prepIT®•MAX

Maximizing *Mycobacterium tuberculosis* DNA yield for molecular methods with prepIT®•MAX

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Challenge

Significant research and funding have been invested in developing molecular diagnostic methods for *Mycobacterium tuberculosis* (MTb), including the roll-out of Cepheid® GeneXpert®, line probe assays for drug-susceptibility testing and sequencing for epidemiological research. These technologies promise more rapid diagnosis and faster drug-susceptibility profiling. While molecular technologies are being adopted more widely, they have not yet been able to match the sensitivity of culture testing in clinical settings. The sensitivity of molecular tests can be reduced by poor sample quality and inefficient MTb DNA extraction techniques. Standard MTb DNA extraction methods, such as bead beating and sonication, are time-consuming, can generate hazardous aerosols and provide inconsistent DNA yields with high rates of inhibition.

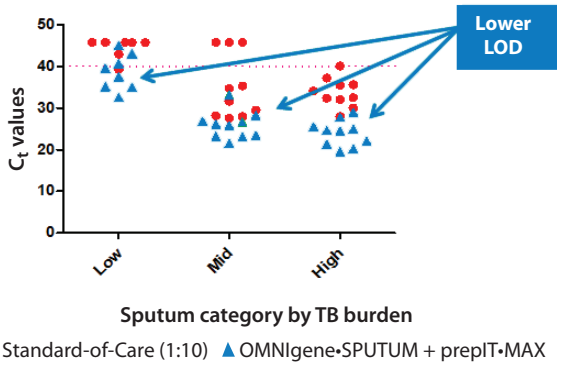
Solution

prepIT•MAX is a simple and quick extraction method that increases MTb DNA yield without bead beating or sonication.

Technical notes

prepIT•MAX is an easy-to-use DNA extraction kit that increases the yield of MTb DNA and can improve detection of low- and moderate-positive samples. The kit includes a liquid lysis reagent, MAX Lysis, which has been shown to release more MTb DNA than bead beating and to reduce PCR inhibition. The prepIT•MAX protocol can be completed in as little as 35 minutes when starting with sputum sediment. Three steps are involved: a 70°C heat step, a cold impurity-precipitation step and a centrifugation step. Further, the MAX Lysis reagent can be easily integrated with various other extraction methods, including ethanol precipitation and column-based purification techniques.

Methods and results



	% samples with MTb detected		
	Low	Mid	High
prepIT•MAX	88%	100%	100%
Bead beating (1:10 dilution)*	25%	70%	100%

Low n=8; Mid and High n=10
** 1:10 dilution results reported due to inhibition in neat samples*

***Figure 1**: Increased DNA yield lowers the limit of detection (LOD) of a CLIA/CLEP-approved PCR assay.*

Method: DNA from duplicate low-, mid- and high-positive sputum samples (provided by FIND) was extracted 1) using prepIT•MAX after treatment with OMNIgene®•SPUTUM, or 2) according to standard-of-care protocol (NaOH and bead beating). DNA was analyzed by a TaqMan® real-time PCR assay.

Results: PCR C_t values were consistently lower for samples extracted using prepIT•MAX compared to the standard-of-care method. Further, 100% of the standard-of-care samples exhibited significant inhibition when tested undiluted, whereas the prepIT•MAX samples had 0% inhibition.

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	Standard-of-care NaOH decon + bead beating	OMNIgene•SPUTUM + prepIT•MAX
Duplicate sputum aliquots	6	
MTb-positive by smear	50%	nd
MTb-positive by MGIT	50% (10–19 days)	nd
MTb-positive by PCR	**50%**	**100%**
Day 0 pyrosequencing success	**0%**	**100%**
MGIT pyrosequencing success	**50%**	n/a
Rifampicin-resistant	0	1
Isoniazid-resistant	2	3

nd = not done, n/a = not applicable

***Table 1**: Pyrosequencing from primary specimens.*

Method: Duplicate sputum samples (provided by FIND) underwent DNA extraction using prepIT•MAX or standard-of-care protocol.

Results: All (100%) of the prepIT•MAX/extracted samples generated successful pyrosequencing results for rifampicin and isoniazid resistance markers on Day 0 (i.e., directly from the primary specimen).

In contrast, 100% of the standard-of-care samples failed Day 0 pyrosequencing and required an average of 14 days of culture before 50% of samples were successfully pyrosequenced. The remaining 50% of samples were unable to be pyrosequenced for drug resistance due to low DNA yield from the primary specimen and due to no growth in culture.

Conclusion

The results indicate the following:

- prepIT•MAX is simpler, faster and safer for laboratory technicians than traditional bead beating or sonication methods.
- Compared to bead beating techniques, prepIT•MAX yields consistently more DNA from a primary sputum sample.
- The MTb DNA that prepIT•MAX extracts from a primary sputum specimen is of sufficient quantity and quality for immediate use in routine PCR testing as well as pyrosequencing for drug susceptibility profiling. Using prepIT•MAX may reduce the time to results by eliminating the need to wait for culture isolates to grow.

Compatibility: MTb DNA extracted using prepIT•MAX can be analyzed with current molecular techniques, including PCR (see Figure 1), LAMP (data not shown), line probe assays[1], pyrosequencing (see Table 1) and whole-genome sequencing (data not shown).

Reference:

1 prepIT•MAX (PT-MAX) data sheet. DNA Genotek. PD-BR-00196.