

sediment was inoculated into MGIT culture and then split into two aliquots. MTb DNA was extracted from aliquot #1 using the GenoLyse extraction method and from aliquot #2 using the prepIT•MAX extraction method. Each DNA sample was then analyzed using the LPA.

Results

The GenoType MTBDR*plus* (v2.0) LPA detected significantly more of the smear-negative or scanty specimens when prepIT•MAX was used (Figure 1, Tables 1 and 2). Specifically, the LPA detected 82% of the 11 smear-negative/scanty specimens when prepIT•MAX was used for DNA extraction, whereas only 64% were detected when GenoLyse was used. Detection of smear-negative/scanty specimens using GeneXpert and MGIT culture was identical, at 55%. For smear samples graded above 1+, detection of MTb-positive samples by all four diagnostic methods was high (Tables 1 and 2).

For specimens that were low-positive and had matched results available (samples marked * in Table 2), the frequency of indeterminate results on the LPA with prepIT•MAX-extracted DNA was comparable to that observed with GenoLyse-extracted DNA. Use of prepIT•MAX allowed two low-positive specimens to be identified as MTb complex (MTBC)-positive (samples marked † in Table 2), whereas these specimens were not

detected using the GenoLyse-extracted DNA. One of these samples (ID 1666) was also MTb-positive on GeneXpert, which supports the conclusion that this sample was a low-but-true positive and the prepIT•MAX extraction method was able to recover DNA in such quantity that it was detectable by the LPA. Regarding specimen ID 2315 (Table 2), it is unclear whether this was a true positive that was below the GeneXpert limit of detection but became detectable with prepIT•MAX extraction, or whether this LPA result was a false-positive. A larger cohort of samples is needed to determine rates of false-positive and false-negative results and the true impact of sample splitting for low-positive sputum specimens.

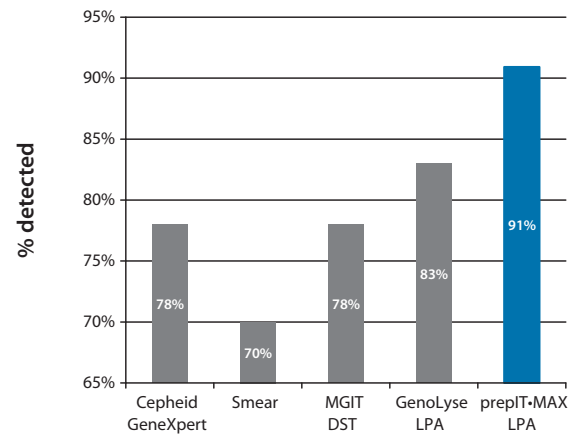


Figure 1: Comparison of overall MTb detection rates for the methods.

	Smear category	# of specimens	Cepheid GeneXpert	Smear	MGIT DST	GenoLyse LPA	prepIT•MAX LPA
% detected	Neg/Scanty	11	55%	36%	55%	64%	82%
	1+	3	100%	100%	100%	100%	100%
	2+	7	100%	100%	100%	100%	100%
	3+	2	100%	100%	100%	100%	100%
	All	23	78%	70%	78%	83%	91%

Table 1: Proportions of samples in each smear category detected by each diagnostic method.

Lab ID	CPHD GXP	Smear	DST by MGIT			GenoLyse			prepIT-MAX		
			MGIT	INH	RIF	MTBC	RIF	INH	MTBC	RIF	INH
2667	R	NEG	POS	R	R	POS	R	R	POS	R	R
2320*	NEG	NEG	NEG	----	----	POS	I	I	POS	S	S
1039*	R	NEG	POS	R	R	POS	R	R	POS	I	I
1923*	NEG	NEG	NEG	----	----	POS	I	I	POS	I	I
2250	NEG	NEG	NEG	----	----	NEG	----	----	NEG	----	----
2261	NEG	NEG	POS	----	----	NEG	----	----	NEG	----	----
2315 [†]	NEG	NEG	NEG	----	----	NEG	----	----	POS	I	S
1666 [†]	S	scanty	NEG	----	----	NEG	----	----	POS	I	I
1794	R	scanty	POS	R	R	POS	R	R	POS	R	R
2536*	R	scanty	POS	R	R	POS	I	I	POS	I	I
3232	S	scanty	POS	----	----	POS	S	S	POS	S	S
1034	S	1+	POS	S	S	POS	S	S	POS	S	S
1926	S	1+	POS	S	S	POS	S	S	POS	S	S
2173	S	1+	POS	----	----	POS	S	S	POS	S	S
1036	R	2+	POS	R	R	POS	R	R	POS	R	R
2019	S	2+	POS	S	S	POS	S	S	POS	S	S
2088	R	2+	POS	----	----	POS	R	R	POS	R	R
2703	R	2+	POS	----	----	POS	R	R	POS	R	R
2258	R	2+	POS	R	R	POS	R	R	POS	R	R
2318	R	2+	POS	R	R	POS	R	R	POS	R	R
2504	R	2+	POS	R	R	POS	R	R	POS	R	R
848	S	3+	POS	S	S	POS	S	S	POS	S	S
2161	S	3+	POS	S	S	POS	S	S	POS	S	S

CPHD GXP: Cepheid GeneXpert MTB/RIF assay; DST: drug susceptibility test; I: indeterminate; INH: isoniazid; MGIT: Mycobacterium growth indicator tube; MTBC: Mycobacterium tuberculosis complex; NEG: negative; POS: positive; R: resistant; RIF: rifampicin; S: sensitive; ----: not done.

Table 2: Summary of results by sample for detection of MTb using the molecular, smear and culture methods evaluated.

The results indicate that the prepIT•MAX DNA extraction kit can offer distinct advantages over the GenoLyse method for use in an MTb LPA:

- The combination of prepIT•MAX extraction with the Hain Lifescience GenoType MTBDR*plus* LPA performed better for detecting low-positive sputum specimens than all other TB diagnostic methods evaluated.
- Use of prepIT•MAX to extract MTb DNA resulted in detection of 82% of low-positive sputum samples, whereas the corresponding results for other methods were 36% (smear microscopy), 55% (Cepheid GeneXpert MTB/RIF and MGIT) and 64% (GenoLyse DNA preparation with the LPA).

- Overall, prepIT•MAX increased the MTb case detection rate by 8% to 21% (depending on diagnostic method) compared to the standard methods currently employed by the TB National Reference Laboratory at the Phthisiopneumology Institute, Chisinau.

References

- ¹ World Health Organization Regional Office for Europe. Tuberculosis, Country Work, Republic of Moldova. www.euro.who.int/en/health-topics/communicable-diseases/tuberculosis/country-work/republic-of-moldova
- ² World Health Organization. Laboratory services in TB control. Part III: Culture. Geneva, WHO, 1998 (WHO/TB/98.258. Available at: <http://www.who.int/tb/dots/laboratory/resources>)
- ³ Laboratory indicators from TB NRL, Institute of Phthisiopneumology, Chisnau, Republic of Moldova
- ⁴ World Health Organization Post-2015 Global TB Strategy. http://www.who.int/tb/post2015_strategy/en/

*OMNigene and prepIT are registered trademarks of DNA Genotek Inc. All other brands and names contained herein are the property of their respective owners.