ABSTRACT

Background: The emergence of multidrug resistant tuberculosis (MDR-TB) is a major threat to global TB control. Rapid diagnosis of drug resistance is of vital importance in high TB and drug resistant TB incidence settings.

Aim: To test and validate a new lab-on-chip-based platform (In-Check™) for rapid identification of TB and resistance to rifampicin (RIF) and isoniazid (INH) on mycobacterial cultures and primary specimens collected in high TB incidence area.

Methods: A total of 459 patients with bacteriologically confirmed pulmonary TB have been recruited into the study in Samara, Russian Federation. Baseline epidemiological data was collected using the structured questionnaires. Molecular drug susceptibility tests (DST) for RIF and INH were performed on decontaminated sputum using the Hain GT MTBDRPlus. Phenotypic DST was performed on cultures derived from these specimens using standard methods on solid and liquid media. Crude DNA specimens extracted from sputum and cultures were tested using the In-Check™ TB platform according to manufacturers protocols.

Results: A total of 160 and 116 culture DNA extracts were analysed using two versions of chips, TB1.0 and TB4.0, respectively. In addition, 18 and 10 DNA specimens extracted from sputum were analysed using the same versions. Strains tested included in the most common mutations (S313L, H526Y, H526D, D516V in the rpoB gene, and C15T, A16G in the regulatory region of the katG gene, and C15T, A16G in the regulatory region of the inhA gene). Each chip was read at 250, 500, 250 ms acquisition times to adjust signal intensity and provide data for setting cut-off values and the development of interpretation rules. Evaluation of the TB1.0 version demonstrated that many probes needed to be re-designed and PCR and hybridization conditions required adjustment to equalize signal intensities. Using the TB4.0 version, the best probes have been identified, including S315T, H526Y, H526D, D516V in the rpoB gene, S313L in the katG gene, and C15T, A16G in the regulatory region of the inhA gene. Each chip was read at 250, 500, 1000 ms acquisition times to adjust signal intensity and provide data for setting cut-off values and the development of interpretation rules.

Conclusion: In-Check™ TB platform has the potential to be used as a new, non-invasive and reliable test for the rapid diagnosis of MDR-TB in high TB incidence settings. Further validation and development is needed to assess the suitability of the platform for routine testing on clinical specimens.

RESULTS

Two versions of chips tested in London:

TB1.0 (RIF+INH) - initial validation, optimization of protocols and probes selection

TB4.0 (RIF+INH+ID) – further validation

Four PCR and/or hybridization (developed by the HSR) tested and validated

- Development and implementation of infrastructural changes in Samara, Russian Federation (middle TB incidence and high TB drug resistance site)
- Ethical permissions obtained in London and Samara, study protocol developed and implemented
- Personnel recruited and trained in Samara and London
- Databases and analysis algorithms developed and validated
- Procedures for specimens transportation (Samara-London) established
- InCheck instruments (x2) were installed in London
- Three staff members from Samara have been trained and performed testing in London on Samara specimens

- Proportion of MDR strains in the collection
- Genotyping results available (24 loci VNTR + spoligotyping)
- Phenotypic DST results available
- Molecular DST results available

Patients recruitment and drug susceptibility testing on isolates

Samara, Russian Federation

| Patients | 459 |
| M.tuberculosis cultures | 459 |
| MTB DNA (crude extracts from cultures) | 459 |
| MTB DNA (crude extracts from sputum) | 459 |
| Proportion of MDR strains in the collection | 30.3% |
| Genotyping results available | (24 loci VNTR + spoligotyping) |
| Phenotypic DST results available | 459 |
| Molecular DST results available | 459 |