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## Diagnostic Approaches For Paediatric Tuberculosis By Use Of Different Specimen Types, Culture Methods, And Pcr:

### A Prospective Case-Control Study

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## Abstract

**Background**—The diagnosis of pulmonary tuberculosis (PTB) presents challenges in children, because symptoms are non-specific, specimens are difficult to obtain, and *Mycobacterium tuberculosis* (MTB) cultures and smears are often negative. The primary objective was to evaluate new diagnostic approaches for TB in children in a resource-poor country.

**Methods**—MTB culture by two techniques and a heminested IS 6110 polymerase chain reaction (PCR) assay were performed on specimens from 218 Peruvian children with symptoms suggestive of PTB and 238 healthy controls. Cases were grouped into moderate- and high-risk categories by Stegen-Toledo score. Two specimens of each type (gastric aspirate [GA], nasopharyngeal aspirate [NPA], and stool specimens) from each case were examined by 1) auramine smear microscopy, 2) broth culture by Microscopic-Observation Drug-Susceptibility (MODS) technique, 3) standard culture on Lowenstein Jensen (LJ) medium, and 4) PCR. Specimens from controls included a single NPA and two stools, examined with the same techniques. Subjects were enrolled 2002 to 2007 at two hospitals in Lima, Peru. Controls were enrolled from a low income shantytown community in south Lima.

**Findings**—Twenty-two case subjects (10%) had at least one positive MTB culture (from GA in 22 cases, NPA in 12 cases, and stool in 4 cases). Laboratory confirmation of tuberculosis was more frequent in high-risk than moderate-risk cases. MODS was significantly more sensitive than LJ culture, diagnosing 20/22 vs. 13/22 patients ( $P=0.015$ ), and MTB isolation by MODS was faster than by LJ culture (mean 10 days vs. 25 days,  $P<0.001$ ). All 22 culture-confirmed cases had at least one culture-positive GA, and the addition of the second GA specimen increased detection of culture-positive cases by 37%. In high-risk children duplicate GA PCR identified half of all culture-positive cases.

**Interpretation**—MODS culture increased PTB diagnostic sensitivity and speed compared with LJ culture. Although most children treated for presumed PTB were culture-negative in all specimens, MODS culture of duplicate GAs considerably improved recovery by culture. PCR was insufficiently sensitive or specific for routine diagnostic use, but in high risk children duplicate GA PCR provided same-day identification of half of all culture-positive cases. Collection of duplicate GA specimens from high-risk children for MODS culture was the optimal diagnostic test.

## Keywords

tuberculosis; culture; polymerase chain reaction; diagnosis; children; Peru

## INTRODUCTION

While tuberculosis (TB) control programs have made major strides in reducing TB incidence in adults in many high-burden countries, assessing program impact on pediatric TB remains difficult because of the lack of a dependable “gold standard test”. Children comprise an estimated 20% of the total TB caseload in high-incidence communities,<sup>1,2,3</sup> but most surveillance programs only count acid-fast bacillus (AFB) smear-positive cases, excluding over 95% of presumptive TB cases among children under 12.<sup>4</sup> Adequate diagnosis of pediatric TB is difficult due to lack of sputum production and paucity or absence of organisms in respiratory secretions, because TB bacilli typically remain confined to perihilar nodes that do not rupture into the bronchus.<sup>5</sup>

Technical and economic factors compound these diagnostic problems. The best available diagnostic tests are costly, while traditional methods are slow and insensitive. Even under optimal circumstances, *Mycobacterium tuberculosis* (MTB) is isolated in fewer than 50% of children thought to have TB clinically.<sup>6,7,8,9,10</sup> Consequently, physicians often rely on poorly validated scoring systems.<sup>11,12,13</sup> Even in 2010, we still depend primarily on tools available

since the 1950s to presumptively diagnose pediatric TB: PPD skin test, chest X-ray, history and physical exam, usually without bacteriologic confirmation.

The primary goal of this study was to address these problems by evaluating new methods to diagnose PTB in children, including a heminested PCR assay (for potential rapid results), improved culture methods, and alternative clinical specimens. Our study evaluated these outcomes among Peruvian children with suspected PTB:

1. **MTB recovery from non-invasive specimens, such as nasopharyngeal aspirates (NPA) and stool, as compared to gastric aspirates (GA), from single vs. duplicate specimens.** Our previous data suggest that NPAs are a useful and less invasive alternative to GAs for diagnosis of TB in children<sup>14</sup>, and stool is potentially useful for TB PCR.
2. **The speed and sensitivity of MTB isolation by Microscopic-Observation Drug-Susceptibility (MODS) culture as compared to conventional Lowenstein-Jensen (LJ) culture, and determine the added benefit of repeated testing.** The study allowed us to expand our previous experience in children using the MODS technique<sup>15</sup>, to determine the extent to which the enhanced microbiological sensitivity of this technique translates into clinical utility in diagnosing children.
3. **The sensitivity, specificity, and predictive values of a heminested PCR assay as compared to MTB culture.** PCR technology may be available in reference labs in resource-poor countries, providing results within 6 hours that can be used in initial clinical decision-making. An age- and sex-matched control group was included to determine test specificity.

## MATERIALS AND METHODS

The study design is summarized in Figure 1.

### Subjects

This study included cases with clinical evidence suggestive of pulmonary tuberculosis, and age and sex-matched healthy controls. Children with evidence of HIV infection or AIDS were excluded. Cases were enrolled between April 2002 and January 2007 at the Instituto de Salud del Niño (ISN) and the Hospital Nacional Cayetano Heredia in Lima, Peru. Pediatricians evaluating children with respiratory illness in clinics at the two participating hospitals referred patients that they suspected of having PTB to a physician co-investigator or study nurse, who then assessed their eligibility for participation based on criteria described below. Subjects were classified according to the clinical criteria of Stegen and Jones<sup>16</sup> (i.e. “Jones Score” in Africa) for diagnosis of pediatric TB as revised by Toledo,<sup>17</sup> with modifications as described below. This “Stegen-Toledo” (ST) system is widely used throughout Latin America, and criteria used to determine ST score are shown in Table 1. Inclusion criteria for cases were 1) age  $\leq 12$  years, 2) ST score  $\geq 5$  points, and 3) absence of antituberculous therapy. Although positive MTB culture is one of the Stegen-Toledo criteria, culture results are a primary outcome of this study and were not available at enrollment, so this criterion was **not included** in our modified scoring system for subject eligibility. Cases were classified as either moderate-risk (ST score 5–6) or high-risk (ST score  $> 7$ ).

Subjects were enrolled by parental informed consent. The protocol and consent forms were approved by the Institutional Review Boards of Tulane Medical Center, Johns Hopkins Bloomberg School of Public Health, Asociacion Benefica PRISMA, the US Naval Medical Research Detachment (Lima, Peru), Hospital Nacional Cayetano Heredia, and the Instituto Nacional de Salud del Niño of Lima, Peru. Empiric treatment for tuberculosis was administered

according to standard guidelines of the World Health Organization and the Peruvian Ministry of Health. Therapeutic decisions including inpatient versus outpatient management and treatment protocols used were determined by local hospital physicians. The study procedures did not necessitate hospitalization.

**Age- and sex-matched control group subjects** were enrolled on an ongoing basis from the Pampas de San Juan, a low-income shantytown community in south Lima that is within the area served by one of the hospitals from which cases were recruited. Inclusion criteria were: a) absence of chronic cough, fever, or evidence of pulmonary disease; b) absence of prior clinical TB; c) no prior or current TB treatment; and d) absence of recent (past 2 months) household contacts with known or suspected PTB.

### Screening for HIV infection

Cases and controls were screened for HIV infection by duplicate commercial assays. HIV DNA PCR tests were performed in children less than 18 months old. Screening for HIV infection was requested, but not required if parents refused the test. Cases and controls who declined HIV testing were included and classified as HIV-negative if they had no history of HIV exposure and no clinical evidence of HIV/AIDS.

### Data and Specimens Collected

Demographic and clinical data, including tuberculin skin test (TST) results, were collected from cases and controls. Data from only cases included medical history, physical exam findings, and a chest radiograph read by a pediatric radiologist. Specimens collected from cases for TB cultures and PCR were:

1. Gastric aspirates (GAs) were collected on 2 successive early mornings (6–7 am) by brief (<10 minute) nasogastric intubation following an overnight fast. The volume of gastric aspirates was augmented as needed by injecting 5 ml. sterile water and aspirating back.
2. Nasopharyngeal aspirates (NPAs) were collected daily for 2 days by inserting a soft flexible nasopharyngeal tube into the nasopharynx, lavaging with 5 ml. of saline solution, and aspirating with an electrical suction device or hand-held aspirator. The NPA procedure induces a cough and sputum production, which is then aspirated from the nasopharynx.
3. Stool specimens were collected daily for 2 days.

All specimens from presumptive PTB cases were collected in the hospital within 5 days of enrollment, before starting anti-TB therapy. Blood samples were collected from cases for serum albumin, as an indicator of nutritional status.

Specimens collected from controls for TB cultures and PCR assays were one NPA specimen and two stool specimens. No gastric aspirates were collected from controls due to the invasive nature of the test, and only single NPAs were collected from controls because this test is unpleasant for some children, and repeat NPA testing of controls was not acceptable to many parents.

### Tuberculosis diagnostic techniques

Specimens were decontaminated with 0.5% N-acetyl-L-cysteine, 2% NaOH, and 1.45% sodium citrate, as described, and the centrifuged pellet was re-suspended in 2 milliliters 0.2% bovine serum albumin.<sup>18</sup> Prior to decontamination, stools were prepared by suspending 0.1 gram in 6 ml. phosphate buffered saline, homogenizing, and settling for 10 minutes to separate, after which the supernatant was processed. This stool processing technique was found in pilot

work to provide the optimal compromise between detection sensitivity versus culture contamination. Specimens were tested by:

1. **Microscopic-Observation Drug-Susceptibility (MODS) Method**<sup>19,20</sup> 500 µl of each decontaminated specimen were inoculated into modified Middlebrook 7H9 media and separated into four 1.2 ml aliquots that were cultured in a sterile 24-well plate. Plates were placed in a plastic resealable bag, incubated at 37°C, and examined every other day without opening for up to 30 days by inverted light microscopy. Presumptive MTB isolates with cording morphology were reported as positive and all were subsequently confirmed by hemi-nested IS 6110 MTB PCR. Direct concurrent drug susceptibility testing was not done.
2. **Lowenstein-Jensen Agar Culture:** 250 µl of each decontaminated specimen was inoculated onto a Lowenstein-Jensen (LJ) slant, incubated at 37°C, and examined twice weekly from the first to eighth week after inoculation.
3. **Auramine stain (Smear test):** Two drops of each decontaminated specimen were dried on a microscope slide, stained with 0.1% Auramine O, and examined at 100X magnification. Positive tests had ≥ 5 bacilli counted in 300 fields.
4. **N2 IS6110-PCR Procedure (referred to as “PCR testing”).**<sup>21</sup> DNA was extracted from 500 µl of each decontaminated specimen, and two consecutive nested IS 6110 PCRs were performed using outer and inner primers. To reduce the risk of false positive PCR reactions, separate rooms were used for DNA extraction, PCR mix preparation, amplification and electrophoresis with protective clothing. Every hemi-nested PCR assay included positive controls of genomic *M. tuberculosis* DNA and negative controls of water added to the PCR reagents in place of the sample DNA.

### Data Analysis

Demographic, clinical data and culture and PCR results were compared among cases by ST risk group (5–6 points = “moderate-risk”; ≥ 7 points = “high-risk”) and controls. Culture and PCR results were compared by sample and by person. A negative culture result was considered “valid” (i.e. readable and contamination-free) if at least one valid LJ and one valid MODS result was available. A case with negative cultures was valid if all three types of specimen (GA, NA, and Stool) had valid results for duplicate specimens. For controls, NA and stool cultures required valid results for inclusion in analysis. Chi-square and McNemar’s tests were used for categorical variables; two-tailed T-test or Wilcoxon Rank Sum Test for continuous variables. Multiple logistic regression was used for adjustment for potentially confounding variables. Recovery rates for MTB were compared by culture method for subjects and specimens, grouped by specimen type (GA, NPA, stool) and by auramine stain result. Subjects with at least one clinical specimen that was culture-positive for MTB by any method were included in the “culture-positive” group. Data analysis was performed using STATA Version 11 (STATA Corp., College Station, TX) and EpiInfo Version 6 (Centers for Disease Control and Prevention; Atlanta, GA) software programs.

## RESULTS

The study population constituted of 456 analyzable subjects with complete demographic information and most laboratory data complete, including 218 presumptive TB cases (20% inpatients; 80% outpatients) and 238 controls. Eighty-one potential case subjects were not enrolled because the parents declined to participate, and three controls without valid LJ results were excluded. These children were similar in age and sex distribution to enrolled subjects.

Demographic characteristics of cases and controls and distribution of the characteristics used to determine the modified Stegen-Toledo criteria used are shown in Table 1. Among cases, 153 subjects (70%) had an ST score of  $\geq 7$  (high-risk), and 65 cases had ST scores 5–6 (moderate risk for PTB). Most of the high risk group patients had abnormal CXRs, and the most common findings were interstitial markings (54% of cases) and hilar adenopathy (44% of cases). In 22% of cases a primary Ghon complex was present. Among moderate risk group subjects, 40% had suggestive CXRs (all interstitial markings and/or hilar adenopathy), and none had a primary Ghon complex. BCG vaccination rate was lower in the cases than in controls ( $p=0.006$ ). As compared to high-risk cases, the controls demonstrated lower income and lower maternal education level. HIV test results were negative in 179 cases (82%) and the test was declined in 39 cases (18%). No cases or controls were found to be HIV-positive based on screening tests.

### TB Culture Results in cases and controls

Overall, 22 case subjects (10%) had at least one positive MTB culture, based on MODS and LJ results combined. MTB was isolated from GA in all 22 cases, NPA in 12 cases, and stool in 4 cases. In all, 58 culture-positive specimens were obtained from case subjects, including 33 GAs, 20 NPAs, and 5 stool specimens. Seventeen of these 58 specimens (29%) were auramine microscopy-positive.

There was a significant association between high-risk group and positive TB culture results (Table 2). One of 61 moderate risk subjects with complete specimen collection was culture-positive (1.6%) versus 21 of 149 (14.1%) high-risk subjects. The modified ST scores of case subjects with a positive culture for MTB [median: 9 points; range: 6–13] were significantly greater than for culture-negative subjects [7 points; range 5–14 ( $p < 0.001$ )]. Thirteen of 153 high-risk patients and 7 of 65 moderate-risk patients were hospitalized, and hospitalization was associated with higher rates of positive MTB cultures (7/19 inpatient [36.8%] versus 15/191 outpatient [7.9%]; OR 6.8 [2.3–19.9],  $p < 0.001$ ). None of the samples from the 238 controls were MTB culture-positive.

Culture-positive subjects were similar to culture-negative subjects based on sex distribution and age. Mean serum albumin (4.2 for culture-positives vs. 4.5) and prevalence of serum albumin  $< 4.5$  (81% of culture-positives vs. 44%) were both significantly different between groups ( $p=0.0015$ ).

Mean time from sample processing to isolation of MTB was significantly shorter for MODS cultures (mean 10 days [25%–75% range 8–11 days]) as compared to LJ cultures (mean 25 days [25%–75% range 20–30 days];  $p < 0.001$ ). MODS cultures were uninterpretable because of bacterial/fungal contamination significantly less often than LJ (0.96% vs. 7.71% respectively,  $P < 0.001$ ) and there were no significant differences between the contamination rates between stool, NPA and GA specimens.

### MTB recovery from single vs. duplicate specimens

As in our preliminary analysis<sup>15</sup>, which presented data that are expanded in the current manuscript, the MODS technique was significantly more sensitive than other tests. Here we extend previous results to determine how this test performance affected patient diagnosis. Figure 2 shows recovery of MTB from the 22 culture-positive subjects by culture method and specimen type. While all 22 subjects were culture positive in at least one GA, 11 of these 22 subjects only had positive cultures from one of two GA specimens (Figure 2). When results were analyzed based on the order of collection for duplicate specimens, MTB was isolated from the first GA specimen collected in 16/22 patients, while in 6/22 patients only the second

GA specimen was culture-positive (37% greater yield by adding a second specimen). Addition of a second NPA specimen increased yield by 50%.

### PCR and Culture Results by Study Group

PCR results and culture results for high- and moderate-risk cases and controls are shown in Table 2. The proportions of patients with at least one positive PCR result were similar between high-risk and moderate-risk cases, and both groups had significantly higher proportions of positive PCR compared to the control group ( $p < 0.001$ ).

Fifteen of 238 controls had at least one positive PCR result (5 from NPA; 10 from stool). No control subject had more than one positive PCR result. Fourteen controls were found to have a positive PPD skin test, but only one of these 14 subjects also had a positive PCR result (NPA specimen). All controls with a positive PPD were evaluated by a physician, examined by CXR, assessed not to have tuberculous disease, and given preventive therapy. None of these controls with positive PPDs or PCRs developed further evidence of clinical TB during the year after enrollment. Controls had twice as many stool samples as NPA samples per subject, and since 4.2% of controls had a PCR-positive stool and 2.2% of controls had a PCR-positive NPA, this suggests that the rate of false-positive PCR results in any individual specimen (from these highly selected children with minimal risk of TB) was approximately 2%.

### Comparison of PCR results among culture-positive and culture-negative cases

Table 3 compares PCR results among the 21 culture-positive subjects versus the 128 culture-negative subjects and includes all specimens from high-risk cases. Culture-positive cases were three times more likely to have at least one PCR-positive specimen than culture-negative cases. Similar results were found in analyses limited to each specimen type. To assess the potential for PCR to be used to identify high-risk pediatric subjects likely to be culture-positive, PCR sensitivity, specificity, and predictive values were analyzed using culture as the reference standard (Table 3). In this high-risk group, a positive PCR from a GA was associated with a positive predictive value (for a positive culture result) of 50%. Sensitivity of PCR in this subgroup was 62% for GAs and for any specimen, but false-positive PCR results (using culture as the reference standard) equaled or exceeded true positives for all specimen types. However, both specificity and positive predictive values must be reduced by the limited sensitivity of culture as a reference standard.

## DISCUSSION

This study presents a direct comparison of multiple culture methods and PCR, using multiple specimen types for both approaches, for diagnosing pediatric TB in a resource-poor area. In these Peruvian children, MODS approximately doubled the diagnostic sensitivity of culture and halved the time required as compared to traditional LJ agar. Seventy percent more pediatric subjects were diagnosed as having culture-positive TB by MODS, and all specimens from these additional cases were auramine smear-negative. The speed and sensitivity of the MODS technique results from the use of culture broth with microscopic detection of positive cultures before they are large enough to be visible to the naked eye and the capacity of this technique to culture a large volume of each clinical specimen.<sup>20</sup> This increased diagnostic yield in pediatric patients with culture-proven TB by adding MODS greatly exceeds the 10–20% additional yield observed in high-risk Peruvian adults<sup>20</sup>, as would be expected for diagnostic samples from children that usually contain few mycobacteria. Our results support WHO recommendations, which suggest that liquid TB culture techniques are superior to agar-based techniques.<sup>18</sup> MODS performed significantly better than LJ for auramine-negative specimens, which include the vast majority of culture-positive pediatric specimens.

MTB recovery by culture from gastric aspirates (22/22 cases) was clearly superior to recovery from stool (4/22 cases) and from NPAs (12/22 cases). The 37% additional yield of culture-positive patients identified by a second gastric aspirate greatly exceeded the 5–10% additional yield from a second sputum culture in Peruvian adults.<sup>20</sup> One explanation of this major incremental benefit from a second specimen in children is that adults tend to already be smear-positive and with high bacillary loads at detection, whereas children with paucibacillary disease benefit much more from a sensitive method like MODS. In almost half of the patients studied, GAs were the only specimens that were culture-positive, and in 5 of these cases only a single specimen was culture-positive, demonstrating the importance of testing at least duplicate specimens.

We found that “high-risk” clinical scores were associated with higher likelihood of a positive MTB culture. Larger numbers of moderate-risk subjects would be useful to substantiate this finding, as only one moderate-risk culture-positive case was identified in this study. While this result suggests that the ST score may correlate with rates of MTB isolation from children, it also emphasizes the fact that, even with intensive culture methods, 85% of high-risk cases were still culture-negative. It remains difficult to determine how many of these children have culture-negative PTB, but data from cases with positive cultures (e.g. the predominance of smear-negative specimens, the high proportion of cases confirmed by a single MODS culture-positive GA) suggest that many of these cases are in fact TB. Other new techniques, such as induced sputum with increased recovery of MTB by culture in African children,<sup>22,23</sup> may help to confirm these suspicions.

The N2 IS6110 PCR was positive more often in subgroups with greater clinical suspicion of TB or positive cultures. Our findings corroborate those of a prior retrospective study of IS6110 stool PCR<sup>24</sup>, and this larger prospective study also demonstrates that sensitivity of PCR to detect culture-positive children was greater for both NPAs and GAs as compared to stool specimens. However, several healthy controls had positive PCR results despite having no evidence of TB exposure or disease, implying that these were false-positives, occurring in approximately 2% of specimens despite extensive precautions to prevent PCR contamination. These results also raise the possibility that the PCR assay is detecting the acquisition or presence of latent tuberculosis or early, asymptomatic disease. Our one year follow-up of control group children for signs of clinical TB did not suggest this, but additional studies may be needed to better assess this possibility.

In spite of these non-specific positive results, in selected cases we found that PCR may be useful to identify children likely to have culture-positive PTB. Among high-risk children, a positive PCR from a GA was associated with a positive predictive value (for a positive culture result) of 50%, i.e. it identified a subgroup of high-risk patients with a 50% rate of positive cultures using a test result that can be available within hours. Tempering these encouraging results, 8 of 21 children (38%) in the high-risk, culture-positive group were PCR-negative in all specimens. Thus our results do not support the routine use of PCR for pediatric TB diagnosis, but demonstrate that the same-day GA PCR test may be useful as a screening test for high-risk children, because it rapidly identifies those children in the high-risk group who are three times more likely to have culture-proven TB (See Table 3).

The results reported in this study are subject to several limitations. Firstly, cases were enrolled based on Stegen-Toledo score, which although widely used is poorly validated. The association between ST risk group and proportion of culture-confirmed cases was notable, somewhat diminishing this limitation. Secondly, it is possible that potential subjects who did not enroll were different than those who did enroll in the study. Most of the potential subjects whose parents declined to participate were unwilling or unable to return to the clinic for subsequent sample collection, because participation required several days’ attendance. This was usually

due to parental work demands or to distance from home to the clinic. These subjects were not different from enrolled subjects based on age or sex, but there could have been differences in other variables. Third, we chose to include cases and controls who declined HIV testing and lacked evidence of HIV/AIDS. While we cannot exclude occult HIV infection, there is significant cultural stigma associated with HIV testing and a very low HIV seroprevalence in Lima (approximately 0.5% among adults, <0.1 % among children under 12)<sup>25</sup>, so including these children in the analysis seemed appropriate. Fourth, control subjects were only evaluated with a single NPA specimen and no GA specimens, while two NPAs and two GAs were collected from case subjects. The extended sampling strategy for cases is justified from a standard of care perspective, since there is potential direct benefit to the subject from the extended sampling. We did not believe that the discomfort of gastric aspiration was justified for a well child. NPA collection is unpleasant for some, and many parents were unwilling to agree to repeated NPA sampling. While our sampling strategy therefore differed between cases and controls, the study provided the most extensive data to date on specificity of the NPA culture for TB in children, providing samples from over 200 children in a population where MTB infection is common. Among cases with positive NPA cultures, approximately two-thirds were culture positive in both NPA specimens collected, suggesting that examination of a single NPA specimen was reasonable given the logistical and human subjects considerations.

In conclusion, MODS culture increased PTB diagnostic sensitivity and speed compared with conventional LJ culture. Gastric aspirate cultures improved case detection as compared to NPA cultures. Although most children treated for presumed PTB were culture-negative in all specimens, MODS culture of duplicate gastric aspirates considerably improved the yield of laboratory confirmation. PCR was insufficiently sensitive and specific for routine diagnostic use, but in high risk children duplicate GA PCR rapidly identified a subgroup with a 50% rate of positive cultures. Collection of duplicate GA specimens from high-risk children for MODS culture increased microbiologic diagnosis of TB by more than one third..

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## List of Abbreviations

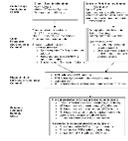
<b>PTB</b>	pulmonary tuberculosis
<b>MTB</b>	<i>Mycobacterium tuberculosis</i>
<b>LJ</b>	Lowenstein Jensen culture
<b>GA</b>	gastric aspirate

<b>NPA</b>	nasopharyngeal aspirate
<b>MODS</b>	Microscopic Observation Drug Susceptibility technique
<b>WHO</b>	World Health Organization
<b>AFB</b>	acid-fast bacillus
<b>ST</b>	“Stegen-Toledo” criteria
<b>HIV</b>	Human Immunodeficiency Virus
<b>PCR</b>	Polymerase Chain Reaction

## References

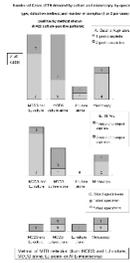
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**Figure 1. PROJECT FLOW CHART**

\*Patients were classified according to the ST score as moderate risk = ST score = 5–6 pts and high risk = ST score  $\geq 7$



**FIGURE 2.**  
Number of Cases of TB detected by culture and microscopy, by specimen type, detection method, and number of samples (1 or 2 per case)

Table 1

Demographic Features and Modified Stegen-Toledo (ST) Criteria<sup>18,19</sup>  
 Demographic features of subjects in the high-risk case group, moderate-risk case group, and the control group. For case groups, the number of subjects positive for each of the factors used to determine the Modified Stegen-Toledo (ST) Criteria are reported. Part A shows the number of subjects in each group with the characteristic described, along with the percentage of each group with each characteristic (when data were not available from all subjects, only those with data for that characteristic are included for determining percentages). Part B includes all characteristics not reported based on number of subjects, and measurements used are indicated for each characteristic.

	High Risk (ST>7)		Mod. Risk (ST 5-6)		Control		p <sup>†</sup>
	n = 153 subjects	(% of total)	n = 65 subjects	(% of total)	n = 238 subjects	(% of total)	
<b>A. Characteristics by # of subjects</b>							
<b>Modified Stegen -Toledo (ST) Criteria<sup>#</sup></b>							
Primary lesion by radiography (4 points) <sup>α</sup>	34	22.2	0	0.0	NA	NA	NA
Tuberculin skin test positive (>10 mm) (3 points)	144	95.4	53	81.5	14	5.9	<0.001
Contact with TB patient past 2 yrs. (2 pts.)	141	92.2	42	64.6	8	3.4	<0.001
Suggestive X-ray (2 points) <sup>α</sup>	118	77.1	26	40.0	NA	NA	NA
Cough > 2 weeks (2 points)	80	52.3	21	32.3	NA	NA	NA
Hospitalized	13	8.5	7	10.8	NA	NA	NA
Income:							
< 400 soles	68	48.2	28	47.5	190	79.8	
≥ 400 soles	73	51.8	31	52.5	48	20.2	<0.001
Mother's education:							
Primary or less	34	22.2	10	15.6	82	34.5	
Secondary or more	119	77.8	54	84.4	156	65.5	0.010
BCG vaccination							
Yes	124	82.7	55	85.9	216	91.9	
No	26	17.3	9	14.1	19	8.1	0.006
<b>B. Other characteristics</b>							
Median ST score [range]	7 [7-14]		5 [5-6]		NA	NA	NA
Median tuberculin skin test size [range]	15 [12-20]		13 [10-18]		0 [0-2]		<0.001
Age, in years [range]	3 [0-12]		4 [0-11]		4 [0-12]		NS
Gender (# male subjects: # female subjects)	78: 75		34: 31		128: 110		NS
Median %weight-for-age [range]	97 [88-106]		94 [85-104]		94 [87-104]		0.058
Median % height-for-age [range]	98 [95-101]		98 [95-103]		96 [93-99]		<0.001

\* P<sub>1</sub> - P-value comparing high- and moderate-risk groups

<sup>f</sup> P: P-value comparing high-risk group and control group

<sup>a</sup> In each case points were assigned for only one of these radiographic criteria

<sup>#</sup> Standard interpretation for ST score is high-risk for TB:  $\geq 7$  pts.; moderate risk for TB: 5–6 pts.; low risk for TB: 3–4 pts.; unlikely TB: 0–2 pts.

NA: Not applicable

NS: Not significant

**Table 2**  
**PCR and culture results among high- and moderate-risk cases and controls**

Number of subjects (percent) with at least one positive MTB culture or PCR result in a clinical specimen, grouped by type of specimen. Analyses by specimen type limited to cases with complete data for specimens of the indicated type by the method used (culture or PCR). “Any specimen” indicates all specimen types combined (for cases, limited to those with 2 GA, NP, and stool specimens; for controls, limited to those with 1 NP and 2 stool specimens).

	ST Score Risk Groups					
	High Risk (ST≥7)		Mod. Risk (ST 5–6)		Control group	
	n (%)	n (%)	AOR [95%CI]*	p*	n (%)	AOR [95%CI]*
Total # subjects per group	153	65			238	
A. Culture	21 (14.1)	1 (1.6)			0 (0)	
Any Spec.	128 (85.9)	60 (98.4)	12.1 [1.5–97.1]	<b>0.02</b>	227 (100)	-
NPX	11 (7.2)	1 (1.6)				
Any Spec.	141 (92.8)	62 (98.4)	6.2 [0.7–53.6]	0.10		
GAX	21 (13.8)	1 (1.6)				
Any Spec.	131 (86.2)	63 (98.4)	13.1 [1.6–105.6]	<b>0.02</b>		
STX	3 (2.0)	1 (1.6)				
Any Spec.	145 (98.0)	61 (98.4)	1.3 [0.1–12.8]	0.82	15 (6.6)	
B. PCR	40 (26.3)	15 (23.4)			212 (93.4)	5.0 [2.6–9.9]
Any Spec.	112 (73.7)	49 (76.6)	1.2 [0.6–2.4]	0.67	5 (2.2)	<b>&lt;0.001</b>
NPX	22 (14.4)	4 (6.2)			223 (97.8)	7.2 [2.5–20.2]
Any Spec.	131 (85.6)	61 (93.8)	2.8 [0.9–8.6]	0.07		<b>&lt;0.001</b>
GAX	27 (17.8)	8 (12.3)				
Any Spec.	125 (82.2)	57 (87.7)	1.6 [0.7–3.8]	0.30		
STX	13 (8.7)	4 (6.2)			10	
Any Spec.	137 (91.3)	60 (93.8)	1.5 [0.5–5.1]	0.50	227	2.4 [1.0–5.9]

\* AOR – Adjusted odds ratio. Odd ratios and *p* values were adjusted for “hospitalized/out patient” for the comparison between the high-risk (ST ≥ 7) group and the moderate-risk [ST 5–6] group and for “Income” and “Mother’s education” for the comparison between the high-risk (ST ≥ 7) group and the control group).

<sup>†</sup> *p* values obtained from chi-square tests stratified by “income” and “instruction” were < 0.01

XNP – Nasopharyngeal Aspirate, GA – Gastric Aspirate, ST – Stool sample.

Agreement of PCR and Culture results, and predictive values of PCR for detecting subjects with at least one positive culture among High-Risk Cases

**Table 3**

PCR	Culture		Positive n = 21 (62%)	Negative n = 128 (20%)	Sensitivity	Specificity	PPV	NPV	Kappa
	Any Spec.								
NP	+	-	13 (62%)	26 (20%)	61.9	79.7	33.3	92.7	0.306
	-	+	8 (38%)	102 (80%)					
GA	+	-	9 (43%)	13 (10%)	42.9	89.8	40.9	90.6	0.321
	-	+	12 (57%)	115 (90%)					
ST	+	-	13 (62%)	13 (10%)	61.9	89.8	50.0	93.5	0.471
	-	+	8 (38%)	115 (90%)					
	+	-	4 (20%)	9 (7%)*	20.0	93.0	30.8	88.1	0.152
	-	+	16 (80%)	119 (93%)					

NP - Nasopharyngeal, GA - Gastric Aspirate, ST - Stool sample

$\chi^2 p < 0.0001$  comparing culture-positive and culture-negative groups

\*  $p \approx 0.05$  comparing culture-positive and culture-negative groups