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HEALTH

Filter-based kits developed for TB diagnosis, drug-resistance testing

R. Prasad

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The kits improve the sensitivity of smear microscopy and transport of sputum samples

To address the TB diagnostic challenges, a multi-institutional team has developed three cost-effective kits that improve the sensitivity of smear microscopy, enable transport of sputum samples at ambient temperature without using bio-safe containers, and extract DNA for diagnosing drug-resistant TB. The three kits are — TB Detect, TB Concentration & Transport, and TB DNA Extraction.

The TB Detect kit is for diagnosis using LED fluorescence microscopy, while the TB Concentration & Transport, and the TB DNA Extraction kits together are for detection of drug-resistance. The TB Detect kit helps increase the positivity of LED fluorescence microscopy by about 5%, while the TB DNA Extraction kit allows the detection of drug-resistant TB bacteria with a high level of sensitivity. The results of the study were published in *PLOS ONE*.

The TB Detect kit currently costs Rs.100 per sample, Rs.100 for the TB transport kit and Rs.85 for the DNA extraction kit.

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Department of Biotechnology at AIIMS used a simple filter-based system to separate TB bacteria from the sputum to improve the sensitivity of LED fluorescence microscopy. In this, the sputum, which is viscous, is first liquefied using a proprietary reagent and pre-filtered. The bacteria in the liquefied sputum pass through a paper-like filter while some of the cell debris are retained on the filter.

The liquefied sputum containing the TB bacteria then comes in contact with a highly porous, plastic membrane filter underlain by multiple fibrous pads kept at the base of the filter device. While the sputum gets sucked by the fibrous pad due to capillary forces, the bacteria remain on the membrane.

"The membrane concentrates the bacteria in a small surface, which is about 1 cm in diameter. This leads to an increase in the detection limit," says Mr. Nalini Kant Gupta from the Advanced Microdevices Pvt Ltd, Ambala, who developed the membrane filter, the filter device and the kits. He is a co-author of the paper.

The limit of detection was found to be higher when the bacteria were separated from sputum prior to examination under a microscope. "By separating the bacteria from sputum, it becomes possible to detect TB bacteria even when they are fewer in number. Even when only 1,000 bacteria are present per ml of sputum it is possible to detect them compared with 10,000 bacteria per 1 ml of sputum in the conventional system," says Prof. Tyagi.

It also takes less time to examine a sample under a microscope — one minute vis-a-vis three-five minutes when the sputum is spread on a glass slide. This is because the sputum on a glass slide covers a large area requiring more number of fields to be viewed.

Diagnosis of drug-resistant TB is carried out only at central laboratories, and so samples have to be transported. The TB concentration & transport kit now makes it easy and simple to transport samples — transport it in sealed covers at ambient temperature making containment at low temperature redundant. This is because the bacteria are killed using a disinfectant.

"Since the bacteria are killed it is not possible to culture them. But for molecular testing to diagnose drug-resistant TB, only the DNA is needed and so it does not matter if the bacteria are dead or alive," says Prof. Tyagi.

The third kit — TB DNA Extraction — allows DNA to be isolated from the bacteria present on the filter paper. "To extract the DNA, the filter paper containing the bacteria are heated at 90 degree C in a lysis solution. The cell membrane ruptures and the DNA gets released from the bacteria, which is purified for molecular testing," says Dr. Sagarika Haldar from the Translational Health Science and Technology Institute (THSTI) and another corresponding author of the paper.

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molecular testing. Here we looked for mutations in the resistance-conferring genes by amplifying and sequencing the DNA," says Divya Anthwal from THSTI and first author of the paper.

Compared with culture, the sensitivity of the kit for drug-resistance was found to be high — 90% for rifampicin, 84% for isoniazid, and 83% for fluoroquinolones. Test specificity was 88-93%. "More importantly, 89-92% of samples tested positive for drug-resistance also tested positive when a standard method was used," says Prof. Tyagi.

"The developed kits are to be evaluated for operational feasibility and performance in field settings under RNTCP. If found suitable, they can be used in the programme," says Prof. Tyagi.

The kit for TB diagnosis was tested at the TB Hospital, Ambala and the National Institute of Tuberculosis and Respiratory Diseases (NITRD), Delhi using 1,190 samples. The kit for diagnosing drug-resistance was tested on 148 samples at NITRD and THSTI.

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