Supplementary material

Contents

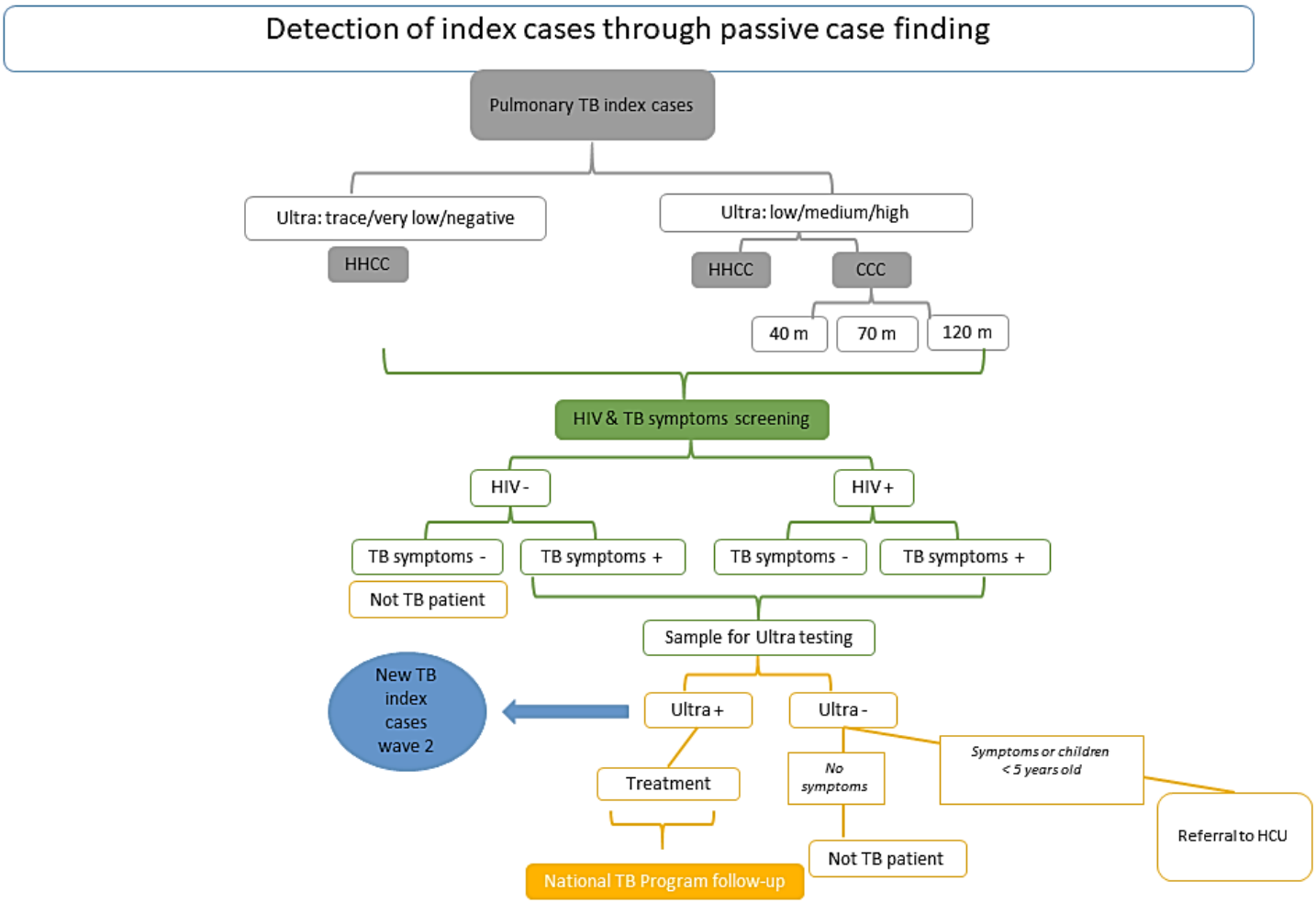
Appendix 1. Xpatial-TB study algorithm.

The target population was all household contacts (HHCC) and community-close contacts (CCC) of pulmonary TB (PTB) cases who started treatment in the district of Manhiça during the study period.

Index TB cases were identified by the routine procedures of the national TB control programme (passive case finding, PCF strategy). PTB patients who started treatment (bacteriologically confirmed or not) were offered to participate in the study. After informed consent, contact investigation (active case finding, ACF) was activated among households (HHCC) or HHCC and community-close-contacts (CCC), depending on the following study algorithm.

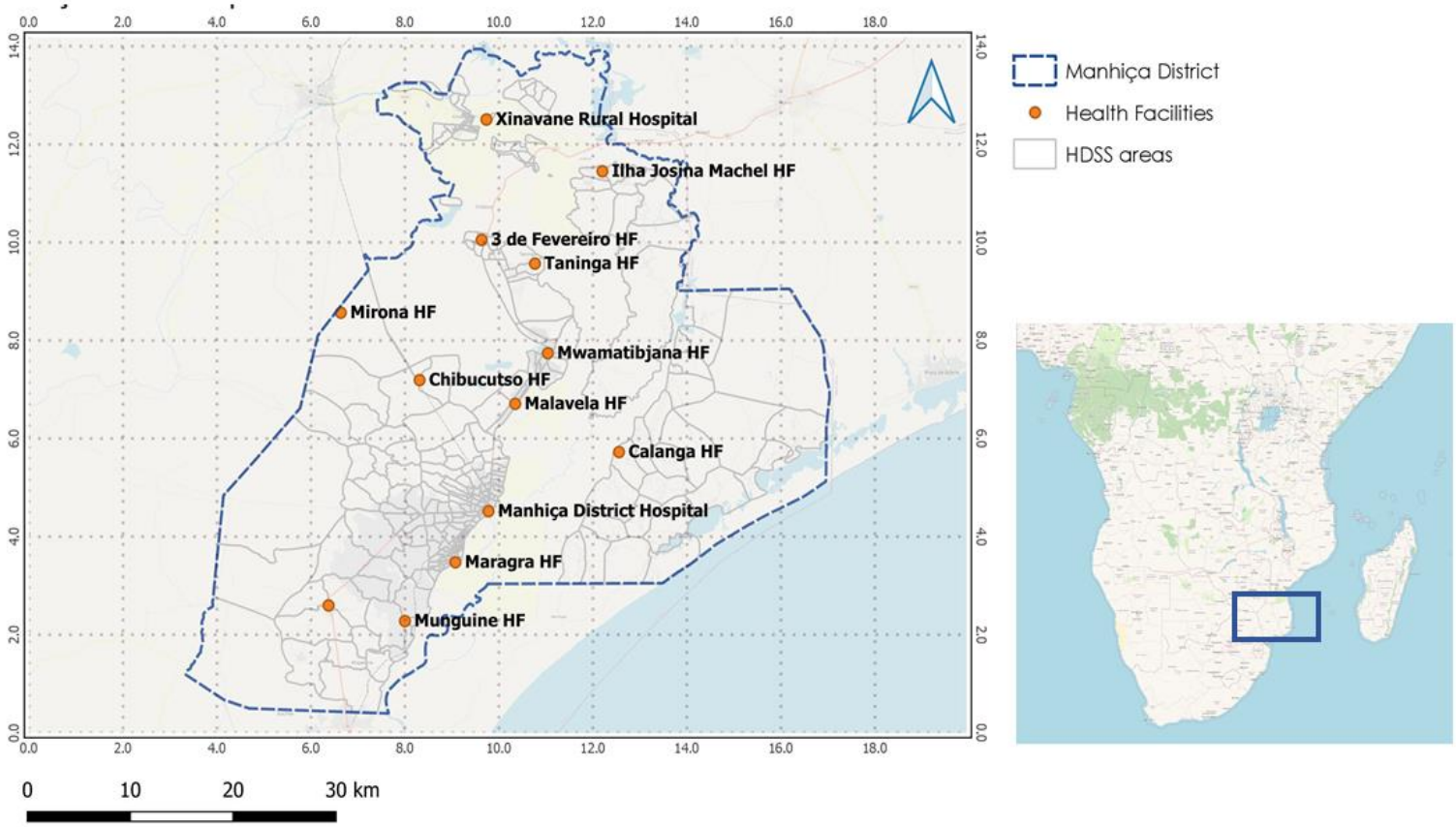
- For those cases with *negative, trace or very low* Ultra result, only household contacts were investigated.
- For those cases *with low, medium or high* Ultra result, also neighbour contacts were investigated. The number of CCC screened was defined by an established screening radius (SR) which depended on neighbourhood density. A screening radius (SR) of 40, 70 or 120 meters corresponded to high, medium or low population density, respectively.

All derived contacts were invited to participate in the study, and those who agreed, fulfilled a baseline questionnaire collecting data on demographic, social and economic factor, and clinical assessment.



Abbreviations: TB: tuberculosis; HHCC: household contacts; CCC: community close contacts; HCU: health care unit

Appendix 2. Map of Manhiça District and location of health care facilities and hospitals from where samples received for testing.

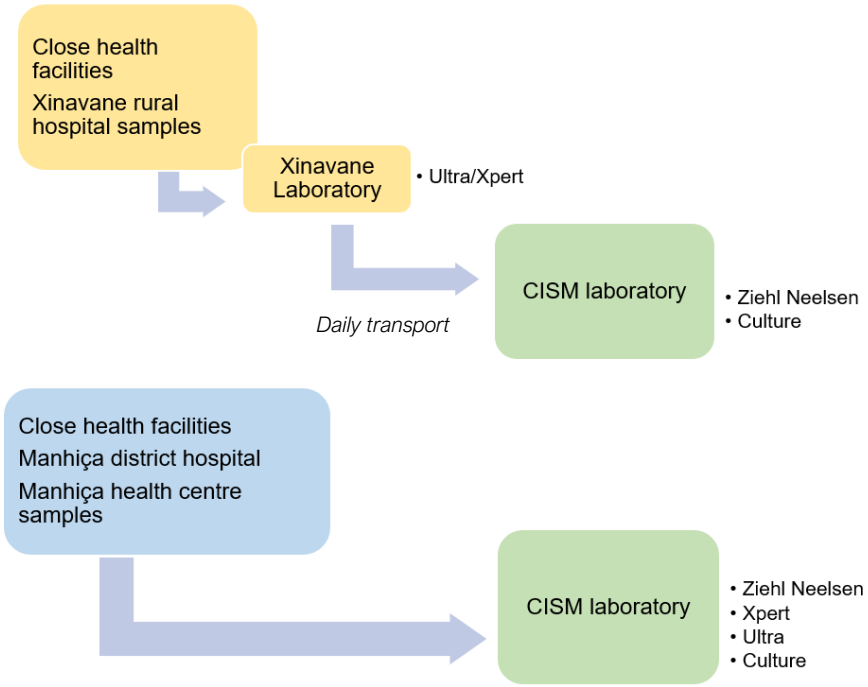


Abbreviations: HF: Health Facility

Appendix 3. Laboratory workflow and flowchart

Samples from suspected TB patients were received through two different pathways.

Passive Case Finding (PCF) route: per the National Tuberculosis Programme protocol, all suspected patients provided a sputum sample to be tested by Ultra at the Xinavane or CISM laboratory, only regarding criteria of proximity. Below, the laboratory flowchart for this pathway.



Abbreviations: CISM: *Centro de Investigação em Saúde de Manhiça*

Active Case finding (ACF) route: All TB contacts who met criteria to be included in the Xpatial-TB study (all household contacts, HIV-positive community close contacts (CCC) and any other CCC who was symptomatic irrespective of HIV status), provided spot sputum samples at screening , which were transported refrigerated directly to the CISM laboratory by the field workers. There they were tested for Zielh- Neelsen, Xpert, Ultra and Culture.

Appendix 4. Sample size consideration

The study was designed to detect a difference of , at least, 5% in sensitivity and 3% in specificity (based on previous works) (26) ; alpha of 5%, desired power of 80%, assuming 2.5% of invalid results by any test, and 12% of microbiological confirmed TB among presumptive cases (based on local previous studies). With those estimations we aimed to recruit 1450 patients. We aimed to reach this sample size by PCF procedures and then increase the sample with the patients enrolled by the ACF strategy. Sample size for the ACF depended on the testing algorithm for TB cases' contacts identified through PCF in the the specified time period. In this way, we tried to ensure enough power for the analysis and to reach required sample size within time.

Appendix 5. Overall diagnostic accuracy results

Results have also been summarised for the entire group of participants to evaluate whether test parameters varied independently of the source of patients. Out of the samples positive according to the gold standard (n=163) ,142 were Xpert positive (sensitivity: 0.87, 95% CI: 0.81,0.92) and 153 were positive by Ultra (sensitivity: 0.94, 95% CI: 0.89, 0.97) p<0.001). Test values are displayed in **the table below.** When stratified by smear microscopy, among smear negative patients (n=1558) Ultra sensitivity was still higher compared to Xpert (0.82 (95% CI: 0.70,0.91) *versus* 0.62 (95% CI: 0.49, 0.75) p<0.001). Conversely, Xpert specificity was slightly higher than that of Ultra, and this difference was statistically significant (0.98 (95% CI: 0.97, 0.99) *versus* 0.96 (95% CI: 0.95, 0.97) p-value= 0.001, respectively).

Logical relations of positivity among tests were also displayed in a Venn diagram **(Figure 2).** Overall, 29.7% of all Ultra positive (60/202) were negative by culture. Ultra yielded 30 additional cases over Xpert and 66.6% (20/30) of them relied on the trace grade.

Trace reclassification:

Twenty-nine patients obtained trace-call results. Only one patient (3.4%) was lost-to-follow-up, 6 patients died, 4 before starting treatment (13.4%) and 2 during treatment (6.9%). Twenty-two could be re-assessed (75.9%) of whom, 13 (59.1%) were HIV positive and 6 (27.3%) had been previously treated for tuberculosis. All those 22 suspected patients started treatment because they fulfilled the criteria for clinically diagnosed TB.

When those results were categorized as negative, Ultra sensitivity maintained its superiority over Xpert (0.90 (95% CI: 0.85,0.94) *versus* 0.87 (95% CI:0.81,0.92) p=0.04), and the specificity increased up to 0.98 (95% CI: 0.97,0.98), reaching Xpert's specificity (0.98 (95% CI: 0.97,0.99) p-value=0.53). Moreover, conditional recategorization strictly for patients who had been treated previously , lead to similar results.

Table comparing smear microscopy, Xpert and Ultra accuracy for the entire group of patients (n=1671) and stratified by smear microscopy and trace recategorization.

	Sensitivity (95% CI[1])	Specificity (95% CI)	PPV [2] (95% CI)	NPV [3] (95% CI)
Smear microscopy	0.66 (0.58, 0.73)	1.00 (0.99, 1.00)	0.95 (0.89, 0.98)	0.96 (0.95, 0.97)
	(107/163)	(1502/1508)	(107/113)	1502/1558)
Xpert MTB/RIF	0.87 (0.81, 0.92)	0.98 (0.97, 0.99)	0.82 (0.76, 0.87)	0.99 (0.98, 0.99)
	(142/163)	(1477/1508)	142/173	1477/1498)
Xpert Ultra	0.94 (0.89, 0.97)	0.96 (0.95, 0.97)	0.72 (0.65, 0.78)	0.99 (0.99, 1.00)
	(153/163)	(1448/1508)	152/213)	1448/1458)
Statistical test[4]	p-value <0.001	p-value=0.006		
Smear negative (n=1558)				
Xpert MTB/RIF	0.62 (0.49, 0.75)	0.98 (0.98, 0.99)	0.58 (0.45, 0.71)	0.99 (0.98, 0.99)
	(35/56)	(1477/1502)	35/60)	1477/1498)
Xpert Ultra	0.82 (0.70, 0.91)	0.96 (0.95, 0.97)	0.46 (0.36, 0.56)	0.99 (0.99, 1.00)
	(46/56)	(1448/1502)	46/100)	1448/1458)
Statistical test[4]	p-value <0.001	p-value=0.001		
Trace recategorization as negative				
Xpert Ultra	0.90 (0.85, 0.94)	0.98 (0.97, 0.98)	0.80 (0.73, 0.85)	0.99 (0.98, 0.99)
	(147/163)	(1471/1508)	147/184)	1471/1487)
Statistical test[4]	p-value=0.04	p-value=0.53		
Trace conditional recategorization [5]				
Xpert Ultra	0.93 (0.87, 0.96)	0.96 (0.95, 0.97)	0.74 (0.67, 0.80)	0.99 (0.99, 1.00)
	151/163)	1454/1508)	151/205)	1454/1466)
Statistical test[4]	p-value <0.001	p-value= 0.15		

[1]95% CI: 95% Confidence interval; [2]PPV: Positive predictive value ; [3]NPV: Negative predictive value; [4]McNemar´s test for evaluation of differences in test parameters among Xpert and Ultra; [5]Trace conditional recategorization: recategorization of trace results as negative, if patients had been previously treated.

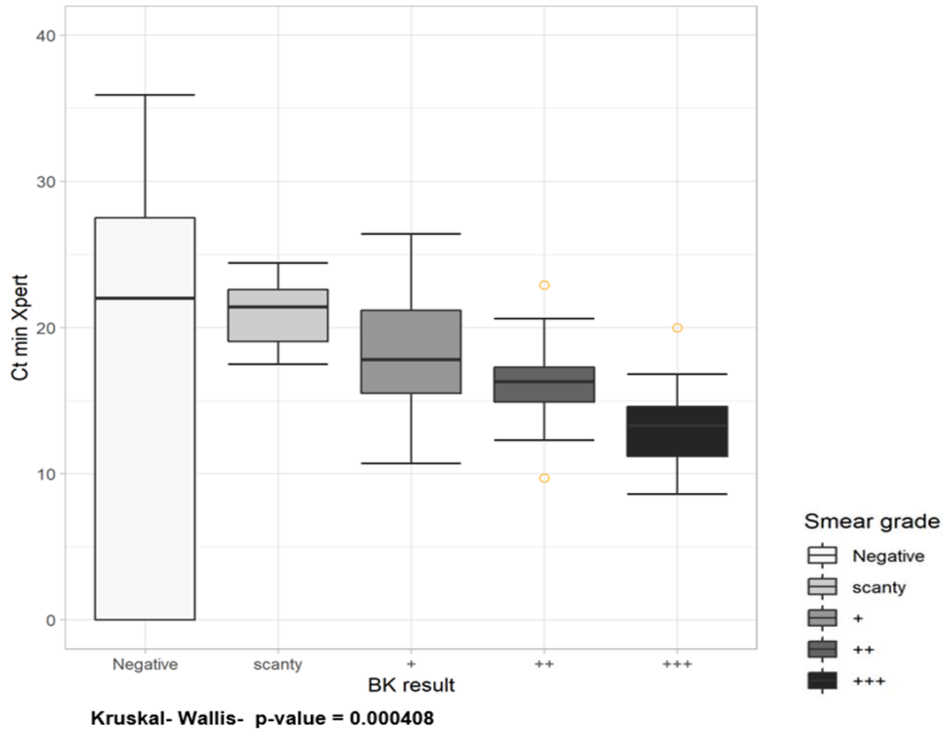
Appendix 6. Cross- tabulation of Xpert and Ultra results, displayed by categories (relying on bacillary burden and rifampicin resistance detection), and stratified by cohort. n(%)

	Ultra results- PCF [1] cohort - n= 1419									
	High R	**High S**	**Medium R**	**Medium S**	**Low R**	**Low S**	**Very low R**	**Very low S**	**Trace**	**Negative**
Xpert MTB/RIF										
High R[2]	4(0.3)	-	-	-	-	-	-	-	-	-
High S[3]	-	42(3.0)	-	24(1.7)	-	1(0.1)	-	-	-	-
Medium R	3(0.2)	-	4(0.3)	-	-	-	-	-	-	-
Medium S	-	6(0.4)	-	25(1.8)	-	2(0.1)	-	-	-	-
Low R	-	-	1(0.1)	-	1(0.1)	-	-	-	-	-
Low S	-	-	1(0.1)	17(1.2)	1(0.1)	12(0.8)	-	2(0.1)	-	-
Very low R	-	-	-	-	-	-	1(0.1)	-	-	-
Very low S	-	-	-	1(0.1)	-	2(0.1)	-	9	4(0.3)	-
Very low I[4]	-	-	-	-	-	-	-	2(0.1)	1(0.1)	-
Negative	-	-	-	-	-	1(0.1)	-	15(1.1)	19(0.6)	1218(85.8)

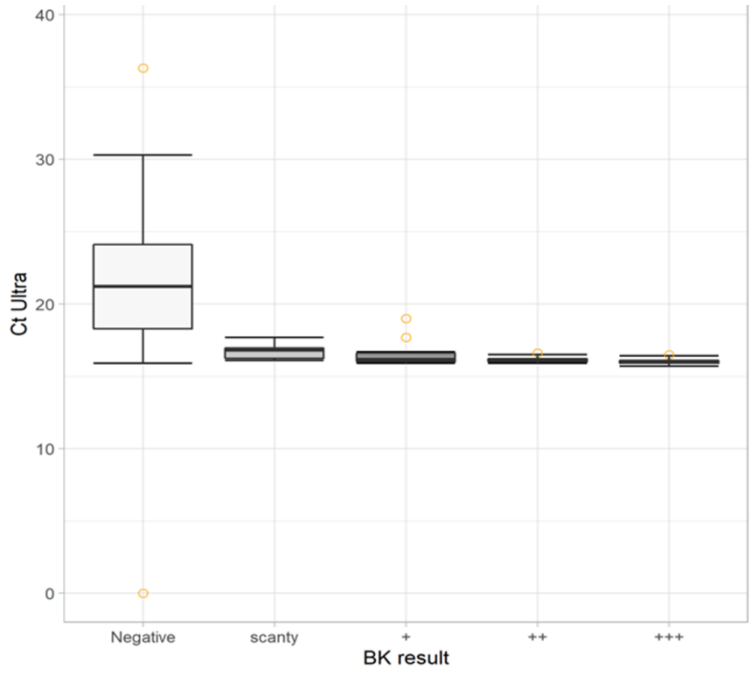
	Ultra results- ACF[4] cohort - n=252									
	High R	**High S**	**Medium R**	**Medium S**	**Low R**	**Low S**	**Very low R**	**Very low S**	**Trace**	**Negative**
Xpert MTB/RIF										
High R	-	-	-	-	-	-	-	-	-	-
High S	-	-	-	1(0.4)	-	-	-	-	-	-
Medium R	-	-	1(0.4)	-	-	-	-	-	-	-
Medium S	-	-	-	-	-	-	-	-	-	-
Low R	-	-	-	-	-	-	-	-	-	-
Low S	-	-	-	-	-	2(0.8)	-	-	-	-
Very low R	-	-	-	-	-	-	-	-	-	-
Very low S	-	-	-	-	-	-	-	1(0.4)	-	-
Very low I	-	-	-	-	-	-	-	-	1(0.4)	1(0.4)
Negative	-	-	-	-	-	-	-	2	4(1.6)	239(94.8)

Results are displayed in absolute numbers and percentages (in brackets) , and cross-tabulated by burden of Xpert and Ultra outputs: High, Medium, Low, Very low, Negative and Trace (only in the case of Xpert Ultra test). [1]PCF: Passive Case Finding ; [2]R: rifampicin resistance detected; [3]S: rifampicin resistance not detected; [4]I: results on rifampicin resistance indeterminate; [5]ACF: Active Case Finding.

Appendix 7. Additional Figure 1. Correlation of Xpert and Ultra Cts with traditional smear grade.



Kruskal- Wallis- p-value = 0.000408



Kruskal- Wallis- p-value < 0.0001

Comparison between traditional measures of bacillary load (international grade for smear microscopy) and cycle thresholds (Cts) for Xpert and Ultra. We have used the Kruskal-Wallis test to assess whether associaton among measures is present. X-axis represents smear grade. Y-axis represents Ct values: Ct min Xpert = minimum Ct of rpoB for Xpert results. Ct Ultra= Ct for IS6110/1081 probe.

Appendix 8. Contingency table showing the distribution of results by test (absolute numbers)

PCF [1] cohort - n= 1419			
	Culture +	**Culture -**	**Total**
Smear +	106/157	6/1262	112/1419
Smear -	51/157	1256/1262	1307/1419
Xpert MTB/RIF +	138/157	28/1262	166/1419
Xpert MTB/RIF -	19/157	1234/1262	1253/1419
Xpert Ultra +	149/157	52/1262	201/1419
Xpert Ultra -	8/157	1210/1262	1218/1419
Smear negative=1307			
	Culture +	**Culture -**	**Total**
Xpert MTB/RIF +	32/51	22/1256	54/1307
Xpert MTB/RIF -	19/51	1234/1256	1253/1307
Xpert Ultra +	43/51	46/1256	89/1307
Xpert Ultra -	8/51	1210/1256	1218/1307
Smear positive=112			
	Culture +	**Culture -**	**Total**
Xpert MTB/RIF +	106/112	6/0	112/112
Xpert MTB/RIF -	0/112	0/0	0/112
Xpert Ultra +	106/112	6/0	112/112
Xpert Ultra -	0/112	0/0	0/112
ACF[2] cohort - n=252			
	Culture +	Culture	Total
Smear +	1/6	0/246	1/252
Smear -	5/6	246/246	251/252
Xpert MTB/RIF +	4/6	3/246	7/252
Xpert MTB/RIF -	2/6	243/246	245/252
Xpert Ultra +	4/6	8/246	12/252
Xpert Ultra -	2/6	238/246	240/252

The table shows cross-tabulation of results of Xpert and Ultra, dissagregated by strategy and, in case of the PCF cohort, by smear results, against the gold standard (aggregated culture). Denominators indicate column figures (culture results) and numerators indicate row figures (results from comparator tests). [1]PCF: Passive Case Finding ; [2] ACF: Active Case Finding.