive and specific and is 100% concurrent to AFB.**
- **There is almost 6% under diagnosis with current AFB method of detecting MTB.**
- **Intrepid Nepal Pvt.Ltd. has also successfully detected MTB in other specimens like prostatic fluid, senovial fluid etc.**