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Use of WGS in *M. tuberculosis* routine diagnosis

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ABSTRACT

Whole Genome Sequencing (WGS) is becoming affordable with overall costs comparable to other tests currently in use to perform the diagnosis of drug resistant tuberculosis and cluster analysis. The WGS approach allows an "all-in one" approach providing results on expected sensitivity of the strains, genetic background, epidemiological data and indication of risk of laboratory cross-contamination.

Although ideal, WGS from the direct diagnostic specimen is not yet standardized and up today the two most promising approaches are WGS from early positive liquid culture and targeted sequencing from diagnostic specimens using Next Generation Technology. Both have advantages and disadvantages. Sequencing from early MGIT requires positive cultures while targeted sequencing can be performed from a specimen positive for *M. tuberculosis* with a consistent gain in time to information. Aim of this study is to evaluate the feasibility and cost to use WGS with a centralized approach to speed up diagnosis of tuberculosis in a low incidence country.

From March to September 2016 we collected and processed by WGS 89 early positive routine MGIT960 tubes. Time to diagnosis and accuracy of this technique were compared with the standard testing performed in the routine laboratory.

An aliquot of 2 ml of early positive MGIT was processed, starting with heat inactivation. DNA was then isolated by using the Maxwell 16 Cell DNA Purification Kit and Maxwell 16 MDx for automated extraction. Paired-end libraries of read-length 75–151 bp were prepared using the Nextera XT DNA Sample Preparation kit, and sequenced on Illumina Miseq/Miniseq platform (based on the first available run). Total variant calling was performed according to the pipeline of the Phyresse web-tool.

The DNA isolation step required 30′ for inactivation plus 30′ for extraction. The concentration obtained ranged from 0.1 to 1 ng/μL, suitable for library preparation. Samples were sequenced with a turn around time of 24–48 h. The percentage of reads mapped to H37Rv reference genome was 83% on average. Mean read coverage was 65×. Main challenge was the presence of non–mycobacterial DNA contamination in a variable amount. Lineage detection was possible for all cases, and mutations associated to drug resistance to antitubercular drugs were examined. We observed high diagnostic accuracy for species identification and detection of full drug resistance profile compared to standard DST testing performed in MGIT.

Two events of recent transmissions including respectively three and two patients were identified and two laboratory cross-contamination were investigated and confirmed based

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on the analysis. Time to availability of report was around 72 h from MGIT positivity compared to up to 6–9 weeks for XDR-TB diagnosis with standard testing.

In addition to speed, main advantages were the availability of a full prediction of resistance determinants for rifampicin resistant cases, the fast detection of potential cross-contaminations and clusters to guide epidemiological investigation and cross border tracing.

Cost analysis showed that the cost per strain was approximately 150 Euro inclusive of staff cost, reagents and machine cost.

WGS is a rapid, cost-effective technique that promises to integrate and replace the other tests in routine laboratories for an accurate diagnosis of DR-TB, although suitable nowadays for cultured samples only.

Conflict of interest

None declared.

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