Supplemental Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

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eMethods.

Study Participants
Children with signs and symptoms of tuberculosis and who were potentially eligible for the study were identified through pre-screening by study nurses and clinical officers at one central hospital and three district hospitals in Kisumu County, Kenya. Children were recruited from inpatient departments, outpatient tuberculosis and HIV clinics, and from households of adults who were diagnosed with laboratory-confirmed tuberculosis (positive result for smear microscopy or Xpert).

Baseline Study Procedures
Participants were admitted to the hospital inpatient ward for 3 days for baseline clinical data collection and specimen collection. Analog chest radiographs were reviewed by study clinicians, and, where available, digital chest radiographs (anteroposterior and lateral) were reviewed by a radiologist to confirm parenchymal abnormalities. HIV testing was performed according to the Kenya national guidelines. Two separate rapid serologic tests were used for children aged ≥18 months. Dried blood spot DNA polymerase chain reaction was used for children aged <18 months with documented HIV exposure (those with HIV-positive mothers or infants with a positive rapid HIV serologic test).

Enrolment Criteria
Study clinical officers confirmed participant eligibility. Children aged <5 years, who weighed >2.5 kg, and who met the following criteria were eligible for the study. Participants had to have a visible cervical lymph node mass measuring >1 cm × 1 cm and persisting for >1 month despite antibiotic therapy for at least 5 days or a parenchymal abnormality on chest radiograph in addition to at least one of the following symptoms: 1) cough or wheezing >4 weeks not resolved after treatment with antibiotics, with cough continuing for at least 2 weeks after starting antibiotics (for hospitalized children only, respiratory distress or diagnosis of severe pneumonia not responding to antibiotics after 5 days or any cough >4 weeks despite at least 5 days of antibiotics), 2) moderate or severe malnutrition (defined as weight-for-height Z score <-2 standard deviations (SD) or -3SD, respectively) not responding after 3 weeks of treatment for malnutrition, and 3) reported fever >7 days not responding after 5 days of antibiotics or antimalarials. Children were excluded from the study if they were currently on tuberculosis treatment or isoniazid preventive therapy (IPT) or had received treatment for tuberculosis in the past year or IPT in the last 6 months.

Specimen Collection
Respiratory specimens were collected in the following order: nasopharyngeal aspirate (NPA), induced sputum (IS), and gastric aspirate combined with the string test (GA/ST). A minimum of 3 hours separated NPA from IS collection and IS collection from GA/ST collection. All respiratory specimens were collected at night, before early morning and first meals of the day. Blood was collected for the QuantiFERON-TB (QFT) assay (Cellestis/Qiagen, Carnegie, Australia) on the first day of hospitalization before placement of the tuberculin skin test. An additional 3– 5 mL of blood was collected directly into Myco/F Lytic bottles (Becton Dickinson, Franklin Lakes, NJ) for mycobacterial culture. Urine and stool specimens were collected whenever available each day during admission.

NPA specimens were collected from all children aged >2 months by placing two drops of saline in each nostril, inserting a 7 or 8 French tubing connected to a mucus trap into one nostril, with length of tubing inserted equal to the distance between the tip of the nose to the lacrimal duct and withdrawing the tubing slowly while suctioning. IS was performed after at least 3 hours (if child received primarily breastmilk or formula) or 4 hours (if on solid food) fasting. One puff albuterol (100 µg/dose) was administered by a metered dose inhaler into a face mask; after 10 minutes, 5 mL hypertonic saline (5%) was administered by nebulization for 15 minutes or until the full volume were nebulized or until the child began coughing. Suction tubing attached to a suction machine was then placed into one nostril the distance from the tip of the nose to the tragus of the ear to obtain sputum from the nasopharynx. Sputum induction was repeated once if the participant did not cough or produce sputum on the first attempt. Oxygen saturation level (SaO_2) was monitored during the IS procedure; sputum induction was deferred if baseline SaO_2 was <92% or if there was evidence of respiratory distress. The procedure was terminated for participants with SaO_2 <90% or respiratory distress.

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Because young children frequently cannot swallow an encapsulated string test, GA and string test specimens were collected using a combined naso-gastric tube and string test (CNGTST) device (Grupo Tuberculosis Valle-Colorado, Medellin, Colombia), which comprises a single-lumen small-bore polyvinylchloride nasogastric tube (NGT, size 6 or 8) with four lateral eyelets and a 100% nylon textured light-weight 100-cm thread (denier count 220 Td) wrapped around the external surface of the NGT. The CNGTST device was placed after a minimum of 3 hours (if child received primarily breastmilk or formula) or 4 hours (if on solid food) fasting. Participants were immobilized using a papoose board; the CNGTST was inserted through the nose to the stomach and secured. Gastric contents were manually aspirated using a 20-mL syringe; the procedure was repeated with the participant in the left and right decubitus positions if required to obtain adequate volume. If <3 mL GA was obtained, the procedure was repeated. Gastric lavage was not performed. After specimen collection, the pH of the GA specimen was measured, adjusted to pH 6.5-7.0 by adding 1.5-mL increments of a 2.5% bicarbonate solution until neutralized. The combined GA/ST apparatus was left in place for 2–3 hours prior to removal of the string; dwelling time was recorded, and strings were processed regardless of actual dwelling time. Using a sterile technique, the string was removed from the nasogastric tube by milking it down to the distal end, then cutting and placing it into a tube with five drops of sterile water.

Lymph node aspirate (LNA) specimens were obtained from children with enlarged cervical lymph nodes using a 23- or 25-gauge needle; two needle passes were used. Two smears were prepared from each needle. The remaining material was inoculated immediately into tuberculosis transport bottles (TTB) containing 2.0 mL of 7H9 broth.[1,2] Urine specimens were collected via collection bags or directly into collection cups. Stools were collected from diapers or diaper pads. All nonsterile specimens were transported to the Kenya Medical Research Institute (KEMRI) tuberculosis reference laboratory at 2°C–8°C using a cold chain transport system within 3 hours of collection. Thermometer logbooks were maintained and reviewed for each transport. LNA inoculated into TTB and Myco/F Lytic culture bottles were forwarded to the laboratory at ambient temperature.

Specimen Processing for Smear Microscopy, Nucleic Acid Amplification (NAA), and Culture

The pH of the GA specimens was measured and confirmed as neutral (pH 6.5–7) before processing and further adjusted if not neutral. Specimens of all types with volume >10 mL were concentrated before processing by centrifuging at $\geq$3,000 × g at 4°C for 10 minutes and decanting to a volume of 5 mL. Stool specimens were prepared for processing as described by Monkongdee et al.[3] String tests were prepared by placing strings into a mesh filter basket (Fitzco, Spring Lake, Minnesota) within a microfuge tube followed by centrifugation in a microfuge for 15 minutes at 14,000 RPM; the resulting pellet was used for processing.

Nonsterile specimens (i.e., all but lymph node aspirate and blood) were processed using the standard N-acetyl-L-cysteine/4% sodium hydroxide-sodium citrate (NALC/NaOH Na-citrate, final concentration of NaOH 1%) method.[4] Following centrifugation, specimens were decanted and resuspended in 2.0 mL of fresh phosphate buffer (pH 6.8). The concentrated pellet was mixed completely and divided for testing by culture and Xpert. A total of 0.5 mL of processed specimen was inoculated into one mycobacterial growth indicator tube (MGIT; BD, Franklin Lakes, NJ). LNA specimens submitted in TTB were inoculated (0.5 mL) directly into MGIT media without processing. Myco- F/Lytic blood cultures were placed directly into the BD BACTEC 9000 instrument.

Microscopy

Concentrated smears were prepared from processed specimen pellets, stained using Auramine O fluorescence microscopy (FM) staining methods and evaluated for acid-fast bacilli (AFB) in accordance with World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease grading for FM.[5] Two LNA smears were prepared from each needle at the time of collection; one smear was stained with DiffQuick (cytoplasmic) stain for bedside microscopy evaluation for adequate cellular material and subsequent Ziehl-Neelsen staining, and the second smear was evaluated by a pathologist for cytopathology using Papanicolaou staining and bright-field microscopy. AFB microscopy was not performed on blood samples.

Nucleic Acid Amplification

We performed Xpert (Cepheid, Sunnyvale, CA) on all processed specimen pellets according to the manufacturer's protocol. LNAs were prepared for Xpert by aspirating 0.7 mL from the inoculated TTB and adding 1.4 mL of Xpert sample reagent. Xpert was not performed on blood samples because the large blood volumes needed for this test in adults were not feasible in children; more recently, use of smaller volumes of

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blood for Xpert testing in children has been described.[6,7]

Mycobacterial Culture and Identification

MGIT cultures were incubated in the MGIT 960 instrument for up to 6 weeks. MGIT cultures flagged as positive were inoculated to brain heart infusion (BHI) media to determine the presence of contamination by non-mycobacterial species; smears were also prepared and stained using Ziehl-Neelsen and bright-field microscopy to confirm the presence of AFB. Cultures demonstrating growth consistent with *Mycobacterium* species were identified using the MGIT TBc Identification Test (Beckton Dickinson, Franklin, NJ) and the Genotype Mycobacterium MTBC or CM line probe assay (Hain LifeScience, Nehren, Germany). Cultures with both *Mycobacterium* species and contaminating species were decontaminated using the standard method described above. Blood cultures that were negative for AFB and had growth on BHI were tested for bacterial identity.

Data Analysis

Participants were eligible for this study if they had at least one Xpert or MGIT result for each of four of the following specimen types (standard panel: GA, NPA, IS, ST, stool, and urine. Of the 300 participants, 32 children had laboratory evidence of tuberculosis on the standard panel, and 31 met the study criteria (one participant had only a single NPA, which is positive on more than one test). This study criterion for number of available specimens also reduced the number of participants without evidence of tuberculosis from 268 to 263 (five children had three or fewer specimen types available).

To determine the cumulative yield of various combinations of specimen types as well as the incremental yield between pairs of combinations, we characterized the complex relationships among six specimen types, two test types (Xpert, MGIT, or both), and up to two results per combination of specimen type and test. We included 31 participants with laboratory evidence of tuberculosis and 263 participants with no evidence of tuberculosis. To recover as much statistical information as possible under these constraints, we applied a nonparametric resampling scheme that takes maximum advantage of the covariability among multiple specimen results for a given participant.

The primary analysis created 10,000 pseudosamples of 294 pseudoparticipants each by resampling analysis-eligible participants with replacement from the analytic set of 294 participants. For a given specimen type and test (Xpert or MGIT), the analysis randomly sampled the specimen order (first vs second specimen) without replacement. Thus, for a given pseudosample, each participant's original record appeared a random number of times. Among the pseudosamples in this analysis, each participant's sampled frequency ranged from 0 to (rarely) 8 times within each pseudosample. The number of tuberculosis-positive pseudoparticipants ranged from 14 to 51, with a mean and median of 31.

For each pseudosample, the sensitivity of a given combination of specimens and test types (Xpert, MGIT, or both) was calculated as the percentage of pseudoparticipants for which that combination is positive, among pseudoparticipants within the same pseudosample for which any specimen was positive on Xpert or MGIT. Pseudosample-specific yield was calculated as sensitivity × 31/100 to achieve a scale comparable to the original sample. Resampling distributions were obtained by assembling sensitivity and yield values across all 10,000 pseudosamples. When aggregated across the 10,000 pseudosamples, this analysis produced a distribution of the sensitivity and yield of each test/specimen type combination, as well as the covariability of sensitivity and yield estimates across test/specimen type combinations. Figures 2, 3, and S1 depict the means of resampling distributions of the yield values, together with their 10th, 25th, 75th, and 90th centiles. The 10th and 90th centiles (1st and 9th deciles) of the sensitivity estimates are presented in the text as the interdecile ranges of each resampling distribution. Analyses were conducted using R v 3.5.2 (R Core Team, Vienna, Austria). Data were managed using Microsoft SQL Server (Microsoft, Redmond, WA) and REDCap (Vanderbilt University, Nashville, TN).

Quality Control

All specimen collection procedures were witnessed by an observer who cross-checked patient and sample information. Electronic barcoding was used for all collected samples. Mycobacterial culture control procedures included inoculation of MGIT with a processed artificial sputum (AS) spiked with *M. tuberculosis* H37rv as a positive control and AS without H37rv as a negative control every 2 months.

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eTable 1. Comparison of yield of first and second specimens, Xpert or MGIT

Specimen Type	Specimen 1 Positive (%)	Specimen 2 Positive (%)	Difference in % Yield
GA	4/26 (15)	2/26 (8)	8 (-4, 20)
NPA	3/26 (12)	2/26 (8)	4 (-7, 15)
IS	1/25 (4)	5/25 (20)	-16 (-28, -4)
String	3/25 (12)	2/25 (8)	4 (-7, 15)
Stool	3/24 (13)	0/24 (0)	13 (4, 22)
Urine	0/11 (0)	0/11 (0)	0 (—)

The difference in % yield is the percentage of specimen pairs in which the first specimen is positive by either Xpert or MGIT minus the percentage in which the second specimen is positive, together with an 80% interval representing the first and ninth deciles from the resampling distribution over 10,000 pseudosamples. All observed pairs of urine specimens produced the same result, such that there is no variability in the resampling distribution for the difference in yield of first and second urine specimens.

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eTable 2. Specimen yield using smear, Xpert, and MGIT among children with confirmed tuberculosis in Kisumu County, Kenya (October 2013–August 2015), by HIV status

	Evaluated	Smear Positive/Tested (%)	Xpert Positive/Tested (%)	MGIT Positive/Tested (%)	Any Positive/Tested (%)
Children with HIV					
Gastric aspirate	39	3/7 (43)	6/7 (86)	6/7 (86)	6/7 (86)
Nasopharyngeal aspirate	42	2/7 (29)	6/7 (86)	6/7 (86)	7/7 (100)
Induced sputum	30	1/5 (20)	4/5 (80)	4/5 (80)	4/5 (80)
String test	33	1/6 (17)	4/6 (67)	4/6 (67)	5/6 (83)
Stool	39	1/7 (14)	5/7 (71)	5/7 (71)	6/7 (86)
Urine	27	1/6 (17)	2/6 (33)	2/6 (33)	2/6 (33)
Blood	3	0	0	1/3 (33)	1/3 (33)
LN FNA	0	0	0	0	0
Children without HIV					
Gastric aspirate	129	3/23 (13)	10/23 (43)	16/23 (70)	18/23 (78)
Nasopharyngeal aspirate	132	4/25 (16)	11/25 (44)	16/25 (64)	17/25 (68)
Induced sputum	132	3/24 (12)	10/24 (42)	15/24 (62)	16/24 (67)
String test	132	2/24 (8)	7/24 (29)	8/24 (33)	10/24 (42)
Stool	125	3/23 (13)	8/24 (33)	2/24 (8)	8/24 (33)
Urine	93	0/23 (0)	2/23 (9)	0/23 (0)	2/23 (9)
Blood	11	0	0	0/11 (0)	0/11 (0)
LN FNA	10	0	2/4 (50)	3/4 (75)	3/4 (75)

Abbreviations: MGIT, mycobacteria growth indicator tube; LN FNA, lymph node fine needle aspiration.

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eTable 3. Comparison of yield among children with and without HIV, Xpert or MGIT, on up to 2 specimens

Specimen type	HIV+ children	HIV- children	Difference in % yield
GA	6/7 (86)	18/23 (78)	7 (-14, 27)
NPA	7/7 (100)	16/24 (67)	33 (21, 46)
IS	4/5 (80)	16/24 (67)	13 (-16, 40)
String	5/6 (83)	10/24 (42)	42 (16, 65)
Stool	6/7 (86)	8/24 (33)	52 (30, 73)
Urine	2/6 (33)	2/23 (9)	25 (-5, 53)

The difference in % yield is the percentage of children with HIV for whom up to 2 specimens were positive by either Xpert or MGIT minus the corresponding percentage among children without HIV, together with an 80% interval representing the first and ninth deciles from the resampling distribution over 10,000 pseudosamples.

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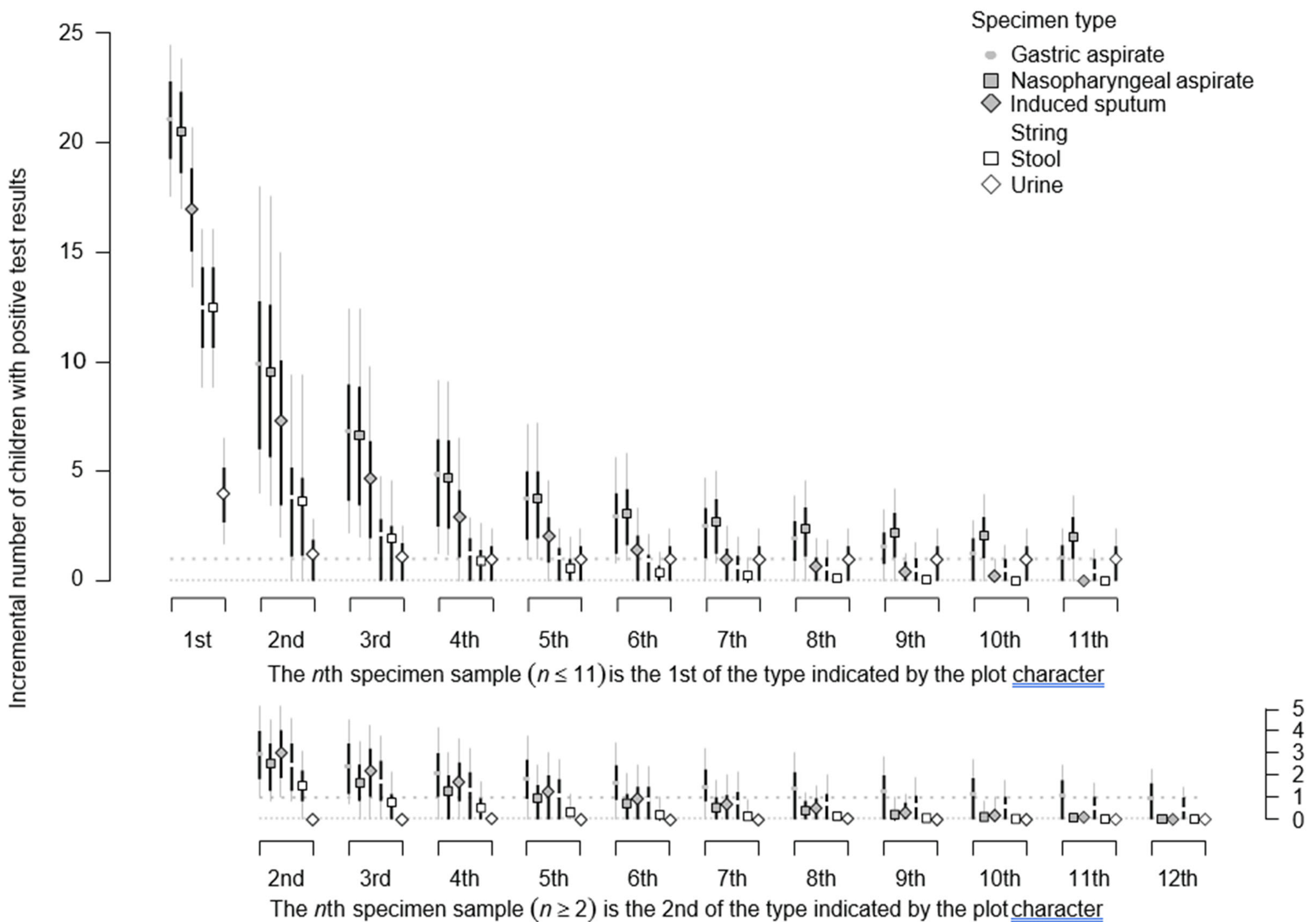
eFigure. Incremental yield by specimen type and cumulative number of specimens from children in Kisumu County, Kenya (October 2013 to August 2015), tested for tuberculosis

This figure shows the joint effects of the number of specimens, the increment of one additional specimen by specimen type, and whether that incremental specimen was the first or second of its type. This figure combines Xpert and MGIT results. The upper panel shows incremental yield for each of the six specimen types when added to a base combination of 0–10 specimens not containing that type (for example, a base combination of three specimens without gastric aspirate compared to that combination of four specimens containing the base three plus gastric aspirate.) The lower panel shows incremental yield for each of the six specimen types when added to a base combination of 1–11 specimens already containing one specimen of that type (for example, a base combination of four specimens, including one gastric aspirate sample, compared to that combination of five specimens containing the base four plus one more gastric aspirate sample). Each panel contains a horizontal dotted line at one for visual reference. The points depict average yield over the resampling distribution, black bars represent the interquartile range (25th to 75th centile), and gray bars represent the interdecile range (10th to 90th centile). Abbreviations: MGIT, mycobacteria growth indicator tube.



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