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Intra-gastric string test: an effective tool for diagnosing tuberculosis in adults unable to produce sputum

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SUMMARY

SETTING: Tuberculosis (TB) diagnosis is challenging in patients who are unable to produce sputum. The string test, a method for retrieving enteropathogens, is a potential alternative diagnostic tool.

OBJECTIVES: To compare the TB detection yield and tolerability of the string test and that of sputum induction in adults with presumed TB in Uganda.

DESIGN: Cross-sectional study. String test and sputum induction were performed consecutively in patients unable to produce sputum. The string was removed after a 2-h intra-gastric downtime. Sputum induction used nebulised 5% saline for 20 min. Light-emitting diode fluorescence microscopy, Löwenstein-Jensen and MGIT culture were performed on all specimens, and the Xpert® MTB/RIF assay on a subset. Tolerability questionnaires were administered.

RESULTS: Of 210 patients included in the study, 59% were human immunodeficiency virus (HIV) positive and 50 (23.8%) were diagnosed with TB. Of these, 48 (96.0%) were detected with the string test and 46 (92.0%) with sputum induction. In patients with specimens collected using both methods for paired analysis, the yield of microscopy detection with the string test was 13.8% (26/188) vs. 13.3% (25/188) with sputum induction ($P = 1.0$). The yield increased to 22.9% (42/183) using culture for string test vs. 24.6% (45/183) for sputum induction ($P = 0.37$). Xpert detected TB in 15/96 (15.6%) patients with the string test vs. 17/96 (17.7%) with sputum induction ($P=0.62$). Tolerability was comparable.

CONCLUSION: The string test was well tolerated and provided similar yields to sputum induction, offering a viable alternative in resource-limited settings with minimal risk of transmission.

KEY WORDS: diagnosis; specimen collection; mycobacterial disease; detection

THE DIAGNOSIS of tuberculosis (TB) is mostly based on the bacterial examination of sputum. The decision to treat patients unable to expectorate is thus often based on less rigorous evidence, such as clinical examination and/or chest radiograph (CXR).[1–5] This is particularly common among human immunodeficiency virus (HIV) infected patients and children. Bronchoalveolar lavage, although considered the gold standard for patients unable to expectorate, is used only infrequently in low-resource countries due to its complexity, cost and safety risk.[6] While sputum induction and gastric aspiration both show good detection yields, the need for stringent infection control measures for sputum induction and clinicians' reluctance to use gastric aspiration impede their broad implementation, especially at low-level health facilities.[6–11] A simpler, inexpensive method of specimen retrieval suitable for remote areas in low-resource settings is therefore needed.

One little-explored option is the 'string test', a procedure used to retrieve enteric pathogens *Giardia* and *Helicobacter pylori*.[12–15] The string test uses a weighted gelatine capsule containing a coiled absorbent nylon string, with one end of the string protruding from a small hole in the capsule. The trailing part of the string is taped to the patient's cheek and the capsule is swallowed. After retrieving the string by gentle pulling, it is placed in 0.9% saline solution and processed the same way as induced sputum. The diagnostic value of the string test for TB has been assessed only in one study in smear-negative HIV-positive adults with a culture detection yield of 8.7% compared to 5% for sputum induction ($P = 0.03$).[16] Further evaluation of the performance of the string test for diagnosis of TB is therefore required.

In this study, we compared the string test and sputum induction in terms of TB detection yield using microscopy and culture among adults with presumed

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TB who were unable to produce sputum. The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was performed in a subgroup of patients. We also compared the tolerability and acceptability of the two methods.

STUDY POPULATION AND METHODS

Study design and population

In this cross-sectional, prospective study, all consecutive patients (aged ⩾18 years) presenting to the Mbarara Regional Referral Hospital, Mbarara, Uganda, with clinical suspicion of TB (presence of at least one of the following signs: cough of at least 2 weeks, chronic unexplained weight loss, fever, or recent CXR showing radiological features compatible with TB), who were unable to produce sputum spontaneously were eligible for the study. Patients were excluded if they had received anti-tuberculosis treatment or a fluoroquinolone for at least 1 week within the month before enrolment, had contraindications to sputum induction (asthma, chronic obstructive pulmonary disease, restrictive airway disease or oxygen saturation <94%) or were too unwell to tolerate the procedures. After signing an informed consent form, patients were given a questionnaire on symptoms and underwent a physical examination and CXR. CXR findings were recorded as normal, abnormal but not suggestive of TB and abnormal suggestive of TB.

Sample collection and laboratory procedures

A string test followed by a sputum induction procedure were performed at enrolment ('spot' sample), and both procedures were repeated in the same order the next morning (morning sample). For the string test, 2 h or overnight fasting was required prior to sample collection. The string test from the HDC Corporation (San Jose, CA, USA) was used according to the manufacturer's instructions, except that the intra-gastric down time was reduced from 4 h to 2 h to improve the feasibility of the test.[17] String test failure was defined as failure to swallow the capsule despite two attempts. For sputum induction, 5% hypertonic saline solution was nebulised using an ultrasonic nebuliser for a maximum of 20 min. A failed sputum induction procedure was defined if it resulted in the collection of <2 ml of specimen within 20 min. All procedures were performed in a dedicated, well-ventilated room to limit the risk of TB transmission.

For both sputum induction and the string test, samples were processed within 2 h after collection. Smear microscopy was performed on freshly induced sputum and on sediment after decontamination and centrifugation (string test samples). Specimens were decontaminated for 20 min using the *N*-acetyl-L-cysteine 0.5%-sodium hydroxide 1.5% (NALC-NaOH) method (final NaOH concentration of 1.5%), followed by the addition of 45 ml of phosphate buffer solution (PBS) for neutralisation to make a total of 50 ml, centrifugation for 20 min at 3000 x *g* and re-suspension of the sediment in 2.5 ml PBS. Starting in March 2011 (midpoint of the study), sediment remaining after decontamination and centrifugation of one string test and one sputum induction sample per patient was frozen at –20°C and then tested using Xpert at the end of the study.

Auramine-stained smears were read with a light-emitting diode (LED) fluorescence microscope (FluorescenS® LED system, Bergman Labora, Danderyd, Sweden) under 200/400 magnification.[18] For each sample collected, two drops of re-suspended sediment were inoculated into two tubes with Löwenstein-Jensen (LJ) medium and one MGIT tube (BD, Franklin Lakes, NJ, USA). For *Mycobacterium tuberculosis* identification and detection of contamination, culture-positive samples were processed with Ziehl-Neelsen smear microscopy, blood agar inoculation, the *p*-nitrobenzoic acid (PNB) test and then tested with the SD TB Ag MPT64 test (Standard Diagnostics, Gyeonggi-do, South Korea). For the Xpert assay, 0.5 ml of the remaining sediment was added to 1.5 ml of test reagent and processed according to the manufacturer's instructions. Interpretation of Xpert results was performed blinded to culture results. The laboratory is quality assured by the Tropical Medical Institute in Antwerp (Belgium), while the National Health Laboratory Service in Johannesburg, South Africa, ensures external quality assurance for microscopy.

Tolerability and acceptability assessments

Tolerability and acceptability of the induced sputum and string test were assessed using a Behavioural Pain Scale (Campbell, Detroit Medical Centre, Detroit, MI, USA, 2000) to establish the total tolerability (pain) scores as evaluated by a nurse at seven time points for the string test procedure (before insertion, during insertion, 10 min after insertion, 1 h after insertion, 10 min before removal, during removal, and 10 min after removal), and at three time points for sputum induction (before sputum induction, during sputum induction and 10 min after sputum induction). Discomfort was rated by the patient at various time points using a visual analogue scale of 0 to 10 (0 = no discomfort and 10 = worst possible discomfort)[19,20] (Appendix).* Any adverse events were recorded. Patients were also asked whether they preferred the string test or sputum induction. In addition, at each test attempt, nurses assessed ease of

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2015/00000019/00000005/art00012

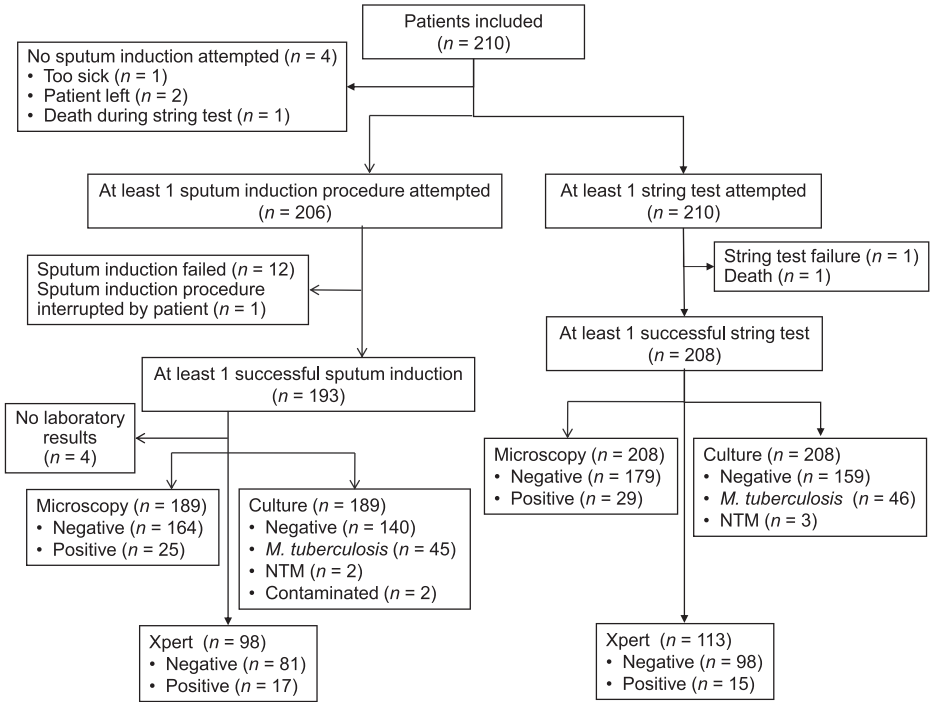

Figure 1 Study profile. NTM = non-tuberculous mycobacteria.

use using a score from 0 to 10 (0 = very easy, 10 = very difficult).

Statistical analysis

We calculated that 189 patients were needed to show equivalence between TB detection yields of string test and sputum induction, with a maximum tolerated difference of 5%, an expected proportion of discordant results of 6%, 80% power and 5% two-sided significance level. Assuming 10% dropouts, we aimed to recruit 210 patients.

Microscopy and culture detection yields, all with 95% confidence intervals (CIs), were calculated for sputum induction and the string test in all patients and by HIV status. A patient was considered positive for sputum induction or string test if there was at least one positive result with any specimen collected for microscopy or culture. A confirmed TB case was a patient with at least one positive result with any detection method on any collected specimen (string test or sputum induction). To calculate the yield of *M. tuberculosis* culture detection, contaminated and non-tuberculous mycobacteria culture results were excluded. Detection yields with string test vs. sputum induction were compared in patients with at least one string test and sputum induction sample, using the McNemar test for matched data. The limits of the 95%CI of the difference of detection yields (using microscopy and culture) between sputum induction and string test were compared with the 5% predefined equivalence margin. Detection yields using the Xpert assay were compared in the subgroup of consecutive patients tested.

Indicators of acceptability and tolerability were described using proportions for dichotomous variables and means with standard deviation (SD) for continuous variables, and compared using χ^2 or Student's *t*-test, respectively. Data were double-entered in a Voozanoo database (Epiconcept, Paris, France) and analysed using Stata® software, v. 11 (StataCorp, College Station, TX, USA).

The study was approved by the Mbarara University Institutional Review Board, Mbarara, Uganda; the Uganda National Council for Science and Technology, Kampala, Uganda; and the Comité de Protection des Personnes Ile de France XI, Paris, France.

RESULTS

A total of 210 patients were enrolled between March 2010 and March 2012 (Figure 1). The cohort mainly comprised out-patients (97.8%); 59% were HIV-infected (Table 1). Nearly all participants had cough (97.1%), half reported unexplained fever in the last 2 weeks, but only 11% had fever ($\geqslant$37.5°C) at inclusion. In 16% of the patients, CXR findings were suggestive of TB, with cavitation, fibrocystic change and consolidation being the most common radiological signs (Table 1). Overall, at least one string test was successfully performed among 208 (99.0%) participants, and 193 (91.9%) underwent at least one successful sputum induction (Figure 1). Among these two groups, 198 (95.2%) had two string test

Table 1 Patient characteristics

Characteristic	*n* (%)
Age, years, median [IQR] ($n = 209$)	34 [26–45]
Sex ($n = 210$)	
Male	101 (48.1)
Female	109 (51.9)
HIV-positive ($n = 198$)	117 (59.1)
Clinical symptoms/signs	
Cough for 2 weeks ($n = 210$)	204 (97.1)
Unexplained fever during the past 2 weeks ($n = 208$)	106 (51.0)
Dyspnoea ($n = 208$)	26 (12.5)
Chest pain ($n = 208$)	152 (73.1)
Suspicious skin lesions ($n = 207$)	5 (2.4)
Night sweats ($n = 206$)	59 (28.6)
Voice hoarseness ($n = 208$)	8 (3.9)
Weight loss* ($n = 208$)	148 (71.2)
Chest X-ray findings ($n = 182$)	
Abnormal, suggestive of TB	29 (15.9)
Cavitation	15 (51.7)
Consolidation	7 (24.1)
Fibrocystic change	7 (24.1)
Lymphadenopathy	1 (3.5)
Miliary	3 (10.3)
Pleural effusion	9 (31.0)
Abnormal, non-suggestive of TB	62 (34.1)
Normal	91 (50.0)

* Subjective weight loss reported by the patient.
IQR = interquartile range; HIV = human immunodeficiency virus; TB = tuberculosis.

results and 161 (83.3%) two sputum induction results. Overall, 50/210 (23.8%) patients were diagnosed with TB and all were started on anti-tuberculosis treatment. In this group, 29 were smear-positive, 49 were culture-positive for *M. tuberculosis* and 5 had a normal CXR. Of the 50 patients, 48 (96.0%) were detected by the string test and 46 (92.0%) by sputum induction. Most patients were diagnosed from the 'spot' specimen (Table 2).

For the paired analysis, both string test and induced sputum samples were available for microscopy for 188 patients and culture for 183 patients. There was no significant difference in detection yields between the string test and sputum induction when using microscopy (13.8% vs. 13.3%) or culture (22.9% vs. 24.6%) (Table 3). The difference in detection yields between induced sputum and the string test was –0.53% (95%CI –3.38 to –0.02) for microscopy and 1.59% (95%CI 0.41–4.94) for culture, with 95%CI limits within the 5% predefined equivalence margin. The detection yields were 13.8% (26/188) and 25.1% (46/183) when the string test was combined with sputum induction using microscopy and culture, respectively. Xpert results for both the string test and sputum induction samples were available for 96 patients. The TB detection yield was 15.6% (15/96) using the string test vs. 17.7% (17/96) using sputum induction ($P = 0.62$). All positive Xpert results were culture-positive.

The number of adverse events was similar between the string test (9/210, 4.3%) and sputum induction (8/206, 3.9%) ($P = 0.83$). With the string test, patients complained of cough ($n = 5$), vomiting ($n = 2$) and bleeding ($n = 1$); with sputum induction, they reported cough ($n = 6$) and wheezing ($n = 2$). The side effects resulted in the interruption of the string test procedure in 3/210 (1.4%) patients vs. 6/206 (2.9%) for sputum induction ($P = 0.73$). One patient collapsed 1 h after the string insertion and died after transfer to the hospital medical department. Oxygen saturation was above 94% at inclusion, and the patient had made no complaint during the string insertion. Post-mortem review of the patient's file revealed recent hospitalisation for blood transfusion due to severe anaemia. Necropsy was not possible, nor was it possible to establish a causal relationship between string insertion and death.

The mean scores for the pain and discomfort of the string test and sputum induction were <2 out of 10 at the different time points of assessment (Figure 2). For the string test, there was a slight increase during insertion and removal of the string. Of 196 patients interviewed, 160 (81.6%) declared a preference for sputum induction over the string test. Regarding ease of use, nurses gave the string test a mean score of 1.9 (SD 1.0, $n = 188$) vs. 1.7 for sputum induction (SD 1.0, $n = 182$) ($P = 0.23$).

DISCUSSION

This is the first study to assess the string test for the diagnosis of TB in a sub-Saharan country. The detection yield of the string test was similar to that of sputum induction. Using either the string test or

Table 2 Smear and culture detection yields from string test vs. sputum induction

	Spot specimen *n/N* (%)	Morning specimen *n/N* (%)	Overall yield	
			n/N	% (95%CI)
String test				
Smear-positive	28/208 (13.5)	26/200 (13.0)	29/208	13.9 (9.2–18.7)
Culture-positive	44/204 (21.6)	40/195 (20.5)	46/205	22.4 (16.7–28.2)
Sputum induction				
Smear-positive	24/180 (13.3)	24/177 (13.6)	25/189	13.2 (8.4–18.1)
Culture-positive	41/173 (23.7)	42/168 (25.01)	45/185	24.3 (18.1–30.6)

CI = confidence interval.

Table 3 Comparison of microscopy and culture detection yields between string test and induced sputum

	All TB suspects		HIV-positive		HIV-negative		*P* value*
Microscopy							
Concordance between string test and sputum induction	String test		String test		String test		
Induced sputum	TB+	TB–	TB+	TB–	TB+	TB–	
TB+	25	0	12	0	12	0	
TB–	1	162	0	92	1	60	
String test detection yield, *n*/*N* (%)	26/188 (13.8)		12/104 (11.5)		13/73 (17.8)		0.24
Sputum induction detection yield, *n*/*N* (%)	25/188 (13.3)		12/104 (11.5)		12/73 (16.4)		0.35
P value using exact Mc Nemar test	1.00		1.00		1.00		
Culture							
Concordance between string test and sputum induction	String test		String test		String test		
Induced sputum	TB+	TB–	TB+	TB–	TB+	TB–	
TB+	41	4	19	2	19	1	
TB–	1	137	0	80	1	50	
String test detection yield, *n*/*N* (%)	42/183 (22.9)		19/101 (18.8)		20/71 (28.2)		0.15
Sputum induction detection yield, *n*/*N* (%)	45/183 (24.6)		21/101 (20.8)		20/71 (28.2)		0.26
P value using exact McNemar test	0.37		0.50		1.00		

* Comparison of microscopy and culture detection yields between HIV-positive and HIV-negative patients (χ^2 test).
HIV = human immunodeficiency virus.

sputum induction, respectively over 10% and 20% of people with presumed TB had a positive microscopy and culture result, results that are similar to TB detection yields obtained in patients able to produce sputum in high TB burden countries.[21] The low rate of culture contamination (1%) can be explained by the use of three cultures (1 MGIT and 2 LJ) per sample, which reduced the risk of a final culture contamination result. The culture detection yield in HIV-infected patients was higher in our study setting

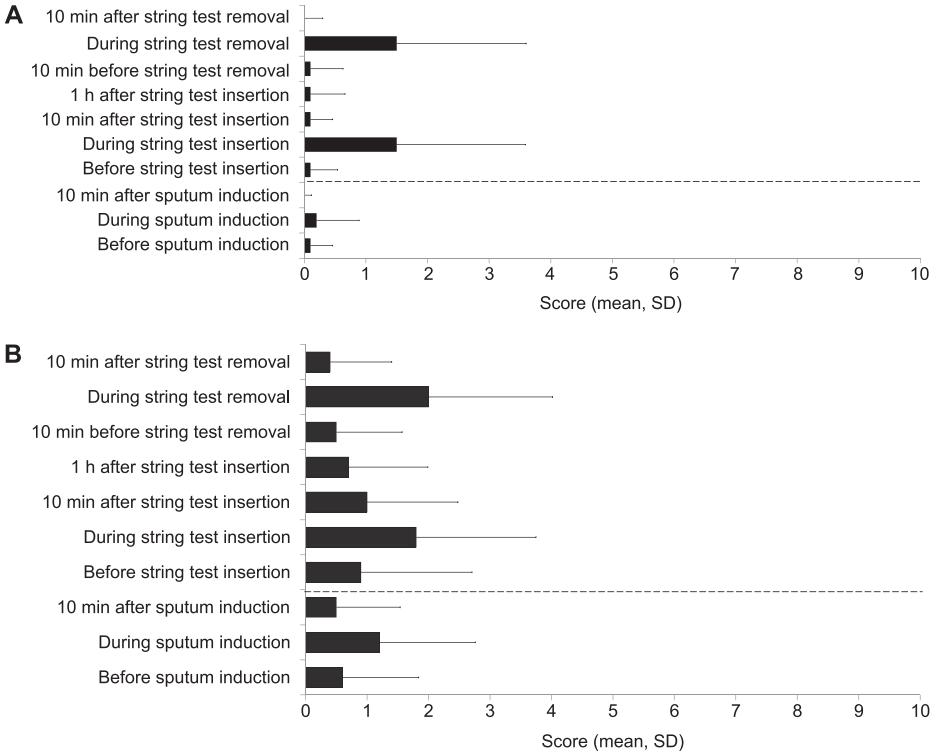

Figure 2 Mean tolerability and discomfort scores during the string test and sputum induction procedures. **A)** Mean tolerability scores at various times during the string test and sputum induction procedures; **B)** mean discomfort scores at various times during the string test and sputum induction procedures. Tolerability score: behavioural pain assessment score (0–10) evaluated by the nurse including the scoring (0–2) of the face, restlessness, muscle tone, vocalisation and consolability (Appendix). Discomfort score: discomfort rated by the patient at various time points using a visual analogue scale of 0–10 (0 = no discomfort; 10 = worst possible discomfort). SD = standard deviation.

(19/101, 18.8%) than in a previous study in Peru (14/160, 8.8%, $P = 0.02$), which could be explained by the lower TB incidence in Peru.[16] Unexpectedly, in our study, most of the TB diagnoses were made from the first specimen collected on the spot. We had expected the first sputum sample to have caused TB-rich sputum to be swallowed, resulting in a higher yield with the second test.

The string test was slightly less well tolerated during insertion and removal of the string. However, both the mean tolerability and discomfort scores remained very low during the different phases of the procedure. Although most patients expressed dislike of swallowing a device, none refused the test. Sputum induction was very well tolerated and well accepted both by patients and by the nursing staff. However, its implementation in our study setting was demanding, requiring modification of the existing infrastructure so that sputum induction could be performed in a separate, well-ventilated room; the purchase and maintenance of equipment such as an ultrasonic nebuliser for induction and an autoclave for disinfecting the nebuliser hoses; oxygen supply in case of bronchospasms; and the assurance of a continuous power supply, measures that are costly and may be difficult to ensure in many out-patient clinics in limited-resource settings.[11] However, other groups in South Africa have shown that sputum induction can be used at primary health care settings.[22,23] In contrast, the string test itself is affordable (US$1.00 per test) and requires limited training of nursing staff.

The string test has several disadvantages that could impede its use in routine practice. It requires 1) sample centrifugation before smear microscopy, probably not necessary for Xpert; 2) a 2-h fasting time and a 2-h intra-gastric downtime, which may be considered too long in out-patient clinics; and 3) the current poor availability, as the string test is currently manufactured by only one manufacturer in the United States and is therefore poorly available. However, the device is extremely simple and could easily be produced locally, as shown by Peru, which developed string capsules made in-house for <$0.20 each using market-purchased gelatine capsules.[19] In addition, it has a reduced risk of TB transmission compared to sputum induction, which is particularly important in patients with presumption of drug-resistant tuberculosis. These advantages for health care workers can also be obtained when using nasogastric aspiration; however, the tolerability and feasibility of nasogastric aspiration in out-patient settings might be lower than with the string test. However, this may deserve further evaluation.

The study has some limitations. It was not initially designed to use Xpert, which became available in the laboratory only after the study began. The Xpert test could only be performed on frozen sediment from a subgroup of patients. However, an earlier study demonstrated no significant difference in sensitivity when the sputum samples were frozen.[24] Further assessment of how the Xpert assay performs on fresh specimens collected with the string test and without prior sample centrifugation is needed. Similarly to what is recommended for gastric aspirates, the need for neutralisation of string test samples should also be further investigated, especially for samples processed with delays in the laboratory. Comparative costing between string test and sputum induction was not done. Finally, due to the exclusion of patients with contraindications for sputum induction, the string test could not be assessed in very sick patients.

CONCLUSION

With the scale-up of the Xpert assay, there is clearly a need to find alternative specimen collection methods that are well-tolerated and easy to use at low-level health care facilities in resource-limited settings.[10,25] This is what the string test device could potentially offer. However, the potential for greatly increased access of the string test, especially given the good prospects for producing the test locally, and the possibility of shortening the duration of the intra-gastric downtime even further (from 2 h to 1 h) would facilitate routine implementation of the test. The application of this device should be also further evaluated in children with clinical suspicion of TB.

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Conflicts of interest: none declared.

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