The current cross-sectional hospital-based test validation study was carried out in the Department of Microbiology, M.M.I.M.S.R, Mullana during the period October 2012- March 2014. This study evaluated the diagnostic accuracy, turnaround time, cost effectiveness, cross contamination rates of direct TTC assay and direct MODS assay with that of conventional indirect proportion method (for INH and RIF drug susceptibility testing directly from the Ziehl-Neelsen smear positive sputum samples). For this study, a total of thousand smear positive pulmonary TB patients [both out patients (OPD) and in patients (IPD) attending chest and TB department] and patients from RNTCP center (Ambala,) were studied and the following inferences were made from the study;

1. Male predominance was 68.2% in this study
2. 50.1% direct AFB smear positive sputum specimens were obtained from the age groups 20-40
3. 76.3% patients were belonged to rural areas
4. 83.9% and 16.1% of samples produced valid and invalid results, respectively.
5. Maximum valid results (85%) were produced by Indirect LJ PM followed by 84.9%, 84.3% direct TTC assay, and direct MODS assays, respectively.
6. The rate of detection of Non Tuberculous Mycobacteria was 3.1% in all the assays.
7. Higher cross contamination rate (4.35%) was observed in direct MODS assay when compared to LJ PM (1.24%) and Direct TTC assay (3.1%)
8. Highest contamination rate (4.2%) was observed in direct MODS assay
9. Highest contamination rate was reported in between June and July (1.3%) and the lowest was in December and January (0%).
10. Among the valid results (N=839), maximum Isoniazid or Rifampicin susceptible isolates (84.74%) were obtained in indirect LJ PM
11. Of the total valid results (N=839), the maximum number of INH or RIF resistant isolates (16.69%) were obtained in MODS assay

12. Among the valid results (N=839), 18.59% INH or RIF resistant isolates were obtained by combination of indirect LJ PM, direct TTC assay and direct MODS assay

13. Among the INH or RIF resistant isolates obtained (indirect PM,N=128, direct TTC assay, N=134, direct MODS assay , N= 140), the maximum INH resistant isolates (32.85%) were detected by direct TTC assay

14. Among the INH or RIF resistant isolates obtained (indirect PM,N=128, direct TTC assay, N=134, direct MODS assay , N= 140), the maximum RIF resistant isolates (10.94%) were identified by Indirect LJ proportion method

15. Among the INH or RIF resistant isolates obtained (indirect PM,N=128, direct TTC assay, N=134, direct MODS assay , N= 140), the maximum INH and RIF resistant isolates (59.7%) were obtained by direct TTC assay

16. Of the18.59% total drug resistance cases, 13.83% and 4.77% cases were previously treated and newly diagnosed TB cases, respectively.

17. Among the previously treated TB patients (N=282), a higher percentage of MTB isolates (24.11%) were resistant to both INH and RIF

18. Among the newly diagnosed TB patients (N=557), the maximum percentage of MTB isolates (3.24%) were resistant to both INH and RIF

19. Among the previously treated (N=116) and newly diagnosed drug resistant TB cases (N=40), 26.72% and 32.5% isolates were resistant to INH, respectively

20. Among the previously treated (N=116) and newly diagnosed drug resistant TB cases (N=40), 11.21% and 10% isolates were resistant to RIF, respectively
21. Among the previously treated (N=116) and newly diagnosed drug resistant TB cases (N=40), 56.03% and 45% isolates were resistant to both INH and RIF, respectively.

22. Of the Isoniazid or Rifampicin susceptible MODS assay isolates (N=699), 41.49% were possessing the smear scores 2+

23. Of the Isoniazid or Rifampicin resistant indirect LJ PM isolates (N=128), 53.91% isolates were possessing the smear scores 1+

24. Among the Isoniazid or Rifampicin susceptible indirect LJ PM isolates (N=711), 41.21% of specimens were having the smear scores 2+ and showed growth within a median time of 62 days.

25. Of the Isoniazid or Rifampicin susceptible direct TTC assay isolates (N=705), 41.42% (having the smear scores 2+) showed growth within a median time of 9.5 days.

26. Of the Isoniazid or Rifampicin susceptible direct MODS assay isolates (N=699), 41.49% (having the smear scores 2+) showed growth within a median time of 7 days.

27. Among the (N=730) Isoniazid or Rifampicin susceptible isolates (obtained by combination of indirect LJ PM, direct TTC assay, direct MODS assay), 40.68% Isoniazid or Rifampicin susceptible isolates (possessing the smear scores 2+) showed growth within a median time of 62 days (for Indirect LJ PM) 9 days (for Direct TTC assay) and 7 days (for direct MODS assay), respectively.

28. Among the Isoniazid or Rifampicin susceptible isolates obtained by indirect LJ PM [(N=184) in the previously treated TB patients], 70.11% (possessing the smear scores scanty)] showed growth within a median time of 89 days.

29. Among the Isoniazid or Rifampicin susceptible isolates obtained by direct TTC assay [(N=180) in the previously treated TB patients], 72.22%
(possessing the smear scores scanty) showed growth within a median time of 16 days

30. Among the Isoniazid or Rifampicin susceptible isolates obtained by direct MODS assay [(N=177) in the previously treated TB patients], 72.88% (possessing the smear scores scanty) showed growth within a median time of 15 days

31. Among the Isoniazid or Rifampicin susceptible isolates [in previously treated TB patients (N=196), detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay], 69.19% sputum specimens (possessing smear scores scanty) showed growth within a median time of 89 days (for Indirect LJ PM), 16 days (for Direct TTC assay) and 14 days (for direct MODS assay), respectively.

32. Among the newly diagnosed INH or RIF susceptible isolates [(N=527) obtained by indirect LJ PM], 53.89% isolates (possessing the smear scores 2+) showed growth within a median time of 63 days

33. Among the newly diagnosed INH or RIF susceptible isolates [(N=525) obtained by direct TTC assay], 53.9% isolates (possessing the smear scores 2+) showed growth within a median time of 9 days

34. Among the newly diagnosed INH or RIF susceptible isolates [(N=522) obtained by direct MODS assay], 53.83% isolates (possessing the smear scores 2+) showed growth within a median time of 7 days

35. Among the (N=534) Isoniazid or Rifampicin susceptible isolates (detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay), 53.93% sputum specimens (possessing the smear scores 2+) showed growth within a median time of 62 days (for Indirect LJ PM), 9 (for Direct TTC assay) and 7 (for direct MODS assay), respectively.
36. Of the Isoniazid or Rifampicin resistant indirect LJ PM isolates (N=128), 53.91% sputum specimens (possessing smear scores 1+) showed growth within a median time of 86 days.
37. Of the Isoniazid or Rifampicin resistant direct TTC assay isolates (N=134), 52.99% sputum specimens (possessing smear scores 1+) showed growth within a median time of 18 days.
38. Of the Isoniazid or Rifampicin resistant direct MODS assay isolates (N=140), 52.14% sputum specimens (possessing smear scores 1+) showed growth within a median time of 21 days.
39. Among the Isoniazid or Rifampicin resistant isolates [(N=156), detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay], 51.92% sputum specimens (possessing smear scores1+) showed growth within a median time of 86 days (for Indirect LJ PM) 18 days (for Direct TTC assay) and 21 days (for direct MODS assay), respectively.
40. Among the previously treated TB patients (INH or RIF resistant Indirect LJ PM isolates, N=98 ), 65.31% sputum specimens (possessing smear scores 1+) showed growth within a median time of 86 days.
41. Among the previously treated TB patients (INH or RIF resistant direct TTC assay isolates, N= 102 ), 67.65% sputum specimens (possessing smear scores 1+) showed growth within a median time of 18 days.
42. Of the previously treated TB patients (INH or RIF resistant Indirect LJ PM isolates, N=105 ), 67.62% (possessing smear scores 1+) sputum specimens showed growth within a median time of 21 days.
43. Among the previously treated Isoniazid or Rifampicin resistant isolates [(N=116) (detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay)], 65.52% sputum specimens (possessing the smear scores 1+) showed growth within a median time of 86 days (for
Indirect LJ PM), 17.5 (for Direct TTC assay) and 21 (for direct MODS assay), respectively.

44. Among the newly diagnosed Isoniazid or Rifampicin resistant indirect LJ PM isolates (N=30), (46.67%, possessing smear scores 2+) showed growth within a median time of 86 days.

45. Of the newly diagnosed Isoniazid or Rifampicin resistant direct TTC assay isolates (N=32), (46.88%, having the smear scores 2+) showed growth within a median time of 24 days.

46. Among the newly diagnosed Isoniazid or Rifampicin resistant direct MODS assay isolates (N=35), (48.57%, possessing the smear scores 2+) demonstrated growth within 21 days.

47. Among the newly diagnosed Isoniazid or Rifampicin resistant direct [(N=40) detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay], 47.5% (possessing the smear scores 2+) demonstrated growth within a median time of 86 days (for Indirect LJ PM) 24 days (for Direct TTC assay) and 16.5 days (for direct MODS assay), respectively.

48. Of the Isoniazid or Rifampicin susceptible indirect LJ PM (N=711), direct TTC assay (N=705), direct MODS assay (N=699) isolates, 82.14%, 80.71%, 80.83% sputum specimens were possessing mucopurulent consistency, respectively.

49. Of the Isoniazid or Rifampicin susceptible isolates [(N=683) (detected by combination of LJ PM, direct TTC assay and direct MODS assay)], 81.84% specimens were possessing mucopurulent consistency.

50. Of the Isoniazid or Rifampicin resistant indirect LJ PM (N=128), direct TTC assay (N=134), direct MODS assay (N=140) isolates, 88.28%, 95.5%, 94.29% sputum specimens were having mucopurulent consistency, respectively.
51. Of the Isoniazid or Rifampicin resistant isolates [(N=156) (detected by combination of LJ PM, direct TTC assay and direct MODS assay)], 88.46% sputum specimens were having mucopurulent consistency.

52. Among the Isoniazid or Rifampicin susceptible indirect LJ PM isolates (N=711), 82.14% of sputum specimens were having the mucopurulent consistency and showed growth within a median time of 61 days.

53. Of the Isoniazid or Rifampicin susceptible direct TTC assay isolates (N=705), 80.71% were having the mucopurulent consistency showed growth within a median time of 12 days.

54. Of the Isoniazid or Rifampicin susceptible direct MODS assay isolates (N=699), 80.83% sputum specimens were having the mucopurulent consistency showed growth within a median time of 9 days.

55. Among the (N=730) Isoniazid or Rifampicin susceptible isolates (obtained by combination of indirect LJ PM, direct TTC assay, direct MODS assay), 81.23% of mucopurulent sputum specimens showed growth within a median time of 54 (for Indirect LJ PM), 12 (for Direct TTC assay) and 9 days (for direct MODS assay), respectively.

56. Among the Isoniazid or Rifampicin susceptible isolates obtained by indirect LJ PM [(N=184) in the previously treated TB patients], 92.39% sputum specimens were having mucopurulent consistency and showed growth within a median time of 83 days.

57. Among the Isoniazid or Rifampicin susceptible isolates obtained by direct TTC assay [(N=180) in the previously treated TB patients], 88.89% sputum specimens were possessing mucopurulent consistency and showed growth within a median time of 14 days.

58. Among the Isoniazid or Rifampicin susceptible isolates obtained by direct MODS assay [(N=177) in the previously treated TB patients],
89.27% were having mucopurulent consistency and showed growth within a median time of 12 days

59. Among the Isoniazid or Rifampicin susceptible isolates [in previously treated TB patients (N=196) detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay], 89.8% sputum specimens were possessing mucopurulent consistency and showed growth within a median time of 86 days (for Indirect LJ PM), 14 days (for Direct TTC assay) and 12 days (for direct MODS assay), respectively.

60. Among the indirect LJ PM ,N=527, direct TTC assay ,N=525,direct MODS assay ,N=522 Isoniazid or Rifampicin susceptible isolates (the newly diagnosed TB patients), 78.56%,77.9%,and 77.9% of the mucopurulent sputum specimens showed growth within a median time of 52,9 and 7 days ,respectively

61. Among the (N=534) Isoniazid or Rifampicin susceptible isolates (detected by combination of indirect LJ PM , direct TTC assay and direct MODS assay), 78.08% sputum specimens (possessing the mucopurulent consistency ] showed growth within a median time of 48 days (for Indirect LJ PM) ,9 days ( for Direct TTC assay) and 7 days(for direct MODS assay), respectively.

62. Of the Isoniazid or Rifampicin resistant indirect LJ PM isolates (N=128), 88.28% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 69 days

63. Of the Isoniazid or Rifampicin resistant direct TTC assay isolates (N=134), 95.52% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 11 days

64. Of the Isoniazid or Rifampicin resistant direct MODS assay isolates (N=140), 94.28% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 9 days
Among the total Isoniazid or Rifampicin resistant (N=156) isolates (detected by combination of indirect LJ PM, direct TTC assay, direct MODS assay), 88.46% resistant isolates (possessing mucopurulent consistency) showed growth within a median time of 76 days (for Indirect LJ PM) 11 days (for Direct TTC assay) and 10 days (for direct MODS assay), respectively.

Among the previously treated TB patients (INH or RIF resistant Indirect LJ PM isolates, N=98), 90.81% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 76 days.

Among the previously treated TB patients (INH or RIF resistant direct TTC assay isolates, N=102), 97.06% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 9 days.

Among the previously treated TB patients (INH or RIF resistant Indirect LJ PM isolates, N=105), 96.19% (possessing mucopurulent consistency) sputum specimens showed growth within a median time of 9 days.

Among the previously treated Isoniazid or Rifampicin resistant isolates [(N=116) (detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay)], 91.38% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 76 days (for Indirect LJ PM), 9 days (for Direct TTC assay) and 9 days (for direct MODS assay), respectively.

Among the newly diagnosed Isoniazid or Rifampicin resistant indirect LJ PM isolates (N=30), 80% sputum specimens (possessing mucopurulent consistency) showed growth within a median time of 61 days.
71. Of the newly diagnosed Isoniazid or Rifampicin resistant direct TTC assay isolates (N=32), 90.63% sputum specimens (having mucopurulent consistency) showed growth within a median time of 11 days.

72. Among the newly diagnosed Isoniazid or Rifampicin resistant direct MODS assay isolates (N=35), 88.57% sputum specimens (possessing mucopurulent consistency) demonstrated growth within 12 days.

73. Among the newly diagnosed Isoniazid or Rifampicin resistant direct [(N=40) detected by combination of indirect LJ PM, direct TTC assay and direct MODS assay], 80% sputum specimens (possessing the mucopurulent consistency) demonstrated growth within a median time of 61 days (for Indirect LJ PM) 11 days (for Direct TTC assay) and 12 days (for direct MODS assay), respectively.

74. The sensitivity and specificity, PPV and NPV of direct TTC assay was:
   a) For newly diagnosed TB patients; 97.54%, 77.78%, 98.85%, 61.76%, respectively (for INH); 99.26%, 83.33%, 99.44%, 78.95%, respectively (for RIF). 98.29%, 76.66%, 98.67%, 71.88%, respectively (for both INH and RIF).
   b) For previously treated TB patients; 93.33%, 90.8%, 95.79%, 85.87%, respectively (for INH); 95.71%, 86.11%, 95.26%, 87.32%, respectively (for RIF); 91.3%, 87.76%, 93.33%, 84.31%, respectively (for both INH and RIF).

75. The sensitivity, specificity, PPV and NPV of direct MODS assay was:
   a) For newly diagnosed TB patients; 98.49%, 85.19%, 99.24%, 74.19%, respectively (for INH); 99.07%, 94.44%, 99.81%, 77.27%, respectively (for RIF). 98.1%, 83.33%, 99.04%, 71.43%, respectively (for both INH and RIF).
   b) For previously treated TB patients; 92.33%, 90.8%, 95.79%, 84.95%, respectively (for INH); 94.76%, 87.
respectively (for INH and RIF).

76. The sputum processing charges (using NALC-NaOH method) were two rupees fifty paisa for all the assays (excludes cost of centrifuge tube, instrument cost, electricity charge, labor charges).

77. The cost of indirect LJ PM was fifteen rupees fifty paisa (excluding the bottle charge, electricity and labor charges).

78. The cost of direct TTC assay was the estimated to be nineteen rupees and eighty eight paisa (excludes the bottle charge, instrument cost, electricity charge, labor charges).

79. For direct MODS assay; the estimated cost was thirty nine rupees one paisa (excludes the instrument cost, electricity and labor charges).