Supplement

Kendall et al., The spectrum of tuberculosis disease in an urban Ugandan community and its health facilities

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Supplemental Methods, data collection:

Estimation of study area population and demographics

Prior to the community-based phase of our study, our study staff conducted a preparatory mapping and census exercise. Working with local community health extension workers to identify zone boundaries, our study staff identifies and traversed the boundaries of the study area, noting important landmarks and reference points and using GPS-enabled devices and to create a map of the target area. Then, with the assistance of local community leadership, our teams conducted an informal census of the study area. Traveling through each zone accompanied by a community leader, staff identified the location of every residential building or compound, marked it on a map, and estimated the number of residents (asking residents or neighbors whenever possible). The population estimated in this manner (48,000) was consistent, within 10%, both with Ugandan Bureau of Statistics data and with our subsequent independent estimate obtained during door-to-door screening (Figure S1).

Then, while conducting door-to-door TB screening, research staff moved systematically through the mapped study area, accompanied by local community health extension workers. They enumerated all residences and collected GPS coordinates for each. If a resident was home and willing to provide information, they demographic information about all residents of the household. If no one was at home or able to provide information, neighbors were asked to provide simple counts of the household's adult and child residents, if possible.

In estimating total population of the study area, we assumed that the mean numbers of child and adult residents in all occupied households was the same as for occupied households where either a resident or a neighbor provided a count of adult and child residents. For residences where no resident was home during our visits, we assumed that the proportion that were unoccupied the same (11%) for residences where a neighbor was able to provide information about occupancy as for those residences with no information from neighbors.

We assumed that the age and sex distribution of the study area's adult population was equal to that of the households (6249 of 15832, Figure S1) in which a resident provided household demographic information. This distribution was used for age and sex adjusted estimates of prevalence as described in Table S1.

Additional details of community-based TB testing:

Staff then invited all available residents 15 years or older (regardless of TB symptoms) to provide expectorated sputum for TB testing.

As research staff moved through the study area, they also conducted recruitment at nonresidential locations. They offered TB testing to employees at shops and places of work, visitors at others' homes, and passersby on the street. In parallel with door-to-door testing, a total of ten "venue-based screening events" were also scheduled to provide additional opportunities for TB testing in each community. These events were held in an open tent in public spaces (e.g. near a market, church, or community hall),

usually for 3-4 hours during a weekend. In all locations, participants who consented to TB testing and reported a residence in the study area were included in data analysis.

Eligible individuals (age ≥15 years, resident of study area, and not known to have previously participated) were recruited using a standard script, instructed in production of high-quality sputum, and asked to expectorate into a sterile container. Specimens were individually packaged, stored in a portable cooler, and transported in batches to an accredited laboratory by motorbike for same-day processing.

Identification of duplicate enrollments

Repeat interactions with the same individual were identified through use of personal identifiers (name, mother's name, year of birth), through participant self report, and through use of a unique iris-based biometric identifier (iRespond, iirespond.org, Seattle).

All participants in community-based TB screening underwent iris scanning. Prior to completing the consent process for TB screening, participants were asked whether they had been screened previously. Those who reported previous participation were asked to undergo iris scanning to identify their previous record but were not enrolled for repeat testing.

For those who were enrolled as a TB case or control, records were searched for any iris matches and for any exact or approximate matches with reported personal identifiers. Any apparent matches were reviewed to determine whether the two records belonged to the same individual. In one instance, an individual tested positive for TB in community-based screening and then was also diagnosed through facility-based care while awaiting their community screening result. This individual was enrolled only once and was analyzed as both a community-based and a health-facility TB case.

Coaching participants in production of expectorated sputum:

All adult community members were asked to provide an expectorated sputum specimen for Ultra testing, and all who were enrolled as a case or control were asked to provide an additional specimen for culture. For consenting participants who could not easily expectorate sputum, we provide the following guidance sequentially:

1. Breath in and out deeply three times, then hold your breath for about four seconds, and then clear your throat.

2. (If process (1) above failed on two attempts) Take some cold water then repeat Process (1) above.

3. (If process (2) above failed) Jump/skip around for about one minute, then repeat Process (1) above.

Additional details of contact investigation:

All individuals enrolled as a TB case during the community-based active case-finding phase of the study were asked to participate in contact investigation. Participants were asked to name, and describe their

frequency and intensity of contact with, all household contacts and any individuals with whom they spent a large amount of time in indoor spaces.

Contact investigation was then conducted, which included sputum Ultra testing for contacts age >15 years (unless already tested within the past 3 weeks), HIV counseling and testing, a TB symptom screen, tuberculin skin testing, and referral to medical care if indicated based on any of: positive Ultra test; TB symptoms; new HIV diagnosis; or preventive therapy eligibility based on age or HIV status.

Due to resource constraints, non-household contact investigations were conducted for the two highest intensity non-household contacts; if more than two contacts had equal amounts of contact in a single location (e.g. in a shared workplace), then all contacts in that location were investigated.

Selection of community controls:

All households in a given zone were enumerated prior to selecting controls for cases from that zone. As a first sampling step, a residence in the zone was randomly selected, weighting each residence by the reported number of adult residents (or, for households with no a resident count reported , assuming the average number of adult residents reported by other households in that zone). Then, the household was visited, all adult residents were enumerated, and one resident was randomly selected and invited to enroll as a community control. Ultra testing was performed at enrollment if not already completed. If the selected control declined to participate or was unavailable, this control selection process was repeated.

Controls were intended to represent the population that participated in screening in each zone, and therefore were not matched based on other characteristics such as age and sex.

CRP measurement

Quantitative analysis of CRP values was restricted to the limits of detection of the test manufacturer with the narrower limits (i.e. a lower bound of 2.5 mg/L, the lower limit of detection for the i-Chroma assay, to an upper bound of 120 mg/L, the upper limit of the Eurolyser assay), and all results were converted to mg/L units.

Supplemental Methods, Data Analysis

Dual enrollment as health facility and community-based cases

A small number of individuals, not yet known to have TB, both submitted TB-positive sputum in community-based testing and, while awaiting their community-based Ultra results, presented to health facilities and were diagnosed with TB through routine care. These are counted as community-based cases in the estimate of prevalence. For comparisons of community-based cases and health-facility cases, they are included in both groups.

Estimated specificity of Ultra

Sputum culture (a single culture of a spot sputum specimen as performed by our study) was taken as a reference standard for this calculation.

Before calculating the specificity, we inferred the culture status of all participants who had an Ultra result but no culture result (either no culture performed, or a contaminated or uninterpretable culture result).

For the relatively small number of participants with a positive Ultra result but no culture result, we assumed that the culture-proportion was the same for those with a culture result as for those without, after stratifying by trace versus non-trace Ultra status. Rounding, we thus estimated $T_{neg,pos}$, the number of participants who would have been culture negative but Ultra positive if culture had been completed for all participants.

For the much larger number of participants with a negative Ultra result and no culture result, we assumed that a proportion *x* culture would have been sputum-culture-negative if tested. Our primary estimate assumed that x = 99%; this corresponds to a 1% prevalence of Ultra-negative, culture-positive TB in the community-based study population who participated in screening, which we felt was a generous estimate. In sensitivity analyses, we assumed that 100%, 99.5%, 98.6%, or 94.6% of those with a negative Ultra result would be culture-negative if tested. The total number of participants who would have been culture-negative and Ultra-negative if cultured was then calculated as $T_{neg,neg} = N + x*U$, where N is the number with a negative Ultra and a negative culture performed by our study, and U is the number with a negative Ultra and unknown culture status.

We then calculated the estimated specificity as $T_{neg,neg}/(T_{neg,neg} + T_{neg,pos})$. The reported confidence interval is based on a binomial distribution and the total number of individuals who completed Ultra testing.

Assuming x = 98.6%, we estimated that of the 12032 participants with an Ultra result, 11825 would have been culture-negative if culture had been performed, and 73 of those culture-negative individuals would have been Ultra positive.

We chose not to calculate and report and estimate of the sensitivity, because the number of individuals who were tested by culture after a negative Ultra result is too small, relative to the expected population prevalence of TB, to estimate sensitivity with reasonable precision.

Table S1: Detailed definitions and methods use for estimation of community TB prevalence

	Numerator	Denominator	Confidence interval	Notes
Primary estimate: Community prevalence among screened adults	Individuals with a positive (including trace) Ultra result in community-wide TB screening, Feb-Nov 2019. *	Individuals with a valid (positive or negative) Ultra result in community-wide TB screening, Feb-Nov 2019	Agresti-Coull binomial 95%CI (R package 'binom')	Excludes tests repeated during contact investigation, or tested during contact investigations and control enrollments that occurred in December 2019 (i.e., that lagged behind the end of the community-wide testing period)
Age- and sex-adjusted adult prevalence	Crude prevalence as for primary estimate, but stratified as shown in Table S2, and adjusted for population proportion among residents enumerated during door-to-door case-finding visits	Crude prevalence as for primary estimate, but stratified as shown in Table S2, and adjusted for population proportion among residents enumerated during door-to-door case-finding visits	Gamma 95%CI[1]	Calculated using R package 'dsr'
Diagnostic yield including adult contacts and controls	Individuals with any positive (including trace) Ultra result during community-based case finding activities.	Individuals with any valid (including trace) Ultra result during community-based case finding activities.	Agresti-Coull binomial 95%CI	Includes those tested during December 2019 control and contact enrollment, and counts as positive those contacts who initially tested negative, but later were retested and identified as TB cases during contact investigations.
Adult prevalence including all who attempted sputum production	As for primary estimate	All who consented for community-wide TB screening, Feb-Nov 2019, including those unable to expectorate or with invalid Ultra results	Agresti-Coull binomial 95%CI	
Adult prevalence excluding culture-negative trace-positives	As for primary estimate, but subtracting estimated number of trace-positive sputum that would be culture negative	As for primary estimate, but subtracting estimated number of trace-positive, culture-negative sputa (excluding these individuals from analysis)	Agresti-Coull binomial 95%CI	The proportion culture-negative was determined among trace positive sputa with a valid sputum culture result, then applied to all trace positive sputa.
Adult prevalence including Ultra-negative, culture-positive cases	First proportion: TB positives excluding culture-negative trace-positives, as above. Second proportion: Number of Ultra-negative individuals with sputum culture positive for *M. tuberculosis*	First proportion: All tested by Ultra excluding culture-negative trace-positives, as above. Second proportion: Number of Ultra-negative individuals enrolled for sputum culture as community controls	Sum of two Agresti-Coull binomial 95%CIs	Excludes estimate of trace-positive, culture-negative individuals
Total proportion of adult population diagnosed with TB during case-finding period	All adults diagnosed with pulmonary TB through community-based case finding (including contacts and controls) at local health facilities, from Feb through Nov 2019	Total estimated adult population of study area	Agresti-Coull binomial 95%CI	Households lacking a count of adult residents were assumed to have the same average size as households for which a household member or neighbor provided a count.
National adult urban prevalence, 2014 prevalence survey				This is crude prevalence of bacteriologically-positive TB measured among urban-dwelling adultsa (Adjusting for pediatric and extrapulmonary TB, TB prevalence is 318 per 100,000 population.)

[1] 1   Fay MP, Feuer EJ. Confidence intervals for directly standardized rates: a method based on the gamma distribution. *Stat Med* 1997;**16**:791–801. doi:10.1002/(sici)1097-0258(19970415)16:7<791::aid-sim500>3.0.co;2-#

* To maintain consistent sampling frames for TB-positive and TB-negative individuals, prevalence estimates were based on residence as reported at time of screening (even if incorrectly reported), but case or control eligibility required a confirmed residence within the study area.

Supplemental Results

Figure S1: Full flowsheet of community population estimation, community screening, and community-based and health-facility case enrollment. *M.tb = Mycobacterium tuberculosis.*
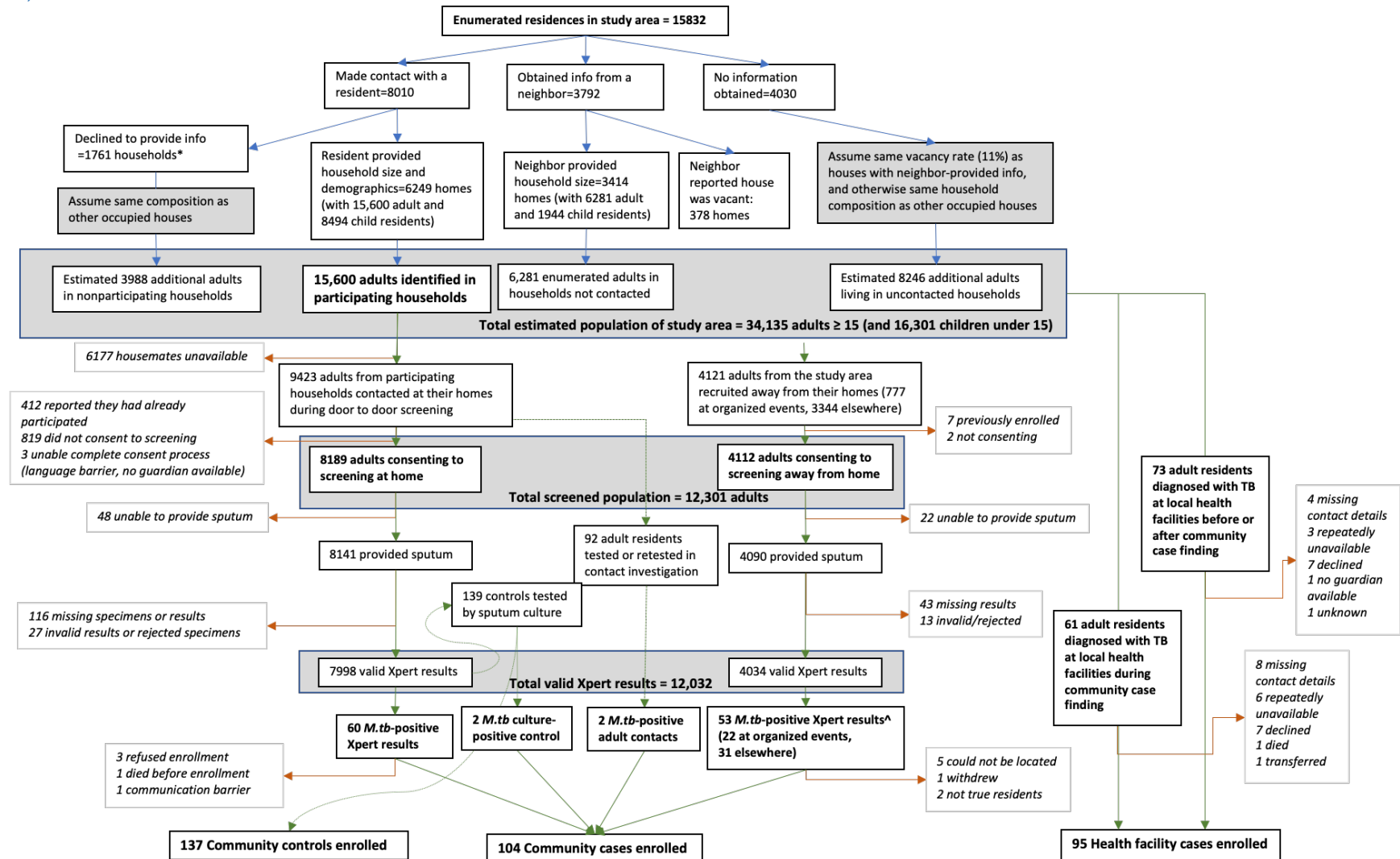
Figure S2: Timeline of health-facility and community-based case enrollment.

At local health facilities, patients from the study population (estimated size 34,000 adults) who were diagnosed with TB were captured throughout the time period shown and are shown in purple. The gray region (Feb-Nov 2019) depicts a 10-month period during which intensive community-based active case finding efforts were undertaken. Of note, health facilities had transitioned to the Xpert MTB/RIF Ultra cartridge in January and February 2019, after which the proportion of health-facility cases diagnosed clinically (with negative or no Xpert result) fell from 21% to 13%. The blue bars show the number of people diagnosed each month through community-based case finding. The higher dotted line indicates the estimate of monthly community-based cases expected if the entire adult population (estimated size 34,000) had been screened during this 10-month period, extrapolating from the prevalence among the 12,000 who completed screening.
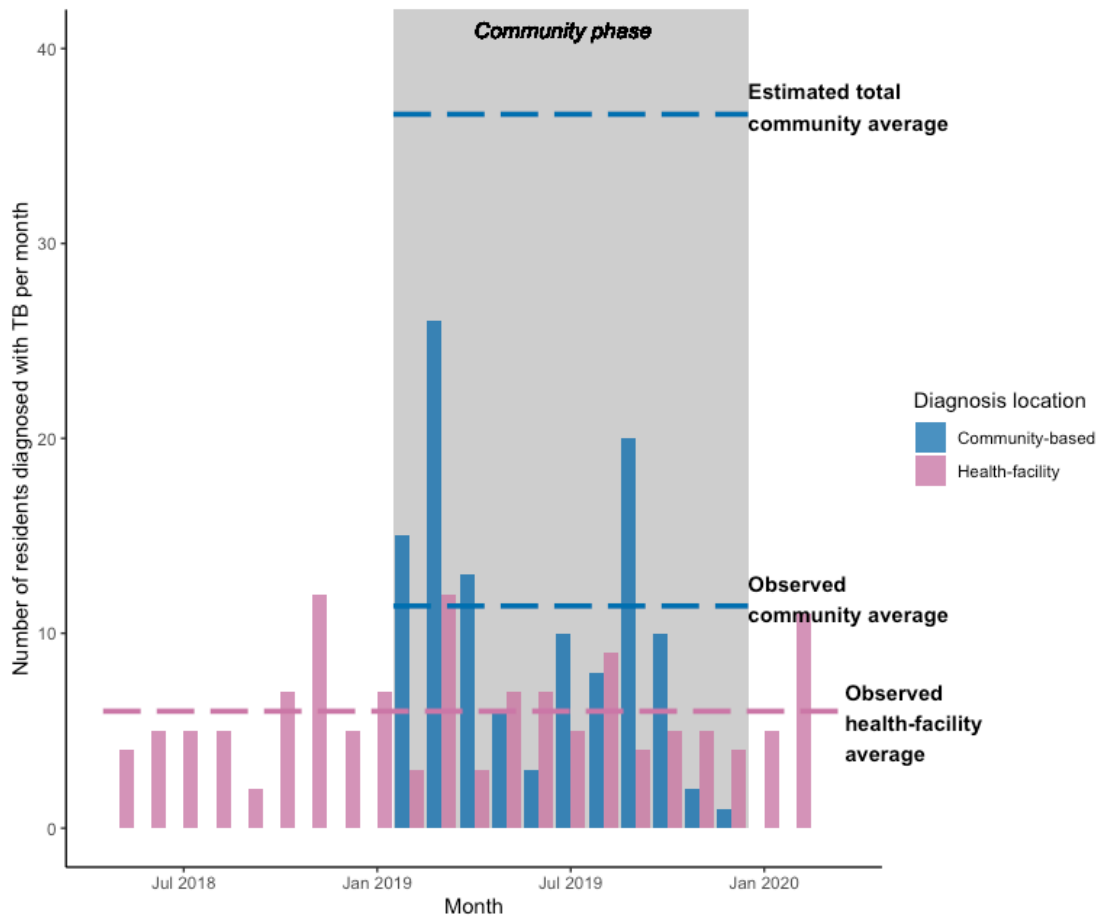
The most common barriers to enrolling at home were: no one was home (estimated 14,527 adults [43% of the adult population]), a specific household member was unavailable (6,177 [18%]), the household declined to provide any information (estimated 3,988 adults [12%]), an individual resident did not consent to screening (819 [2%]), or a resident reported having already participated elsewhere (412 [1%]) (Figure S1).

Among household representatives of individual residents who did not consent to screening, most did not specify a reason, but 104 noted recent TB testing, and although treatment and preventive therapy were not exclusion criteria, 11 noted that they were taking TB medicines as the reason they did not wish to participate.

Demographics

Table S2: Prevalence (per 100,000 adults) of positive Ultra results in the community, stratified by age and sex.

Sex	Age	All residents*	Screened residents	Ultra+ cases	Prevalence estimate	Lower bound	Upper bound
Female	15-24	9132	3336	21	455	89	1396
	25-34	4492	1728	16	829	390	1658
	35-44	2499	965	8	926	558	1511
	45+	1620	659	3	629	406	966
Male	15-24	6740	2442	20	1466	729	2807
	25-34	4682	1448	20	1905	1151	3096
	35-44	2965	840	16	1381	883	2137
	45+	1907	614	9	819	523	1270

* Extrapolated from households where someone reported residents' age and sex

Table S3: Demographics and prevalence of positive Xpert MTB/RIF Ultra results by screening setting.

	Home	Venue-based screening event	Other away-from home location	All locations combined
Successfully tested				
N	7998	753	3281	12032
Female, N (%)	5177 (65%)	262 (35%)	1249 (38%)	6688 (56%)
Age, median (IQR)	25 (21 - 33)	28 (23 - 38)	27 (22 - 36)	26 (21 - 35)
Xpert MTB/RIF Ultra positive				
N	60	22	31	113
Female, N (%)	38 (63%)	8 (36%)	2 (6%)	48 (42%)
Age, median (IQR)	28 (23.75 - 33.25)	30 (25 - 36.25)	35 (25 - 45)	29 (24 - 37)
Prevalence				
Raw prevalence (95%CI) per 100,000 adults	750 (582 - 966)	2922 (1914 - 4407)	945 (662 - 1343)	939 (781 - 1129)
Age- and sex-adjusted prevalence per 100,000 adults*	780 (587 - 1018)	2967 (1758 - 4689)	774 (522 - 1105)	973 (801 - 1172)

* Although men were more likely to participate in away-from-home screening, sex differences did not explain the higher prevalence among venue-based screening participants, as the prevalence among women tested at venue-based screening events was also high (3053 [1453-6010] per 100,000, Supplemental table 1).

Table S4: Yield of Ultra in relation to quality and volume of submitted sputum

	N	Number (%) any positive	Number (%) trace positive	Number invalid
All sputum	12072	113 (0.9%)	71 (0.6%)	40
By quality				
Salivary	7947	59 (0.7%)	37 (0.5%)	0
Non-salivary	3892	53 (1.4%)	34 (0.9%)	1
Missing	233	1 (0.4%)	0	39
By volume				
≤1 ml	6164	53 (0.9%)	35 (0.6%)	0
>1ml	5725	60 (1.1%)	36 (0.6%)	1
missing	184	0	0	39

Table S5: Details of culture results for community-based cases:

	Ultra positive, trace	Ultra positive, not trace	Ultra negative
Liquid no growth, solid no growth*	44	11	0
Liquid contaminated, solid no growth*	4	0	0
Liquid no growth, solid MTBc positive#	0	1	0
Liquid contaminated, solid MTBc positive#	0	0	1
Liquid MTBc positive, solid no growth#	2	0	0
Liquid MTBc positive, solid contaminated#	0	1	0
Liquid MTBc positive, solid mycobacterial growth#	6	25	1
Liquid MTBc negative (Nontuberculous mycobacterium), solid mycobacterial growth	1	0	0
Liquid contaminated, solid contaminated	6	1	0

* Counted as culture-negative; # Counted as culture-positive; MTBc = *M. tuberculosis* complex

Table S6: Sensitivity analysis, specificity of Ultra in the community setting

Assumed proportion culture negative among those with a negative Ultra result	Estimated specificity	95% binomial confidence interval
100%	99.39%	99.23, 99.51
99.5%	99.39%	99.23, 99.51
98.6%	99.38%	99.22, 99.51
94.6%	99.36%	99.19, 99.49

Xpert cartridge changes, trace diagnosis, and empiric treatment in health facilities

The observed proportion of health-facility cases that were by Ultra positive at the trace level was 8% during and after the community phase (i.e., after the switch to Ultra cartridges in February 2019). The proportion of health-facility cases diagnosed clinically (with negative or no Xpert MTB/RIF result) was 21% prior to the community phase and 13% during and after.

Other diagnostics

Table S7: Relationship between CRP level and positive symptom screen within participant groups.

Symptoms	Health facility cases		Community-based cases		Community-based cases, non-trace only		Community-based cases, trace only		Community controls	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
CRP>10	69	0	23	4	13	0	7	2	4	7
CRP<10	21	1	44	40	13	4	26	20	40	67
Fisher's exact p-value	0.2		0.005		0.1		0.3		1	

Table S8: Clinical and bacteriologic characteristics of TB cases, by level of Ultra positivity, and by culture status among those with a trace-positive Ultra

	Health-facility case (N=95)	Community-based case*			Community control (N=137)
		Trace+ culture- (N=48)	Trace+ culture+ (N=8)	Ultra more than trace (N=39)	
Age (Years)					
Median (IQR)	33 (27, 40)	28 (24, 35)	43 (30, 47)	30 (25, 36)	26 (22, 35)
Sex					
Male	65 (68%)	21 (44%)	6 (75%)	27 (69%)	45 (33%)
Female	30 (32%)	27 (56%)	2 (25%)	12 (31%)	92 (67%)
HIV and ART status					
Negative	59 (62%)	43 (90%)	7 (88%)	34 (87%)	123 (90%)
Positive not on ART	10 (11%)	0 (0%)	0 (0%)	3 (8%)	1 (1%)
Positive on ART	26 (27%)	5 (10%)	1 (12%)	2 (5%)	13 (9%)
History of prior TB treatment					
Yes	23 (24%)	5 (10%)	0 (0%)	2 (5%)	4 (3%)
TB contact (same household or past 12 months)					
Yes	39 (41%)	25 (52%)	5 (62%)	19 (49%)	44 (32%)
Smoking status					
Current smoker	16 (17%)	7 (15%)	5 (62%)	10 (26%)	9 (7%)
Former smoker	21 (22%)	1 (2%)	0 (0%)	6 (15%)	4 (3%)
Never smoker	58 (61%)	40 (83%)	3 (38%)	23 (59%)	124 (91%)
Any TB symptom					
Yes	94 (99%)	27 (56%)	6 (75%)	34 (87%)	52 (38%)
Chronic cough (>=2 weeks)					
Yes	88 (93%)	12 (25%)	5 (62%)	30 (77%)	24 (18%)
Duration of TB symptoms (weeks)					
Median (IQR)	8.0 (4.0, 16)	1.5 (0, 4.0)	3.5 (2.3, 9.0)	8.0 (3.0, 16)	0 (0, 4.0)
Serum C-reactive protein (mg/L)					
Median (IQR) †	51 (12, 100)	<LLD(<LLD, 5.1)	4.5 (<LLD, 40)	6.2 (<LLD, 28)	<LLD (<LLD, 3.5)
Missing	4 (4.2%)	2 (4.2%)	0 (0%)	6 (15.4%)	19 (13.9%)

*Trace-positive cases with an uninterpretable culture result are excluded, as are community-basd cases with no Ultra result.

† <LLD: below the lower limit of detection of 2.5 mg/L or less

Table S9: Additional characteristics omitted from Table 1, with community-based cases stratified by trace result

	Health-facility case	Community-based case diagnosed at			Community control
		All cases	Ultra more than trace	Ultra trace	
	(N=95)	**(N=104)**	**(N=39)**	**(N=63)**	**(N=137)**
Education completed					
Less than primary school completion	39 (41%)	31 (30%)	12 (31%)	19 (30%)	36 (26%)
Primary	26 (27%)	42 (40%)	11 (28%)	30 (48%)	44 (32%)
Lower secondary (O-level)	17 (18%)	19 (18%)	10 (26%)	8 (13%)	34 (25%)
Higher secondary (A-level) or higher	13 (14%)	12 (12%)	6 (15%)	6 (10%)	23 (17%)
Employment status					
Regularly employed	45 (47%)	52 (50%)	19 (49%)	32 (51%)	72 (53%)
Irregular work	27 (28%)	33 (32%)	17 (44%)	16 (25%)	18 (13%)
Student or housewife	7 (7%)	13 (12%)	2 (5%)	10 (16%)	39 (28%)
Unemployed	16 (17%)	6 (6%)	1 (3%)	5 (8%)	8 (6%)
Household income (Ugandan shillings, thousands)					
Median (IQR)	350 (200, 610)	380 (200, 650)	300 (200, 650)	400 (210, 600)	400 (200, 500)
Missing	0 (0%)	1 (1.0%)	0 (0%)	1 (1.6%)	0 (0%)
Ever worked in health care					
Yes	5 (5%)	5 (5%)	2 (5%)	3 (5%)	12 (9%)
Ever incarcerated					
Yes	42 (44%)	30 (29%)	16 (41%)	13 (21%)	16 (12%)
Days to seek evaluation for hypothetical cough					
Median (IQR)	5.0 (3.0, 7.0)	6.0 (2.0, 7.0)	7.0 (3.0, 7.0)	4.0 (2.0, 7.0)	3.0 (2.0, 7.0)
Missing	0 (0%)	1 (1.0%)	1 (2.6%)	0 (0%)	2 (1.5%)
Frequency of outpatient visits to any medical facility					
Less than once per year	21 (22%)	38 (37%)	17 (44%)	20 (32%)	31 (23%)
About once per year	13 (14%)	20 (19%)	9 (23%)	11 (17%)	30 (22%)
A few times per year	43 (45%)	33 (32%)	7 (18%)	25 (40%)	57 (42%)
Monthly or more	18 (19%)	13 (12%)	6 (15%)	7 (11%)	19 (14%)
Ever visited traditional healer or herbalist					
Yes	27 (28%)	28 (27%)	13 (33%)	15 (24%)	29 (21%)
Any cough					
Yes	93 (98%)	69 (66%)	37 (95%)	31 (49%)	55 (40%)
Chronic cough (>=2 weeks)					
Yes	88 (93%)	51 (49%)	30 (77%)	21 (33%)	24 (18%)
Fever/chills					
Yes	38 (40%)	34 (33%)	14 (36%)	19 (30%)	19 (14%)
Night sweats					
Yes	39 (41%)	25 (24%)	13 (33%)	12 (19%)	10 (7%)
Weight loss					
Yes	69 (73%)	46 (44%)	24 (62%)	22 (35%)	24 (18%)
Number of TB symptoms reported					
Median (IQR)	2.0 (2.0, 3.0)	1.0 (0, 3.0)	2.0 (1.0, 3.0)	1.0 (0, 2.0)	0 (0, 1.0)
Subjective rating of current health, 0 to 100					
Median (IQR)	60 (50, 70)	70 (50, 80)	70 (50, 80)	70 (50, 88)	80 (70, 90)

*Two community-based cases with a negative Ultra result (diagnosed based on culture) are included only in the "all positives" column