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Xpert MTB/RIF Assay: An Initial Analysis of Errors, Indeterminate and Invalid Results from Central India

Nikita Panwalkar, Ankur Pauranik, Arun Kumar Sharma, Shaina Beg Mirza, Rajeev Ranjan and Prabha Desikan*

Department of Microbiology, Bhopal Memorial Hospital and Research Centre, Raisen Bypass Road, Karond, Bhopal, India

**Corresponding author*

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A B S T R A C T

Early diagnosis and treatment is the key to successful treatment outcome in patients with tuberculosis (TB). Xpert MTB/RIF assay has greatly reduced the lag time for diagnosis and treatment of TB patients. However, despite the high sensitivity and specificity of the Xpert MTB/RIF assay, test failure can occur due to various reasons. We aim to identify the reasons for errors, indeterminate, invalids and no results that may be useful for initiating corrective measures for Xpert MTB/RIF assay. A total of 1052 smear negative samples, including pulmonary(69.9%) and extra-pulmonary(53.3%) samples were tested using GeneXpert MTB/RIF instrument. In pulmonary samples on initial testing: 3.13% indeterminate, 2.17% no results, 2.17% invalids and 11.7% errors were seen. In extra-pulmonary samples on initial testing 2.52% indeterminates, 1.57% no results, 0.31% invalids and 12.6% errors were seen. The reasons for test failures were identified as inadequate amount or quality of reagent, failed fluid transfer, equipment malfunction, power failures, temperature related errors and errors associated with Xpert-computer communication. Invalid tests were mostly associated with quality of sputum samples. For most of the test failure events, the troubleshooting steps are simple and can be curtailed by collecting good quality sample and scrupulously following the test protocol.

Introduction

A successful treatment outcome in patients with tuberculosis (TB), although challenging, can be achieved by early diagnosis and effective treatment. The introduction of Xpert MTB/RIF assay has greatly reduced the lag time for diagnosis and expedited patient management (Gidado *et al.*, 2018). The Xpert MTB/RIF machines are provided at most of the primary health care centres, which are at the bottom of the hierarchical pyramid of the national programme, with an intention to initiate an appropriate treatment without delay, especially in MDR TB patients. The Xpert MTB/RIF assay can provide results in 90 minutes, with minimal biohazard and technical training to the operators. Moreover, the instrument requires nominal infrastructure and can function optimally in an

ambient atmosphere of below 30 degrees centigrade (PMDT, 2021).

The Xpert MTB/RIF assay can detect the presence of *M. tuberculosis* and its resistance to rifampicin from direct or processed samples using specific cartridges. The assay is based on semi-nested real-time fluorescent quantitative PCR technique, targeting the 81-bp rpoB gene. GeneXpert MTB/RIF instrument has been shown to have high sensitivity and specificity; however, test failures may occur due to various reasons. Test failures can negatively impact treatment outcomes. Identifying reasons for errors, indeterminate, invalids and no results can help institute take corrective measures.

Study Design

The study was based on retrospective analysis of test failures reported from Xpert MTB/RIF software and reasons for test failures (indeterminate, no results, invalids and errors) from April 2014-March 2015 at a tertiary care hospital in Central India. All data was collected in an excel sheet and analysed.

Materials and Methods

The samples found to be negative by smear microscopy were subjected to Xpert MTB/RIF assay. Sputum samples were tested directly by XpertMTB/RIF assay, whereas decontaminated sediments were used for other pulmonary (BAL, Bronchial washings) and extra-pulmonary samples. Decontamination was carried out using NaOH-NaLC technique as per the standard protocol under the national programme. Sample processing for the Xpert MTB/RIF assay was performed as recommended by the manufacturer. In short,2 ml of buffer was added to 1 ml of sample, the mixture was shaken vigorously and allowed to stand for 10 minutes at room temperature, the mixture was shaken again and allowed to stand for further 5 minutes, two ml of homogenized mixture was added to the GeneXpert cartridge. In case of decontaminated samples, 1.5ml of buffer was added to 0.5ml of concentrated sediment. The mixture was shaken vigorously and allowed to stand for 10 minutes at room temperature, then again, after a vigorous shake incubation was continued for further 5 minutes. Two milliliters of the processed sample, as detailed above, was transferred to the sample chamber of the Xpert MTB/RIF cartridge. The cartridge was then loaded in the GeneXpert MTB/RIF machine. All results obtained by the Xpert MTB/RIF assay were recorded and analyzed.

Results and Discussion

A total of 1052 samples, including pulmonary and extra-pulmonary samples were tested using GeneXpert MTB/RIF instrument during the study period. Of 1052 samples tested, 631 (59.9%) samples were received from male and 421 (40%) were received from female patients. Of 631 male patients (mean age = 40), 65 patients were under the age of 19 years, 323 patients were between 19 to 45 years, 177 patients were between 46 to 60 years, and 66 patients were over the age of 60 years. Of 421 female patients (mean age = 34), 63 patients were under the age of 19 years, 256 patients were between 19 to 45 years, 72 patients between 46 to 60 years, and 30 patients were over the age of 60 years (Figure-1, Table-1).

There were 735 (69.9%) pulmonary and 317 (30.1%) extra-pulmonary samples. Of 735 pulmonary samples, 392 (53.3%) and of 317 extra-pulmonary samples, 263 (83%) indicated valid results for the presence or absence of MTB by Xpert MTB/RIF assay. In all, the assay displayed valid results for the presence of MTB in 655 (62.2%) samples and for the absence of MTB (MTB not detected) in 202 (19.2%) samples. We got 195 (18.5%) test failures for the various reasons. In pulmonary samples on initial testing: 3.13% (23/735) indeterminate, 2.17% (16/735) no results, 2.17% (16/735) invalids and 11.7% (86/735) errors were seen. Test failures seen from extra-pulmonary samples on initial testing were: 2.52% (8/317) indeterminates, 1.57% (5/317) no results, 0.31% (1/317) invalids and 12.6% (40/317) errors (Figure-2). On analysis of the reasons of test failed events in

pulmonary and extra-pulmonary samples, it was observed that, of the 126 error results (including 86 errors in pulmonary and 40 errors in extra-pulmonary samples), 73% errors were associated with "Inadequate amount or quality of reagent, failed fluid transfer etc", 11.1% errors were associated with "equipment malfunction", 13.49% errors were associated with "Xpert-computer communication error" and 2.38% samples had "temperature related errors". Tests failures showing "No results" due to power failure were the most, and observed on 52.3% (11/27) of the occasions. A detailed description of the reasons of test failures has been depicted in Figure-3.

Given the global drug resistant scenario of TB, use of Xpert MTB/RIF assays are being scaled up to fast-track TB treatment to the eligible patients (PMDT, 2021). Xpert MTB/RIF has been shown to have high sensitivity and specificity. Singh *et al.*, 2016 has demonstrated 100% sensitivity and specificity in pulmonary samples whereas 90.68% sensitivity and 99.62% specificity in extra-pulmonary samples by Xpert MTB/RIF when compared to phenotypic, genotypic and composite reference standards. Similarly, a high sensitivity and specificity was demonstrated by other study groups for Xpert MTB/RIF assay in pulmonary as well as in extra-pulmonary samples (Saeed *et al.*, 2017 and Shah 2016). In our study, 18.5% test failures were observed by Xpert MTB/RIF assay, though it provided significant valid results in pulmonary samples and extra-pulmonary samples with low overall failure. Gidado *et al.*, 2018, experienced 11% unsuccessful tests in their study. Kebede *et al.*, 2019, also found 9.6% unsuccessful results. Our analysis on test failures revealed that the commonest reason for test failure was inadequate sample processing, which could have been due to inadequate amount or quality of reagent, failed fluid transfer or poor sample processing within or outside the cartridge. We observed 2.1% Invalid tests in our study which were mostly associated with quality of sputum samples.

Table.1 Demographic, sample & result details by Xpert MTB/RIF Assay

Characteristics	Category	Total Numbers	%
Gender (n=1052)	Male	631	60.0%
	Female	421	40.0%
Age: Male (n=631)	< 18	65	10.3%
	19 to 45	323	51.2%
	46 to 60	177	28.1%
	>60	66	10.5%
Age: Female (n=421)	< 18	63	15.0%
	19 to 45	256	60.8%
	46 to 60	72	17.1%
	>60	30	7.1%
Sample (n=1052)	Pulmonary	735	69.9%
	Extra-pulmonary	317	30.1%
Result by Xpert MTB/RIF (n=1052)	Positive	655	62.3%
	Negative	202	19.2%
	Test failed	195	18.5%

Fig.1 Demographic, Type of sample & Result Details by Xpert MTB/RIF Assay

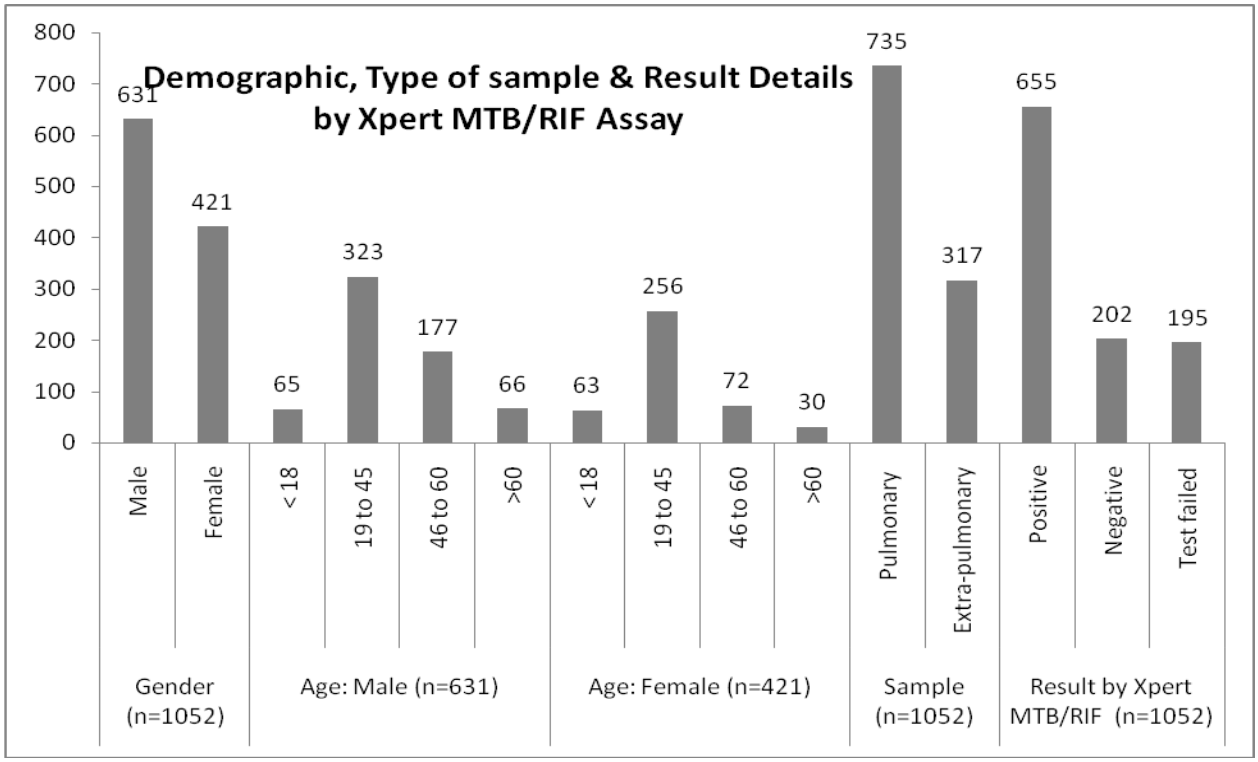


Fig.2 Test failed events in pulmonary & extra-pulmonary samples by Xpert MTB/RIF

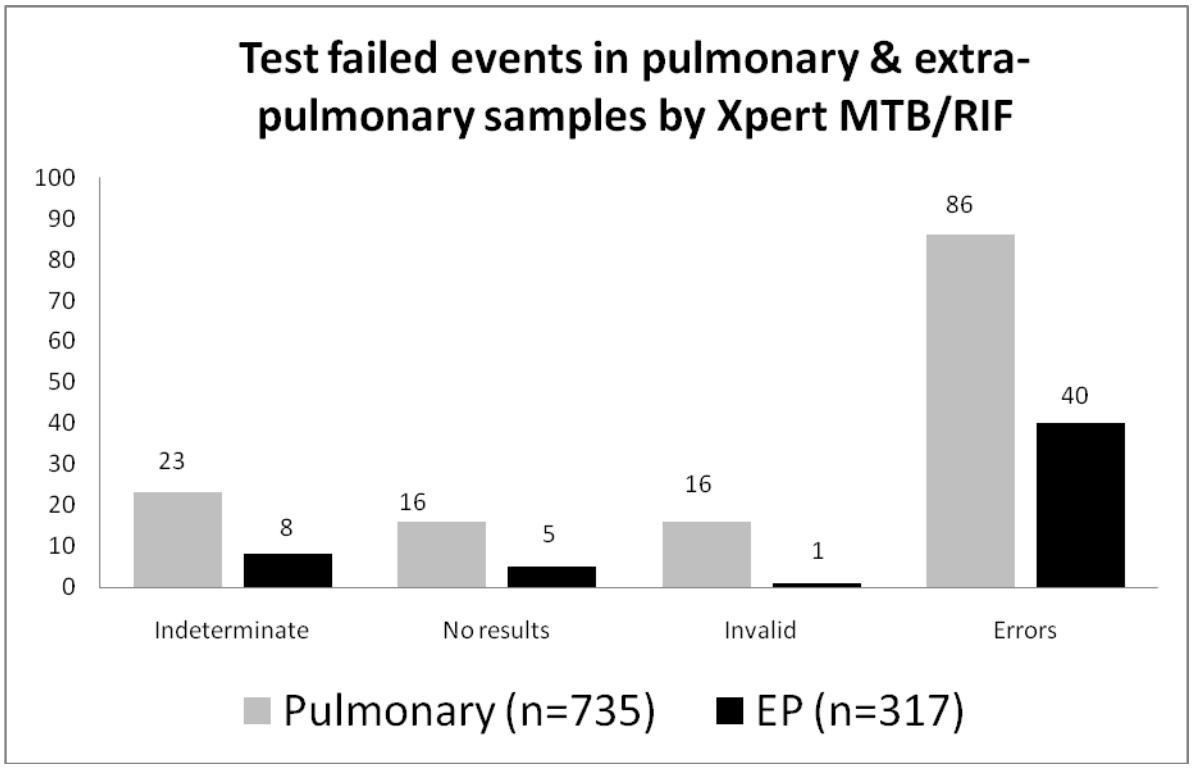
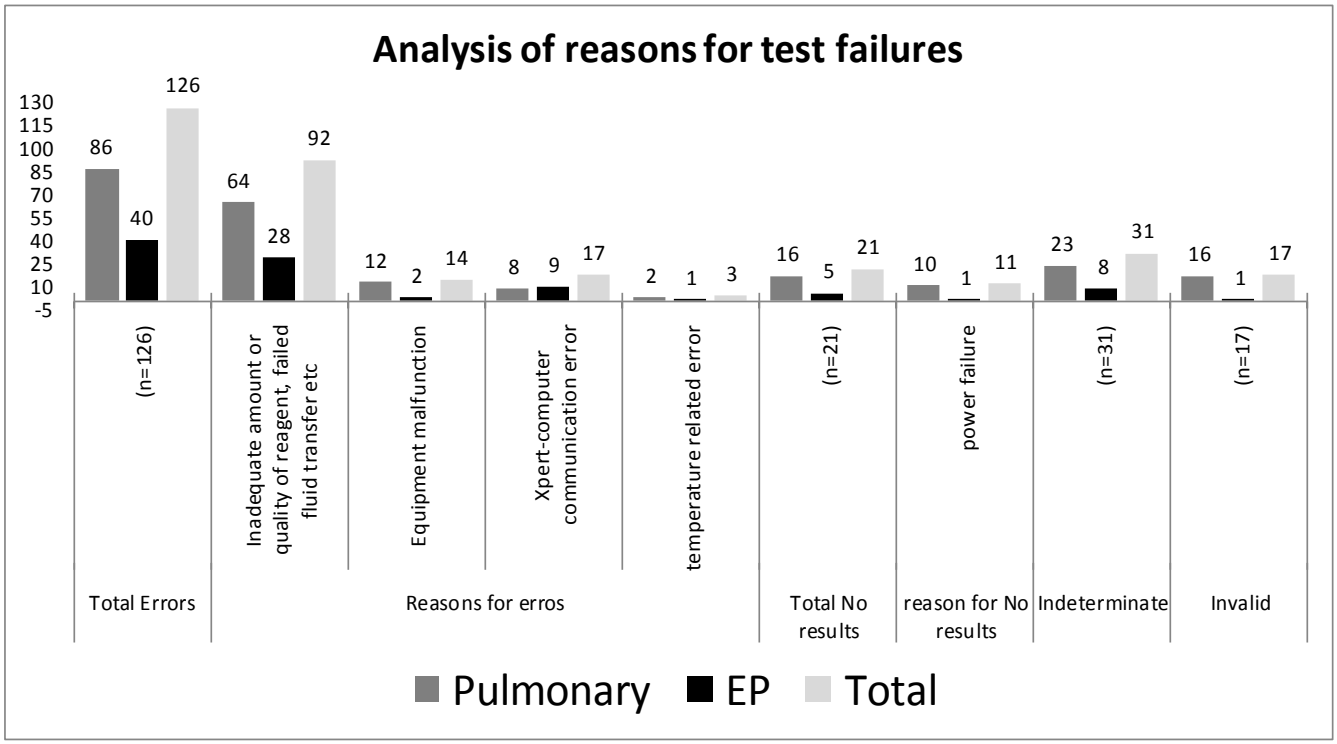


Fig.3 Analysis of reasons for test failures



Retesting of left-over samples can resolve most of issues however, the turn-around time may be compromised (Kebede *et al.*, 2019). Such errors can be avoided initially by scrupulously following the protocol and ensuring appropriate viscosity and homogeneity of the sample. Test failures due to equipment malfunction and Xpert-computer communication errors can be avoided through regular preventive maintenance of the instrument.

Collection of good quality specimen is prerequisite for any diagnostic test which can reduce invalid results. Appropriate instructions to the TB patients before coughing up sputum can reduce the presence of PCR inhibitors in samples like food particles, tobacco, blood etc. which can further lead to reduction in invalid results.

All errors need to be identified, so that they can be addressed effectively. For most of test failure events, the troubleshooting steps are simple. Many test failures can be curtailed by collecting good quality sample and scrupulously following the test protocol.

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