prepIT[•]MAX

Moldova: In-country performance evaluation of prepIT®•MAX with the Hain Lifescience GenoType MTBDR*plus* line probe assay

Introduction

The Republic of Moldova is considered one of the World Health Organization's highest-priority countries in Eastern Europe due to its high rate of tuberculosis (TB) infection and, specifically, its high rate of multidrug-resistant TB (MDR-TB). In Moldova, one-third of newly diagnosed TB patients and two-thirds of those previously treated are MDR-TB-positive¹. Because of the high rate of MDR-TB, all new suspect TB cases must be tested using culture-based drug susceptibility methods that place high demand on already-taxed infrastructures, budgets and personnel. In addition, there are difficulties maintaining standardized cold chain for sample transport, as sputum specimens are collected at peripheral microscopy clinics and shipped to centralized laboratories for confirmatory testing. This method is accepted as standard protocol and recommended by the WHO², and makes good use of specialized resources concentrated at centralized reference laboratories; however, it leads to high rates of culture contamination, especially in the hotter summer months (8% to 10%)³. Contaminated cultures can have serious implications for patient care and TB control. A contaminated culture results in an invalid diagnostic test, which must be reported back to the clinic so that the patient can be found, notified and provide another sputum sample that is sent for repeat testing. Each step of this process can take several days, which translates to delayed treatment, increased TB transmission in the community, greater loss of life and additional costs to the system.

The WHO's End TB Strategy calls for universal access to drug susceptibility testing and systematic screening of contacts and high-risk groups as essential to eliminating TB infections⁴. To achieve these targets, it is necessary to consider at national and global scales how sample transportation and sample quality affect patient access to drug susceptibility tests and confidence in these test results. Increasing pressure is being placed on countries to test more samples using an expanding

number of methods; however, very few evaluations have been done on solutions that will lower costs and increase test quality as priority, resource-constrained nations scale their testing programs.

DNA Genotek has developed two products that will enable TB control programs to effectively scale their national testing programs without disrupting established testing algorithms or workflows. OMNIgene®•SPUTUM is a sample transport reagent that decontaminates and liquefies sputum, and is compatible with gold standard TB tests. prepIT[®]•MAX is a simple chemical DNA extraction method that increases DNA yield from *Mycobacterium* tuberculosis (MTb) and increases the sensitivity of molecular tests. These versatile, reliable and beneficial products can be easily integrated with existing diagnostic algorithms, and will provide TB control programs with concrete solutions that reduce costs, improve sample quality, increase patient access to reliable tests and ultimately help improve patient outcomes.

The National TB Reference Laboratory of the Phthisiopneumology Institute in Chisinau, Republic of Moldova conducted a comparative study in which the Hain Lifescience GenoType MTBDR*plus* (v2.0) line probe assay (LPA) was performed using DNA prepared via two different methods: DNA Genotek's prepIT•MAX method and the Hain Lifescience GenoLyse[®] extraction method.

Methods

Prior to processing, 23 raw sputum samples were initially tested in the Cepheid® GeneXpert® MTB/RIF assay and also assessed by smear microscopy (grading scale: negative/scanty/1+/2+/3+). Each sample was then processed using the standard NaOH/NALC method, which involves fresh preparation of a 6% NaOH + NALC solution, addition of an equal volume of solution to the sputum sample, 15 minutes incubation at room temperature, neutralization using sterile buffer, centrifugation to form a sediment, removal of the supernatant and re-suspension of the sediment in sterile buffer. Each re-suspended

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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 GenoLyseextracted 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.

			DST by MGIT			GenoLyse			prepIT•MAX		
Lab ID	CPHD GXP	Smear	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.

Conclusion

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.

The degree of improvement in case detection that was observed in this study, specifically within the low-positive specimen cohort, could have significant implications for a national testing program that is aiming to increase access to molecular drug susceptibility testing as part of a renewed drug resistance surveillance initiative.

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/

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