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Article *in* The International Journal of Tuberculosis and Lung Disease · August 2016

DOI: 10.5588/ijtld.15.0691

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INT J TUBERC LUNG DIS 20(8):1113–1117

http://dx.doi.org/10.5588/ijtld.15.0691

Xpert® MTB/RIF for detection of *Mycobacterium tuberculosis* from frozen string and induced sputum sediments

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SUMMARY

SETTING: Although it is now widely used for tuberculosis (TB) diagnosis, Xpert® MTB/RIF availability remains inadequate in low-resource settings. Moreover, its accuracy in testing stored samples from non-expectorating patients has not been evaluated.

OBJECTIVE: To assess the performance of Xpert in frozen samples of induced sputum (IS) and sputum from string test (ST) from non-expectorating individuals with presumed TB.

DESIGN: This was a laboratory-based study of 377 ST and IS samples collected between March 2010 and March 2013 at a referral hospital in Uganda. Samples were decontaminated, centrifuged and cultured, and the resultant samples were frozen at –20°C before Xpert evaluation.

RESULTS: TB was detected in ST and IS samples from 19/163 (11.7%) children and 63/201 (29.4%) adults using culture. Xpert sensitivity in frozen sediments from children was 37.5% (95%CI 8.5–75.5) in ST and 41.7% (95%CI 15.2–72.3) in IS samples, with specificities of respectively 100% (95%CI 94.9–100) and 98.6% (95%CI 92.7–100). In adults, sensitivity was respectively 50% (95%CI 31.3–68.7) and 48.5% (95%CI 30.8–66.4) in ST and IS samples, with specificities of respectively 100% (95%CI 95.5–100) and 98.6% (95%CI 92.4–100).

CONCLUSION: Given these results, and particularly the high specificity, the use of Xpert on frozen ST and IS sediment samples from both children and adults is promising.

KEY WORDS: TB diagnosis; string test; induced sputum; non-expectorating; Xpert

TUBERCULOSIS (TB) is one of the leading causes of death due to an infectious agent worldwide in both adults and children.[1,2] TB incidence and prevalence are likely to be underestimated due to the difficulty in obtaining samples among non-expectorating patients,[3] especially children, severely ill adults and patients co-infected with the human immunodeficiency virus (HIV).[4,5]

A number of less invasive but well-tolerated alternative approaches have recently been developed to improve sample collection for TB diagnosis among non-expectorating patients; these include the '*la cuerda dulce*' (sweet string)[6] method and induced sputum. The string test (ST) consists of a coiled nylon string inside a weighted gel capsule.[6] After being swallowed by a patient, the string unravels through a hole at the weighted end of the capsule as it descends into the stomach and the capsule dissolves. The string becomes coated with gastrointestinal secretions containing any pathogens present in the stomach. After retrieving the string by gently pulling, it is placed in 0.9% saline solution.[6]

Induced sputum (IS) is another method of obtaining samples for TB evaluation from non-expectorating patients. Sputum induction involves the administration of an inhaled bronchodilator and nebulised hypertonic 3–5% saline, followed by the nasopharyngeal aspiration or expectoration of mucus from the lower respiratory tract.[7] TB detection yield was recently found to be comparable between ST and SI using microscopy and culture in patients presumed to have TB but unable to produce good-quality sputum.[8,9]

The traditional gold standard for TB diagnosis in samples from all body sites is standard microbiological culture, which is cumbersome in low-resource settings due to the long culture time, expensive reagents and difficult patient follow-up. To circumvent these challenges, alternative tools to diagnose TB have recently been developed, including the Xpert® MTB/RIF test (Cepheid, Sunnyvale, CA, USA). The good performance, simplicity and short turn-around time of the Xpert test[10] make it a feasible assay for wide implementation and potentially considerable

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Article submitted 21 August 2015. Final version accepted 18 March 2016.

impact on TB control, even in resource-limited settings.[11] However, Xpert availability remains inadequate in resource-limited settings. Where it is available, the supplies of power and cartridges are often unreliable, and delays in maintenance and availability, respectively, are common. This leads to occasional crises, necessitating prolonged sample storage in some facilities and even shipment to other facilities.

Cross-sectional drug resistance surveys could be implemented with specimens collected in the field, transferred to centralised laboratories and frozen before testing. Although studies on the performance of Xpert in fresh and stored expectorated sputum samples have been published, there are few data on frozen sediments of IS and ST specimens.[12]

We evaluated the performance of the Xpert assay in frozen ST and IS sediments collected from non-expectorating paediatric and adult patients in south-western Uganda using culture of fresh sputum samples as reference.

STUDY POPULATION AND METHODS

Study setting

This was a cross-sectional laboratory substudy from a large study conducted between March 2010 and March 2013 that evaluated the use of ST and SI methods of specimen collection for TB diagnosis in children and adults unable to produce sputum at Mbarara Regional Referral Hospital in Southwestern Uganda.[8,9]

Specimen collection and laboratory procedures

As part of the main study, we collected fresh specimens using SI with 5% hypertonic saline for a maximum of 20 min and the ST with an intragastric downtime of 2 h.[8,9] The IS and ST specimens were then decontaminated using 0.5% *N*-acetyl-L-cysteine–1.5% sodium hydroxide, followed by neutralisation with phosphate buffer solution, centrifugation and inoculation into BACTEC™ MGIT™ (Mycobacterium Growth Indicator Tube; BD, Sparks, MD, USA) in Löwenstein-Jensen (LJ) medium.[8,9] The remaining sediment was frozen at –20°C for 6–36 months and used in this substudy to evaluate Xpert performance on frozen samples in comparison with the original results on fresh samples.

All laboratory analyses were carried out at the Epicentre Mbarara Research Centre Laboratory, Mbarara, Uganda. The laboratory is quality assured by the Institute of Tropical Medicine in Antwerp, Belgium; external quality assurance for culture is performed at the National Health Laboratory Service, Johannesburg, South Africa.

Data analysis

Data were imported into STATA, version 13 (StataCorp, College Station, TX, USA) for all analyses. The sensitivity and specificity of Xpert in stored ST and IS sediments using culture (LJ and/or MGIT) of fresh samples as reference were reported. Confidence levels of 95% (95%CIs) were used; $P < 0.05$ was considered statistically significant. A specimen was considered culture-positive if *Mycobacterium tuberculosis* was detected on either LJ or MGIT.

Table 1 Patient demographics

Children		Adults	
	n (%)		*n* (%)
Age, years		Age group, years	
<5	16 (18.2)	<25	16 (14.2)
⩾5	72 (81.8)	25–35	40 (35.4)
		36–45	36 (31.9)
		⩾50	21 (18.6)
Sex			
Male	40 (45.5)		61 (54)
Female	48 (54.5)		52 (46)
HIV status			
Positive	27 (30.7)		43 (31.0)
Negative	54 (61.4)		86 (63.0)
Unknown	7 (8.0)		8 (6.0)

HIV = human immunodeficiency virus.

Ethics

Sediments were obtained from participants enrolled in studies approved by the Faculty Research and Ethics Committee Institutional Review Board at Mbarara University of Science and Technology, Mbarara, and the Uganda National Council for Sciences and Technology, Kampala, Uganda. Participants gave consent to the use of products of their samples for other studies.

RESULTS

A total of 377 frozen sediments of sputum samples previously evaluated using standard *M. tuberculosis* culture (MGIT and LJ) were used in this study. The 377 frozen sediments were obtained from samples from 201 patients, 113 (56.2%) adults and 88 (43.8%) children (Table 1). Of the 377 sediments, 214 (56.8%) were from adults and 163 (43.2%) from children. Of the 214 stored adult sediments, 63 (29.4%) were confirmed culture-positive (Figure). Of the 163 sediments from children, 19 (11.7%) were confirmed culture-positive prior to sample storage.

Xpert was positive in 31/214 (14.5%) stored adult sediments. Of these, one was Xpert-positive, while the fresh specimen (IS) was culture-negative (Tables 2 and 3). Xpert was positive in 8/163 (4.9%) stored paediatric sediments. Of these, 11 were Xpert-positive, while the fresh specimens (IS) were culture-negative.

In adults, the sensitivity of Xpert was respectively 50% (95%CI 31.3–68.7) and 48.5% (95%CI 30.8–66.4) for ST and IS samples, with specificities of respectively 100% (95%CI 95.5–100) and 98.6%

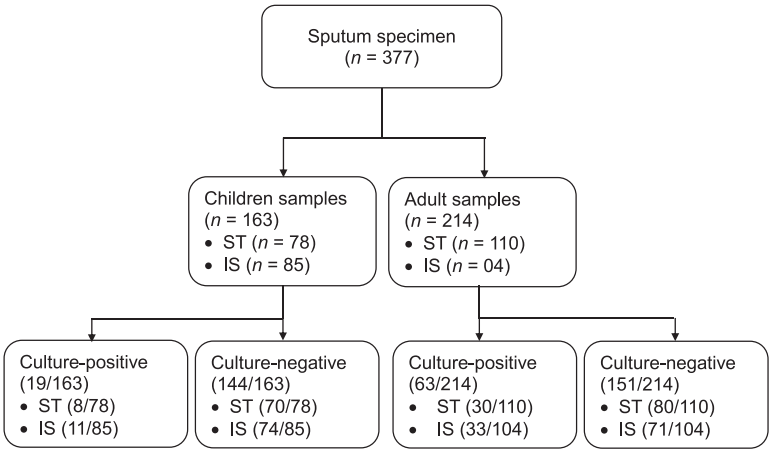

Figure Study profile. ST = string test; IS = induced sputum.

(95%CI 92.4–100). The sensitivity of Xpert in children was 37.5% (95%CI 8.5–75.5) in ST and 41.7% (95%CI 15.2–72.3) in IS samples, with specificities of respectively 100% (95%CI 94.9–100) and 98.6% (95%CI 92.7–100) (Table 4).

DISCUSSION

Our study is the first to evaluate Xpert performance in frozen ST and IS sediments from both adults and children using culture as reference. In adults, Xpert sensitivity and specificity in the frozen ST samples were close to the lower limit of the 95%CIs of pooled estimates reported by the World Health Organization (WHO) in frozen gastric fluid, at respectively 79% (95%CI 57–100) and 98.1% (95%CI 92.3–99.5).[13] Xpert had a much lower sensitivity but similar specificity to the reported WHO estimates of 88% (95%CI 84–92) and specificity of 99% (95%CI 98–99) in fresh IS specimens.[13]

In paediatric samples, Xpert sensitivity in ST was also low, with high specificity compared to pooled fresh gastric fluid estimates of respectively 66% (95%CI 51–81) and 98% (95%CI 96–99) reported in a recent Xpert meta-analysis in children.[14] Xpert in frozen IS sediments had sensitivity and specificity included in the CIs of pooled estimates of respectively 62% (95%CI 51–73) and 98% (95%CI 97–99) in fresh IS samples, as described by the WHO.[13] This suggests that Xpert performance using frozen IS samples from children is comparable to its performance using fresh samples.

The differences between our study results and results from the recent meta-analysis could be due to the nature of the samples tested (ST vs. gastric aspirates), sample conditions (frozen vs. fresh), patient characteristics (only adults unable to produce sputum were enrolled in our study population) and the very different study conditions covered by the meta-analysis compared with our study. This is the first time to our knowledge that frozen ST and IS sediments from non-expectorating patients were tested.

The overall low sensitivity of Xpert using frozen samples in this study underlines the fact that culture remains the best available reference for TB diagnosis to date, and that there is still need for improvement in TB detection using molecular methods, especially in children and adults living with HIV/acquired immune-deficiency syndrome. However, it is also well known that culture is an imperfect reference standard in children, and alternative reference standards, using retrospective case classification by an independent expert panel based on clinical, radiological, and

Table 2 Two-by-two table of Xpert® MTB/RIF and culture on all stored sediments from IS and ST specimens.

Method of collection	Children			Adult		
	Culture			Culture		
	Positive	Negative	Total	Positive	Negative	Total
Xpert on IS						
Positive	5	1	6	16	1	17
Negative	6	73	79	17	70	87
Total	11	74	85	33	71	104
Xpert on ST						
Positive	3	0	3	15	0	15
Negative	5	70	75	15	80	95
Total	8	70	78	30	80	110

ST = string test; IS = induced sputum.

Table 3 Two-by-two table of Xpert® MTB/RIF and culture on paired stored sediments from IS and ST specimens

Method of collection	Children			Adult		
	Culture			Culture		
	Positive	Negative	Total	Positive	Negative	Total
Xpert on IS						
Positive	4	0	4	16	0	16
Negative	0	60	60	8	67	75
Total	4	60	64	24	67	91
Xpert on ST						
Positive	3	0	63	14	0	14
Negative	0	61	61	8	69	77
Total	3	61	64	22	69	91

ST = string test; IS = induced sputum.

Table 4 Performance of the Xpert® MTB/RIF assay in ST and IS samples*

Method	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
Children				
IS	41.7 (15.2–72.3)	98.6 (92.7–100.0)	83.3 (35.9–99.6)	92.4 (84.2–97.2)
ST	37.5 (8.5–75.5)	100.0 (94.9–100.0)	100.0 (29.2–100.0)	93.3 (85.1–97.8)
Adults				
IS	48.5 (30.8–66.4)	98.6 (92.4–100.0)	94.1 (71.3–99.8)	80.5 (70.6–88.2)
ST	50.0 (31.3–68.7)	100.0 (95.5–100.0)	100.0 (78.2–100.0)	84.2 (75.3–90.9)

* Mycobacterial culture of fresh specimen was used as the gold standard.
ST = string test; IS = induced sputum; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

microbacteriological findings, and treatment response, are now recommended by several experts.[15] The highest Xpert specificity was observed using ST sediments compared to IS sediments in both children and adults, which could be explained by the differential impact of decontamination and lengthy storage of the two types of samples evaluated.

Further investigation of the poorer performance observed in children's samples is required, with assessment of the independent effect of decontamination and storage on Xpert performance over time. An assessment of the separate contributions of decontamination and storage conditions on Xpert performance is important in low-resource settings where Xpert is not available; this could allow health facilities to store specimens and transport them to other laboratories for testing. In addition, if reliable analysis of frozen specimens can be confirmed in future studies, this technique could be useful for research activities requiring long-term storage of sputum sediments for future studies or technology not available at sites where samples are collected.

We were not able to stratify Xpert performance using IS and ST samples according to the duration of storage, due to the small number of samples. Another limitation of our study is that we did not test Xpert performance using fresh samples, as Xpert became available at our site only at the end of the study period. Combined culture and Xpert testing of fresh samples would have been a stronger reference standard, and could potentially avoid false culture-negative results due to decontamination, especially of paucibacillary samples. It would also have been interesting to use other methods, such as sequencing, to test samples found positive on Xpert but negative on culture, and identify false culture-negative results. We have not described the clinical characteristics of the patients in this laboratory-based study, as these were published elsewhere.[8,9]

CONCLUSION

Xpert should be used with caution on stored frozen sediments, given its varying performance depending on the type of patient (children vs. adults) and the type of specimen (ST vs. IS). In our study, we found that Xpert performed better on frozen adult ST sediments. Our findings apply mainly to research operations requiring sample storage for future evaluation and/or for rapid resistance surveys using Xpert. The results of our study suggest the need to evaluate the effect of decontamination processes, storage conditions and length of storage on Xpert performance in sputum specimens from non-expectorating TB patients.

Acknowledgements

The authors thank the Mbarara University Microbiology Department staff (L Nkangi and J Mwesigye) and post-graduate students for their technical support; Médecins Sans Frontières and the Epicentre Mbarara Research Centre (Mbarara, Uganda) administration for providing us with sediments and laboratory data and for allowing us to use their laboratory; the TB laboratory staff of the Epicentre Mbarara Research Centre Laboratory, Mbarara, Uganda, for their technical support; G Mugume for technical support in informatics; the Uganda Research Student Support Fund for mentoring; and L Bebel for her critical review of the manuscript.

This work was made possible by Medical Education for Equitable Services to All Ugandans (Mbarara, Uganda), a Medical Education Partnership Initiative (Grant no 5R24TW008886) from the Office of the US Global AIDS Coordinator and the US Department of Health and Human Services (Washington DC, USA), Health Resources and Services Administration (Rockville, MD, USA) and National Institutes of Health (Bethesda, MD, USA). Additional funding was provided by the German Academic Exchange Service, DAAD (Deutscher Akademischer Austauschdienst, Bonn, Germany) (grant number A113/94049). Both grants were awarded to JA.

Conflicts of interest: none declared.

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RESUME

CADRE : Malgré l'utilisation du test Xpert® MTB/RIF pour le diagnostic de la tuberculose (TB), sa disponibilité reste insuffisante et sa performance sur des échantillons congelés à partir de patients non-expectorant reste sous-évaluée.

OBJECTIF : Evaluer la performance de l'Xpert sur des échantillons congelés d'expectoration induite (IS) et du test string (ST) de personnes non-expectorantes présumées d'avoir la TB.

MÉTHODE : Cette étude diagnostique a utilisé 377 échantillons prélevés entre mars 2010 et mars 2013 dans un hôpital de référence en Ouganda. Les échantillons étaient décontaminés, centrifugés et cultivés et ceux restants étaient congelés à –20°C avant analyse avec l'Xpert.

RÉSULTATS : La TB a été détectée par culture dans des échantillons ST et IS de 19/163 (11,7%) enfants et 63/201 (29,4%) adultes. La sensibilité de l'Xpert sur les sédiments congelés d'enfants était de 37,5% (IC95% 8,5–75,5) pour les ST et 41,7% (IC95% 15,2–72,3) pour les IS, avec les spécificités de 100,0% (IC95% 94,9–100,0) et 98,6% (IC95% 92,7–100,0), respectivement. Chez les adultes, elles étaient de 50,0% (IC95% 31,3–68,7) et 48,5% (IC95% 30,8–66,4) pour ST et IS, respectivement, avec des spécificités de 100% (IC95% 95,5–100,0) et 98,6% (IC95% 92,4–100,0), respectivement.

CONCLUSION : Sur la base de ces résultats, en particulier sa spécificité, l'utilisation de l'Xpert sur les échantillons congelés de ST et d'IS est prometteuse.

RESUMEN

MARCO DE REFERENCIA: Pese al despliegue de la prueba Xpert MTB/RIF® para el diagnóstico de la tuberculosis (TB), su disponibilidad sigue siendo insuficiente en los entornos con bajos recursos y no se ha investigado a cabalidad la precisión de la prueba en las muestras congeladas de los pacientes que no expectoran.

OBJETIVO: Evaluar el rendimiento diagnóstico de la prueba Xpert en muestras congeladas recogidas mediante la inducción del esputo (IS) y la prueba de la cuerda dulce (ST), en pacientes con presunción clínica de TB que no expectoraban.

MÉTODO: Se utilizaron en el estudio 377 muestras congeladas, recogidas mediante la ST y el IS de marzo del 2010 a marzo del 2013 en un hospital de referencia de Uganda. Las muestras se descontaminaron, se centrifugaron y una parte se puso en cultivo y el remanente se congeló y se conservó a –20°C hasta la realización de la prueba Xpert.

RESULTADOS: Mediante los métodos de cultivo se había detectado la TB en 19/163 (11,7%) muestras obtenidas por ambos métodos en niños y en 63/201 (29,4%) muestras de adultos. La sensibilidad de la prueba Xpert en los sedimentos congelados provenientes de niños fue 37,5% en las muestras obtenidas con la ST (IC95% 8,5–75,5) y 41,7% en las muestras IS (IC95% 15,2–72,3); la especificidad fue 100,0% en las muestras obtenidas con la ST (IC95% 94,9–100,0) y 98,6% en las muestras de IS (IC95% 92,7–100,0). En los adultos la sensibilidad fue 50,0% con la ST (IC95% 31,3–68,7) y 48,5% con el IS (IC95% 30,8–66,4) y la especificidad fue 100,0% (IC95% 95,5–100,0) y 98,6% (IC95% 92,4–100,0), respectivamente.

CONCLUSIÓN: A la luz de estos resultados, en especial de la especificidad, la utilización de la prueba Xpert en muestras congeladas obtenidas mediante la ST o la IS aparece como un método promisorio en los niños y los adultos.