ERJ Express. Published on December 21, 2018 as doi: 10.1183/13993003.01832-2018

EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

Simple stool processing method for the diagnosis of pulmonary TB using GeneXpert MTB/Rif

Basti Andriyoko, Harini Janiar, Retno Kusumadewi, Eveline Klinkenberg, Petra de Haas, Edine Tiemersma

Please cite this article as: Andriyoko B, Janiar H, Kusumadewi R, *et al*. Simple stool processing method for the diagnosis of pulmonary TB using GeneXpert MTB/Rif. *Eur Respir J* 2018; in press (https://doi.org/10.1183/13993003.01832-2018).

Simple stool processing method for the diagnosis of pulmonary TB using GeneXpert MTB/Rif

Basti Andriyoko [1] basti_dr@yahoo.co.id

Harini Janiar[2] harini.janiar@kncvtbc.org

Retno Kusumadewi[3] retnokusumadewi@yahoo.com

Eveline Klinkenberg[4,5] eveline.klinkenberg@kncvtbc.org

Petra de Haas[4,*] petra.dehaas@kncvtbc.org

Edine Tiemersma[4] edine.tiemersma@kncvtbc.org

[1] Molecular Biology Division, Department of Clinical Pathology, Hasan Sadikin General Hospital, Bandung, Indonesia

[2] KNCV TB Foundation, Indonesia office, Jakarta, Indonesia

[3] National Tuberculosis Program, Jakarta, Indonesia

[4] KNCV Tuberculosis Foundation, The Hague, The Netherlands

[5] Department of Global Health, Amsterdam Institute for Global Health and Development, Academic Medical Centre, Amsterdam, The Netherlands

* Corresponding author

Take-home message:

A simple processing method for stool with Xpert MTB/Rif yielded reliable results for young children, thus providing opportunity for a painless bacteriological diagnosis of TB in children at the lowest health care levels.

The diagnosis of pulmonary tuberculosis (pTB) in young children often relies on clinical diagnosis because young children are usually unable to produce a sputum sample. Sputum induction (SI) or gastric aspiration (GA) can be applied to obtain a sample for microbiological diagnosis, but these methods cause discomfort, stress and pain, and cannot be performed at the lowest levels of the health care system, thus limiting access to pTB diagnosis of children.

However, stool samples can be obtained easily and have been shown to contain *Mycobacterium tuberculosis* (MTB) from swallowed sputum [1]. Although stool has historically received little attention as a sample to detect pulmonary TB [2], recent publications highlight its value for a bacteriological diagnosis in children and persons living with HIV [3-5]. GeneXpert MTB/Rif (Xpert) (Cepheid, France), has produced accurate results on stool samples of children, with a specificity and sensitivity of over 95%, respectively 80%, when compared to Xpert on respiratory samples (RS) [6, 7], which is the primary diagnostic test for TB [8]. However, the stool sample processing methods described so far are complex and often mirror culture processing, requiring equipment for decontamination, neutralization, and centrifugation. Some apply a commercial buffer to increase release of bacteria or flotation with sugar to concentrate the MTB bacilli [5, 6]. Recently described methods not including centrifugation still use addition of glass beads and filtration [6, 9]. Such methods cannot be easily implemented at the lower laboratory level in low income, high burden countries.

Xpert testing is accessible at the lower health care levels where sick children mostly initially present, therefore using it on stool samples could substantially improve access to a bacteriological diagnosis for TB in children. Here, we describe a simple processing method that is almost as simple as sputum processing for Xpert testing with potential for use at primary health care level.

This laboratory study was done between 1 October 2016 and 30 May 2017, following the ethical standards of the Helsinki Declaration (1975), in Dr Hasan Sadikin Hospital in Bandung, Indonesia, a tertiary care hospital for pulmonary diseases. Per standard of care, care takers were asked for informed consent for each of the procedures and subsequent samples taken for diagnosing pTB in the children. One stool sample was collected for consecutive children under 15 years of age with presumptive pTB. Most children also

submitted one respiratory sample (RS) obtained pre-prandial by GA for children aged up to 5 years or by SI using a nebulizer for older children. RS were processed as described previously [10, 11].

Stool samples were processed using a simple stool processing method that approaches the procedure of processing sputum for Xpert testing: approximately 1 gram of stool, picked from the sample using a wooden applicator stick, was added to 10 ml Phosphate Buffered Saline (PBS) of pH 7.4 (gibco™, Germany), mixed by vigorous shaking, and left for at least 10 minutes for stool particles to gravitate, after which 2 ml of the supernatant was mixed with 2-4 ml of the Xpert MTB/Rif sample reagent (SR) provided in the Xpert kit. After 15 minutes, 2 ml of this mixture was transferred into a cartridge for Xpert testing. Inconclusive tests were repeated once with the remaining 2 ml of the mixture. We calculated binomial exact confidence intervals for proportions.

In total, 36 children were included in this study with a median age of 17 months (interquartile range: 5.5-78 months); 20 (56%) children were under 2, and five (14%) were between 2 and 5 years old. MTB was detected in six children (17%), either on one or both samples (Table). Twenty-nine (81%) children also submitted one RS; for 20 (69%) children this was obtained by GA. For 27/29 children, a valid test result was obtained for both the stool and the RS and for 24 (89%; 95% confidence interval (CI): 71-98%) of these, the results were concordant (Table). The three children (8%, 95%CI: 2%-22%) with discordant results all tested MTB-positive on stool, but not on RS. Their median age was 5 months (versus 11 years for children with concordant MTB-positive results on stool and RS). Children with bacteriologically confirmed TB were aged between 3 months and 13 years (median 3.2 years). All children were started on anti-TB treatment.

Using a simple two-step processing method, high concordance between Xpert test results on RS and stool was obtained. We show that testing stool samples with Xpert can increase the number of children with a bacteriological confirmation of TB with three additional TB cases detected. Possibly, these additional cases had extrapulmonary TB, such as miliary/disseminated and gastro-intestinal TB, and shed more bacilli via their stool than RS. Especially in young children, the immune system is not fully mature which may cause disseminated disease and bacteria replicating in multiple tissues. Hematogenous TB

dissemination has been found among patients testing positive on urinary lipoarabinomannan (LAM), which is likely due to mycobacteruria [12]. Urine of patients testing positive on urinary LAM was also Xpert MTB/RIF positive [13]. A similar process may be provoked in the intestines of young children with TB. Another possibility is that the respiratory samples of these children were of inferior quality. Bonnave *et al.* obtained lower diagnostic yields from GA (60%) than from stool (64%) [14].

The rate of inconclusive test results was higher for stool samples (6/40 tests, 15%) than RS (1/30, 3%); for 2/36 (6%) stool samples, repeating the test on the remaining mixture did not lead to interpretative results. Six of the seven inconclusive test results had error codes (2008, 5006, invalid) suggesting solid particles were blocking the cartridge. Thus, there is room for further optimization of the processing method, e.g. by providing clear instructions on every critical step in the process, from the volume of stool (by type of stool sample) to be picked, to mixing of stool and buffer, and transfer of supernatant into the Xpert cartridge.

This small study has several limitations. First of all, it included a limited number of children, of whom only six were found MTB-positive. Second, it was conducted in a tertiary care hospital that receives children in whom TB is highly suspected but not yet diagnosed, part of whom are severely ill. Thirdly, since this was a laboratory study, we had no influence on which children were selected for submitting stool samples, nor do we know if additional children were diagnosed on clinical grounds only. Also, we have no information about the outcome of anti-TB treatment or previous TB episodes.

Although this study was done in a tertiary care hospital, the stool processing method can safely be applied at lower health care levels, as no biosafety cabinet or complex, expensive or difficult-to-obtain equipment is needed, although PBS is not available everywhere in Indonesia. An even simpler and safer variant of the method presented here, the KNCV Simple One-step Stool method for TB detection (KNCV SOS TB) omits the PBS step as approximately 0.5g of stool is directly added to the SR. This method will be tested in more health facilities to demonstrate the feasibility and acceptability of routine implementation. We anticipate that such simple, non-invasive methods may radically improve the access to a bacteriological diagnosis for TB, especially in very young children.

Acknowledgements:

Prof. Heda Melinda Nataprawira (Pediatric Respirology Division, Department of Child Health, Hasan Sadikin General Hospital / Faculty of Medicine Universitas Padjadjaran) is thanked for recruiting children, and Prof. Ida Parwati (Department of Clinical Pathology, Hasan Sadikin General Hospital / Faculty of Medicine Universitas Padjadjaran) for her supervision and support of the laboratory work for this project.

Funding:

This work was partly funded by United States Agency for International Development through Challenge TB under the terms of Agreement No. AID-OAA-A-14-00029 .

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Table. Overview of Xpert testing results in stool and respiratory samples obtained by sputum induction and/or gastric aspiration.*

Stool sample Xpert MTB/Rif result	**Xpert MTB/Rif result on respiratory sample** MTB positive	MTB negative	No sample available	subtotal
MTB positive	3	3	0	**6**
MTB negative	0	21	7	**28**
error/invalid	0	2	0	**2**
subtotal	**3**	**26**	**7**	**36**

*Four stool samples and one GA sample did not yield a conclusive result on the first test. Repetition of the test resulted in 2 additional test results for the stool sample and one additional test result for the GL sample.