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Sensitive and Feasible Specimen Collection and Testing Strategies for Diagnosing Tuberculosis in Young Children

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IMPORTANCE Criterion-standard specimens for tuberculosis diagnosis in young children, gastric aspirate (GA) and induced sputum, are invasive and rarely collected in resource-limited settings. A far less invasive approach to tuberculosis diagnostic testing in children younger than 5 years as sensitive as current reference standards is important to identify.

OBJECTIVE To characterize the sensitivity of preferably minimally invasive specimen and assay combinations relative to maximum observed yield from all specimens and assays combined.

DESIGN, SETTING, AND PARTICIPANTS In this prospective cross-sectional diagnostic study, the reference standard was a panel of up to 2 samples of each of 6 specimen types tested for *Mycobacterium tuberculosis* complex by Xpert MTB/RIF assay and mycobacteria growth indicator tube culture. Multiple different combinations of specimens and tests were evaluated as index tests. A consecutive series of children was recruited from inpatient and outpatient settings in Kisumu County, Kenya, between October 2013 and August 2015. Participants were children younger than 5 years who had symptoms of tuberculosis (unexplained cough, fever, malnutrition) and parenchymal abnormality on chest radiography or who had cervical lymphadenopathy. Children with 1 or more evaluable specimen for 4 or more primary study specimen types were included in the analysis. Data were analyzed from February 2015 to October 2020.

MAIN OUTCOMES AND MEASURES Cumulative and incremental diagnostic yield of combinations of specimen types and tests relative to the maximum observed yield.

RESULTS Of the 300 enrolled children, the median (interquartile range) age was 2.0 (1.0-3.6) years, and 151 (50.3%) were female. A total of 294 met criteria for analysis. Of 31 participants with confirmed tuberculosis (maximum observed yield), 24 (sensitivity, 77%; interdecile range, 68%-87%) had positive results on up to 2 GA samples and 20 (sensitivity, 64%; interdecile range, 53%-76%) had positive test results on up to 2 induced sputum samples. The yields of 2 nasopharyngeal aspirate (NPA) samples (23 of 31 [sensitivity, 74%; interdecile range, 64%-84%]), of 1 NPA sample and 1 stool sample (22 of 31 [sensitivity, 71%; interdecile range, 60%-81%]), or of 1 NPA sample and 1 urine sample (21.5 of 31 [sensitivity, 69%; interdecile range, 58%-80%]) were similar to reference-standard specimens. Combining up to 2 each of GA and NPA samples had an average yield of 90% (28 of 31).

CONCLUSIONS AND RELEVANCE NPA, in duplicate or in combination with stool or urine specimens, was readily obtainable and had diagnostic yield comparable with reference-standard specimens. This combination could improve tuberculosis diagnosis among children in resource-limited settings. Combining GA and NPA had greater yield than that of the current reference standards and may be useful in certain clinical and research settings.

Supplemental content

Editorial

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Tuberculosis is the leading infectious cause of death globally.[1] The risk of tuberculosis infection progressing to disease and from disease to death is especially high for young children.[2,3] The World Health Organization estimates that globally 230 000 children younger than 15 years died of tuberculosis in 2020.[1,4] Tuberculosis-related mortality rates among infants and children younger than 5 years are approximately 9-fold higher than in children aged 5 to 15 years, and these younger children account for almost 80% of all tuberculosis-related deaths in children 15 years and younger.[4] However, detecting tuberculosis among children is challenging, and the vast majority of these deaths (96%) are among children not receiving treatment.[4] Reductions in child tuberculosis-related mortality may be feasible with improved diagnostic methods.

Pediatric tuberculosis is often paucibacillary, making microbial confirmation of tuberculosis disease challenging. Additionally, expectorated sputum, the primary diagnostic specimen for tuberculosis in adults, cannot be obtained for most young children.[5] Aspiration of gastric fluid (GA) and suctioning following sputum induction (IS) are typically recommended for obtaining pediatric specimens for tuberculosis diagnosis.[6,7] However, both these approaches are invasive, and collection of GA generally requires hospitalization for specimen collection.[7,8] Therefore, few children (estimated 30% to 40%) with tuberculosis have disease confirmed by mycobacterial culture even in ideal settings.[9,10] This also hampers scientific research advances in tuberculosis treatment and prevention in children, as the optimal diagnostic approach for measuring an adequate end point in clinical treatment and vaccine trials in children is unknown.[11,12] The self-contained nucleic acid amplification test, Xpert MTB/RIF (Cepheid), provides an attractive alternative to mycobacterial culture in many resource-limited settings with limited laboratory infrastructure.[13] Since 2013, the World Health Organization has endorsed Xpert MTB/RIF as a first-line test for diagnosing tuberculosis in children.[13-15] However, the challenge of collecting an appropriate specimen type from children to test remains.

Several pediatric studies have explored the diagnostic yield of individual specimen types, such as nasopharyngeal aspirate (NPA), stool, and string tests (ST), and compared yield of these alternative specimens with reference-standard specimens by Xpert MTB/RIF or mycobacterial culture.[16-21] However, comparisons were generally pair-wise or included few specimen types. The key question yet to be answered is what combinations of specimens, ideally minimally invasive, provides the highest yield for bacteriologic diagnosis of tuberculosis in young children. We aimed to rigorously answer that question by measuring yield and test sensitivity for a comprehensive range of specimen types and bacteriologic tests in a cohort of HIV-positive and HIV-negative children younger than 5 years.

Key Points

Question What combinations of specimens, ideally minimally invasive, provide the highest yield for bacteriologic diagnosis of tuberculosis in young children?

Findings In this diagnostic study of 300 children, the yield of 2 nasopharyngeal aspirate (NPA) samples (74%), of 1 NPA sample and 1 stool sample (71%), and of 1 NPA sample and 1 urine sample (69%) was similar to the reference-standard samples (up to 2 GA samples [77%] and up to 2 sputum induction samples [64%]), which are more difficult to collect.

Meaning These combinations of specimens could improve and facilitate tuberculosis diagnosis among children younger than 5 years in resource-limited settings.

Methods

Study Participants

In this prospective diagnostic cross-sectional study, we recruited a consecutive series of children younger than 5 years who had persistent cough, fever, or malnutrition despite therapy for other common illnesses and lung parenchymal abnormality on chest radiography or who had persistent cervical lymphadenopathy between October 2013 and August 2015 from inpatient and outpatient settings in Kisumu County, Kenya. Details of study recruitment, eligibility criteria, and enrollment procedures are described in the eMethods in the Supplement. This study was approved by the institutional review boards of the US Centers for Disease Control and Prevention, the Kenya Medical Research Institute, and the Jaramogi Oginga Odinga Teaching and Referral Hospital. Children's Hospital Boston/Harvard Medical School relied on the review and oversight of the Centers for Disease Control and Prevention institutional review board. Written informed consent was obtained by parents or legal guardians of participants. This study followed the Standards for Reporting of Diagnostic Accuracy (STARD) reporting guideline.

Specimen Collection and Testing Procedures

Study nurses and clinical officers collected specimens over 3 days and 2 nights for each child. The primary study specimen panel for testing with both Xpert MTB/RIF and mycobacteria growth indicator tube (MGIT) included 2 each of GA, NPA, IS, ST, and stool specimens and up to 2 urine specimens. In addition, 2 cervical lymph node fine-needle aspirate (FNA) were collected if indicated (ie, met enrollment criteria for cervical lymphadenopathy). A single blood sample was tested by BD BACTEC (Becton, Dickinson and Company) only. Cutoff values of MGIT and Xpert MTB/RIF do not apply; standard methods were used to determine positive results. Positive MGIT or Xpert MTB/RIF results on any of the primary study specimens were used to define the reference standard because this represents a comprehensive approach to bacteriologic detection of *Mycobacterium tuberculosis*. Different combinations of specimens and MGIT and Xpert MTB/RIF were evaluated as index tests. Clinical data were not available to laboratory staff; because index and reference tests are defined as different combinations of the same set of samples and tests, laboratory staff did have access to results of both reference and index tests. We evaluated the diagnostic yield of specimens and tests and discontinued testing for specimen types with low yield. Details of specimen collection, processing, and testing are described in the eMethods in the Supplement.

Table. Characteristics of Children Tested for Tuberculosis Between October 2013 and August 2015 in Kisumu County, Kenya, by HIV Status[a]

Characteristic	No. (%)		
	HIV positive (n = 73)	HIV negative (n = 223)	Total (N = 300)
Age at enrollment, median (IQR), y	2.1 (0.9-3.4)	2.0 (1.1-3.7)	2.0 (1.0-3.6)
Age group, y			
<1	19 (26.0)	52 (23.3)	73 (24.3)
1-<2	16 (21.9)	57 (25.6)	73 (24.3)
2-5	38 (52.1)	114 (51.1)	154 (51.3)
Female	36 (49.3)	113 (50.7)	151 (50.3)
HIV viral load, median (IQR), thousands of copies/mL[b]	350 (32-1200)	NA	NA
CD4, median (IQR), %[c]	18 (13-25)	NA	NA
Immunodeficiency staging[c,d]			
None	11 (17.2)	NA	NA
Mild	11 (17.2)	NA	NA
Moderate	8 (12.5)	NA	NA
Severe	34 (53.1)	NA	NA

Abbreviations: IQR, interquartile range; NA, not applicable.

[a] Four children had unknown HIV status and are not included in the distributions of characteristics by HIV status.

[b] n = 55.

[c] n = 64.

[d] Using definitions from the World Health Organization.[23]

Clinical Care

Children were treated for tuberculosis or provided isoniazid preventive therapy according to the primary treating clinician. Participants were followed-up with at 2 weeks, 2 months, and 6 months.

Statistical Analysis

The analysis of cumulative and incremental yield included only participants with at least 1 evaluable specimen for at least 4 primary study specimen types. Confirmed tuberculosis was defined as having at least 1 Xpert MTB/RIF or MGIT result positive for *M tuberculosis* complex for at least 1 primary study specimen (maximum observed yield). Using this confirmed case definition as the reference, we performed resampling to estimate the sensitivity and yield of the various specimen types and tests alone and in combination (index tests), reported as resampling means and an interdecile range (80% interval between first and ninth decile) of the resampling distribution to represent variability in the estimation procedure. Sensitivity was estimated as the number of children with positive test results from the candidate index combination divided by the number of children with positive test results on the reference panel; difference in sensitivity was calculated as the arithmetic difference of the 2 measures. Statistical variability was assessed using resampling and reporting of interdecile ranges. Indeterminate test results were subjected to rigorous quality review and were analyzed as not positive, since this study focuses on yield. Our primary analysis of sensitivity of specimen types and their combinations included Xpert MTB/RIF and MGIT results in combination; if either was positive, the result of testing for that specimen was positive (eMethods in the Supplement). We determined that a sample size of 290 would allow us to detect a difference of 10% in the sensitivity of paired tests, based on the following assumptions: test 1 has sensitivity of 30%; test 2 has sensitivity of 21%; type I error set at 5%; type II error set at 20%; and the sensitivity of test 2 increases by 5% among persons with positive results on test 1.[22]

Results

Participants

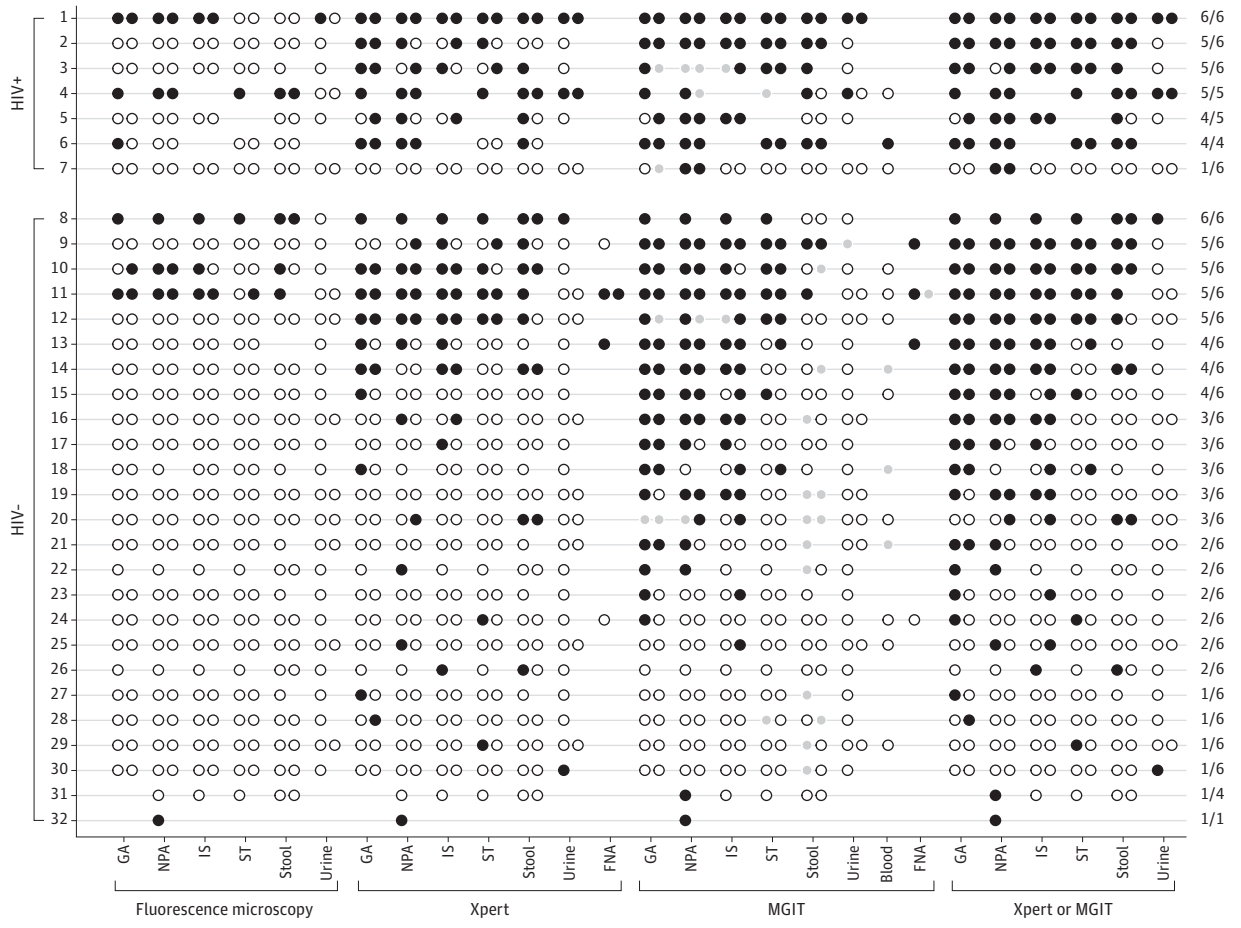
Of the 300 children enrolled in the study, 294 met criteria for the analysis of cumulative and incremental yield. Children were aged 1 to 47 months; the median (interquartile range) age was 2.0 (1.0-3.6) years, and 151 of 300 children (50.3%) were female. Of the 300 children, 73 (24.3%) were HIV positive, 223 (74.3%) were HIV negative, and 4 (1.3%) had unknown HIV status (**Table**). We reduced the number of urine and blood specimens collected based on interim review of assay yields. Of the first 138 participants enrolled, only 2 had a positive Xpert MTB/RIF or MGIT result on the second urine specimen; both were also positive on the first urine specimen. We therefore only collected and tested 1 urine specimen for each of the remaining participants. Of the first 135 participants, only 1 had a blood specimen with positive MGIT result, whereas 96 had negative results, 20 were contaminated, and 18 were not evaluable. We therefore discontinued collecting and culturing blood specimens.

Of the 32 children who tested positive for tuberculosis by either Xpert MTB/RIF or MGIT performed on any of the primary study specimens, 27 (84%) had at least 1 GA, IS, NPA, ST, stool, and urine sample tested by Xpert MTB/RIF, MGIT, or both (**Figure 1**). Among those with confirmed tuberculosis, 14 (44%) had blood collected for MGIT and 4 (13%) had lymph node FNA specimens collected. One patient with confirmed tuberculosis had only NPA collected; this participant was excluded from analysis of cumulative and incremental yield. Of the primary specimens tested, GA, NPA, and IS demonstrated the highest yield (**Figure 2**).

Yield of Individual Specimen Types

Among the 294 children eligible for analysis of cumulative and incremental yield, 31 had confirmed tuberculosis (confirmed cases). Testing of up to 2 of each specimen type by Xpert MTB/

Figure 1. Participant-Level Mycobacterial Results for 32 Children Tested for Tuberculosis Between October 2013 and August 2015 in Kisumu County, Kenya



Dot plot of participant-level results for fluorescence microscopy, Xpert MTB/RIF (Cepheid), and mycobacteria growth indicator tube (MGIT) by sample type and sample number. Up to 2 samples of each type were tested per participant. Black circles indicate a positive result, gray circles indicate an invalid (Xpert MTB/RIF) or contaminated (MGIT) result, and white circles indicate a negative result. The top 7 rows are results for participants with HIV. The column on the right shows the number of positive results by Xpert MTB/RIF or MGIT over the number of evaluable specimens. Participants are sorted vertically in descending order of number of testing results available. FNA indicates fine-needle aspiration; GA, gastric aspirate; IS, induced sputum; NPA, nasopharyngeal aspirate; ST, string test.

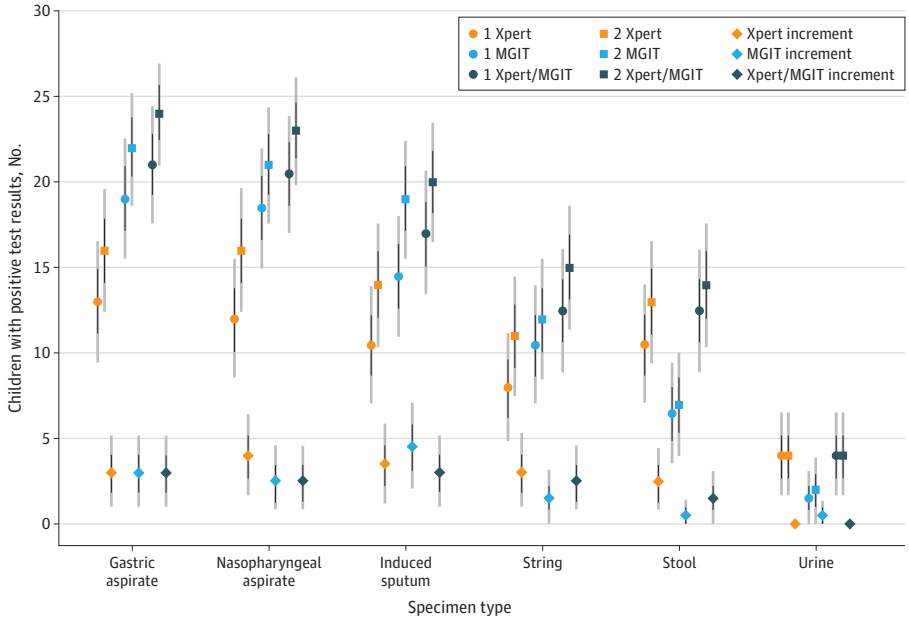
RIF and/or MGIT resulted in an average yield of 24 of 31 children with positive test results (sensitivity, 77%; interdecile range, 68%-87%) for GA, 23 of 31 with positive test results (sensitivity, 74%; interdecile range, 64%-84%) for NPA, and 20 of 31 with positive test results (sensitivity, 64%; interdecile range, 53%-76%) for IS. The performance of ST and stool specimens was lower, with an average of 15 of 31 children with positive test results (sensitivity, 48%; interdecile range, 37%-60%) for ST, 14 of 31 with positive test results (sensitivity, 45%; interdecile range, 33%-57%) for stool, and 4 of 31 with positive test results (sensitivity, 13%; interdecile range, 5%-21%) for urine.

Figure 2 shows the yields of testing each specimen by Xpert MTB/RIF only, MGIT only, or both Xpert MTB/RIF and MGIT among children with confirmed tuberculosis. The average incremental yield for testing up to 2 of each of GA, NPA, or IS specimens by Xpert MTB/RIF or MGIT was an additional 3 children with positive test results (sensitivity, 10%; interdecile range, 3%-17%) for GA, 2.5 with positive test results (sensitivity, 8%; interdecile range, 3%-15%) for NPA, and 3 with positive test results (sensitivity, 10%; interdecile range, 3%-17%) for IS relative to testing only 1 specimen. The number of positive bacteriologic test results increased with the number of specimens tested, although the relative additional yield decreased with increasing number of specimens (**Figure 3**) (eFigure in the Supplement). Yield tended to be higher on the first specimen for GA, NPA, and stool, although we cannot rule out chance as a reason for the difference. In contrast, for IS, yield was consistently higher for the second specimen (eTable 1 in the Supplement). Lymph node FNA had high yield among children with confirmed tuberculosis; 3 of 4 children had a positive Xpert MTB/RIF or MGIT result.

Yield of Combinations of All Specimen Types

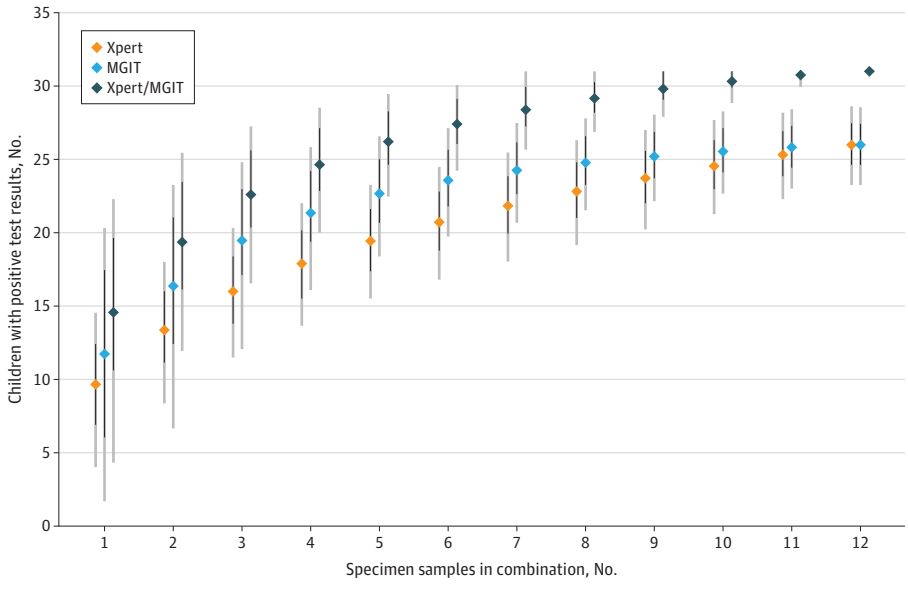
Starting from a baseline average yield from Xpert MTB/RIF and/or MGIT of 21 children with positive test results (1 GA; sensitivity, 68%; interdecile range, 57%-79%) or 20.5 with posi-

Figure 2. Cumulative and Incremental Diagnostic Yield by Specimen Type Among 31 Children With Tuberculosis



The cumulative and incremental yield of each specimen type among 31 analyzable participants is depicted as 3 groups of 3 bars: yield of 1 specimen, yield of 2 specimens, and the incremental yield of the second specimen when added to the first; specimens were tested by Xpert MTB/RIF (Cepheid) alone, mycobacteria growth indicator tube (MGIT) alone, and both Xpert MTB/RIF and MGIT. The points depict average yield over the resampling distribution, black bars represent the interquartile range, and gray bars represent the interdecile range (10th to 90th centile). The specimen types are arranged in decreasing order of yield.

Figure 3. Average Cumulative Yield of Confirmed Cases by Number of Specimen Samples Among 31 Children With Confirmed Tuberculosis



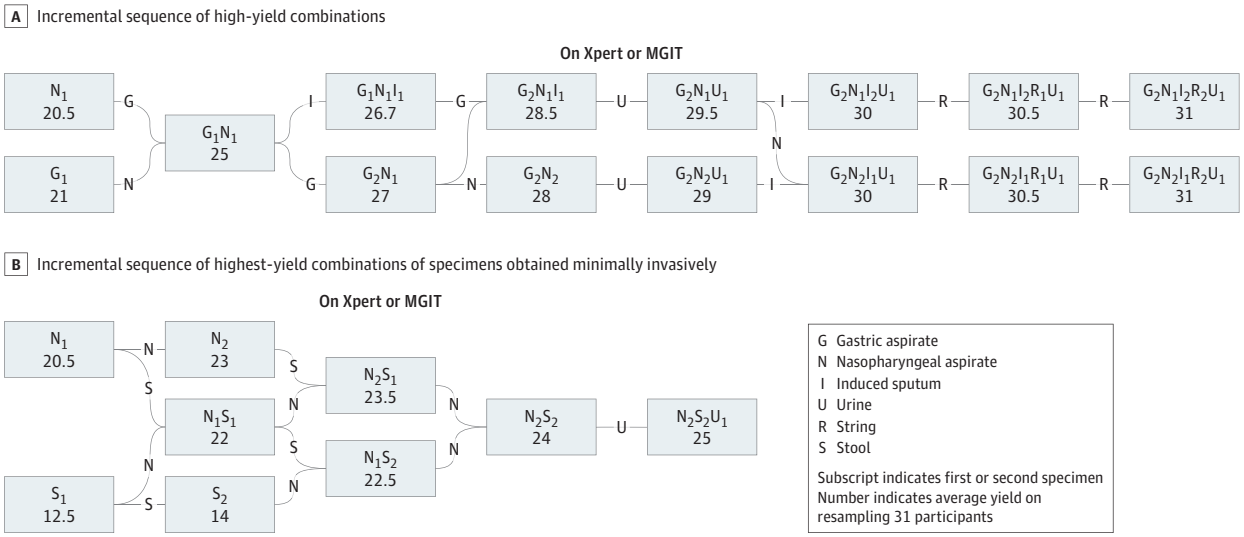
The average yield for 31 analyzable participants over all combinations of specimens is depicted in groups of 3 for all combinations of each number of specimens from 1 to 12: yield for Xpert MTB/RIF (Cepheid) alone, yield for mycobacteria growth indicator tube (MGIT) alone, and yield for both Xpert MTB/RIF and MGIT. The points depict average yield over the resampling distribution, black bars represent the interquartile range, and gray bars represent the interdecile range (10th to 90th centile).

tive test results (1 NPA; sensitivity, 66%; interdecile range, 55%-77%), combining these 2 specimens (ie, 1 GA and 1 NPA) increased yield to 25 of 31 with positive test results (sensitivity, 81%; interdecile range, 71%-90%). Stepwise additions improved yield further: for example, the average yield for up to 2 NPA and 2 GA samples was 28 of 31 with positive test results (sensitivity, 90%; interdecile range, 83%-97%), and adding IS and urine samples increased yield to 30 of 31 (sensitivity, 97%; interdecile range, 92%-100%) (**Figure 4**A). A minimum of 8 specimens was needed for an average yield of 31 with positive test results (100%; 2 example combinations shown in Figure 4A).

Yield of Combinations of Minimally Invasive Specimen Types

When considering only minimally invasive specimens (NPA, stool, and urine) tested by Xpert MTB/RIF and/or MGIT, the average yield of 1 NPA specimen (20.5 of 31 children with positive test results; sensitivity, 66%; interdecile range, 55%-77%) could be further increased with testing up to 2 NPA specimens (23 of 31 with positive test results; sensitivity, 74%;

Figure 4. Incremental Sequence of High-Yield Combinations and Highest-Diagnostic-Yield Combinations for Testing Minimally Invasive Specimen Types From Children for Tuberculosis



Boxes show the average diagnostic yield on resampling participants bacteriologic test results (Xpert MTB/RIF [Cepheid] or mycobacteria growth indicator tube [MGIT]), with corresponding specimens and number of each specimen. The boxes on the left show results for single specimens with the highest yield. Connecting lines between boxes indicate which specimen type is added for the next box in the series. At each step, the specimen or specimens that contribute the greatest number of additional positive bacteriologic results is shown.

interdecile range, 64%-84%) or with also testing stool (22 of 31 with positive test results; sensitivity, 71%; interdecile range, 60%-81%) or urine (21.5 of 31 with positive test results; sensitivity, 69%; interdecile range, 58%-80%). Further testing restricted to these 3 specimens alone yielded an average of 25 of 31 children with positive test results (sensitivity, 81%; interdecile range, 71%-90%) (Figure 4B). Yield of these minimally invasive specimen combinations was comparable with that of current criterion standards (2 GA: 24 of 31 with positive test results; sensitivity, 77%; interdecile range, 68%-87%; 2 IS: 20 of 31 with positive test results; sensitivity, 64%; interdecile range, 53%-76%).

Yield of MGIT Testing vs Xpert MTB/RIF

Total yield from MGIT was higher than Xpert MTB/RIF for all specimen types except stool and urine. Among children with confirmed tuberculosis from primary study specimens, 13 had tuberculosis-positive stool samples via Xpert MTB/RIF (sensitivity, 42%; interdecile range, 30%-53%) compared with 7 via MGIT (sensitivity, 22%; interdecile range, 13%-32%). A total of 4 children had tuberculosis-positive urine samples via Xpert MTB/RIF (sensitivity, 13%; interdecile range, 5%-21%) compared with 2 children via MGIT (sensitivity, 6%; interdecile range, 0%-13%). One child had a single tuberculosis-positive urine Xpert MTB/RIF result, which was the only positive bacteriologic test result from any specimen. Results of each test and each specimen among children with a positive Xpert MTB/RIF or MGIT result on at least 1 sample of any type are shown in Figure 1 and are stratified by HIV status in eTable 2 in the Supplement. When tested by Xpert MTB/RIF alone, an average of 16 of 31 children with positive test results (sensitivity, 52%; interdecile range, 40%-63%) were identified with up to 2 NPA samples, 15 of 31 with positive test results (sensitivity, 48%; interdecile range, 37%-60%) were identified with 1 NPA sample and 1 stool sample, and 13 of 31 with positive test results (sensitivity, 42%; interdecile range, 31%-53%) were identified with 1 NPA sample and 1 urine sample. Yield was consistently higher among children with HIV, with 90% of pseudosample values exceeding 0 for NPA, ST, and stool specimens (eTable 3 in the Supplement).

Discussion

We identified combinations of minimally invasive specimen types with bacteriologic yields comparable with that of reference-standard specimens that are more invasive and less readily available. Specifically, we found that testing 2 NPA samples or 1 NPA sample plus 1 stool sample had similar bacteriologic yield to testing 2 GA or 2 IS samples. These combinations are a novel, less-invasive diagnostic approach for children in standard care settings. Additionally, we identified combinations of specimen types with potentially higher sensitivity than current standards, although these combinations include standard specimen types that are more invasive. These combinations could be appropriate for clinical trials of vaccines or antimicrobials and for complex clinical cases in which bacteriologic confirmation is especially important (for example, for patients with potential drug resistance). Our findings for NPA and stool samples are further supported by data from 4 countries that tested standard specimens (GA and expectorated sputum) and alternative specimens (including NPA and stool) from children with HIV younger than 13 years (median age, 7.2 years).[19]

In our study, bacteriologic yield from the combination of NPA and stool samples was similar to that of NPA alone. However, stool is easily obtainable and increasingly of interest as a diagnostic specimen.[19,24-27] Stool is worth further consideration particularly because its value may increase with a more sensitive test, such as Xpert MTB/RIF Ultra. For stool samples, yield of Xpert MTB/RIF testing was higher than yield of MGIT. Although MGIT may approximate a more sensitive Xpert MTB/RIF modality for other specimen types, it would substantially underestimate testing yield for stool samples. A limitation of methods for testing stool by Xpert MTB/RIF is the need to centrifuge specimens, which requires relatively well-equipped laboratories.[18,19] Additional efforts to simplify use of stool processing for nucleic acid testing may also improve its utility.[28,29]

Urine samples alone had very low yield, but 1 participant had positive test results on a single urine sample via Xpert MTB/RIF, which was the only positive bacteriologic test result from any specimen type. This suggests that bacteriologic diagnosis by urine alone is not a common finding but that urine may add incremental value in combination with other specimen types.

Among children with confirmed tuberculosis, only 4 had cervical lymphadenopathy amenable to aspiration. Consistent with previous reports, the yield by both Xpert MTB/RIF and MGIT was high, suggesting that obtaining lymph node FNA samples should be considered for children with tuberculosis symptoms and with large peripheral lymph nodes.[30,31] Testing both ST and GA samples increased confirmed tuberculosis cases by an average of only 0.5, suggesting that adding ST to the standard GA procedure would not effectively improve yield. However, since the combined nasogastric tube and ST device enables the collection of 2 specimens at different times through 1 device placement procedure, this combination may be considered in situations where GA is being performed, especially if advances in sample processing and testing improve yield.

A total of 5 children tested positive by Xpert MTB/RIF only and not MGIT (2 GA samples and 1 sample each of ST, stool, and urine). All specimens collected as part of the standard diagnostic set were processed and then split between Xpert MTB/RIF and MGIT. Because childhood tuberculosis is generally paucibacillary and *M tuberculosis* complex bacteria frequently clump together, it is possible that bacteria within samples were not adequately distributed into both Xpert MTB/RIF and MGIT. Another explanation for this finding is that bacteria detected by Xpert MTB/RIF were nonviable and therefore did not grow in MGIT. Nonviable bacteria could result from laboratory decontamination processing or by gastrointestinal tract transit (affecting stool and possibly GA and ST). Finally, these results could be false-positive; however, reported specificity of Xpert MTB/RIF for sputum is greater than 99%, and all enrolled children had clinical findings consistent with tuberculosis.[32] In general, yield of Xpert MTB/RIF and MGIT testing was higher among HIV-positive children than HIV-negative children; however, our study included only 7 HIV-positive children with confirmed tuberculosis.

Limitations

Our study had several limitations. Children enrolled may not be representative of the wide spectrum of tuberculosis presentation in children, particularly for those with early disease without pulmonary involvement. However, children included in this study did have symptoms consistent with indicators for a tuberculosis diagnostic evaluation per current international guidelines.[6] MGIT outperformed Xpert MTB/RIF on 4 of 6 primary specimen types, but MGIT is unlikely to be used broadly in most settings because of the required laboratory capacity. However, results of MGIT testing obtained in this study approximate the yield of molecular tests (eg, Xpert MTB/RIF Ultra) that aim for sensitivity comparable with MGIT. The number of children with confirmed tuberculosis was relatively small, but with more than 4 specimens collected per participant, this is, to our knowledge, the most comprehensive study of its kind. The resampling analysis allows us to use the maximum amount of information from this set of children and gives us confidence that many of our observed differences in yield and sensitivity need not be attributed to chance. Additionally, this study was conducted at a single site, and therefore, we are not able to assess reproducibility of results. Most children with bacteriologically confirmed tuberculosis were identified using a limited number of samples, but 8 samples were needed to identify all of these children. It is possible that other combinations (eg, up to 3 of some sample types) could achieve similar results; however, we are not able to assess this in this study.

Conclusions

A simple, sensitive approach to tuberculosis diagnosis in young children is needed and could save many lives. We found that testing 2 NPA specimens or 1 NPA specimen plus 1 stool specimen is simpler than current standards and is equally sensitive. The availability of Xpert MTB/RIF Ultra or a similar molecular modality with sensitivity similar to MGIT could further increase the diagnostic value of these specimen combinations.

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Author Contributions: Drs Heilig and Smith had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Song and Click contributed equally to this work.

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