GENEXPERT:
TOWARDS STANDARD EVALUATION OF
A TB INDEX TEST IN CHILDREN

29 June 2011
A collaborative NDWG Child
Subgroup Protocol
NIH Diagnostics Meeting
Anneke C. Hesseling
Desmond Tutu TB Centre
Stellenbosch University
South Africa
CHALLENGES

1. Paediatric TB: paucibacillary disease (smear negative)
2. Majority (>75%) PTB
3. Physiological challenges: obtaining respiratory samples from infants and young children
4. Culture: limited SENS as ref standard; not rapid
5. Limited use to date of rigorous diagnostic studies using standard approaches in children: importance of standard protocols, reporting, case definitions, design, SOPs
CONTINUUM OF TB INFECTION AND DISEASE STATES IN CHILDREN: WHAT DO WE WANT TO DIAGNOSE?

>60% children 0-5 with TB have household exposure
Delayed diagnosis and initiation of treatment: disease progression

- Exposure
- Infection
- Limited Disease
- Severe Disease
- Disseminated Disease
Exposure

Infection

Limited Disease

Severe disease

Disseminated Disease

Age

HIV

Environment, helminths, strain, nutrition, genetics
<table>
<thead>
<tr>
<th>Disease manifestation</th>
<th>Total (%) N = 439</th>
<th>Bacteriologic yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Not TB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>85 (19.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Intra-thoracic TB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncomplicated LN</td>
<td>307 (69.9)</td>
<td>120/195 (61.5)</td>
</tr>
<tr>
<td>Complicated LN</td>
<td>147 (47.9)</td>
<td>22/64 (34.4)</td>
</tr>
<tr>
<td>Other</td>
<td>106 (34.5)</td>
<td>59/80 (73.5)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>54 (17.5)</td>
<td>39/51 (76.5)</td>
</tr>
<tr>
<td><strong>Extra-thoracic TB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical lymphadenitis</td>
<td>72 (16.4)</td>
<td>31/46 (67.4)</td>
</tr>
<tr>
<td>TBM</td>
<td>35 (48.6)</td>
<td>27/27 (100)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (19.4)</td>
<td>1/10 (10.0)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>23 (31.9)</td>
<td>5/9 (55.6)</td>
</tr>
<tr>
<td><strong>Intra+Extra</strong></td>
<td>25 (5.7)</td>
<td>12/13 (92.3)</td>
</tr>
</tbody>
</table>

*Marais, Hesseling, Clin Infect Dis 2007*
Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Culture positive</th>
<th>Smear positive</th>
<th>Culture or smear positive</th>
<th>Cumulative yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>58 (23%)</td>
<td>29 (12%)</td>
<td>62 (25%)</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Induced sputum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>51 (20%)</td>
<td>25 (10%)</td>
<td>54 (22%)</td>
<td>87%</td>
</tr>
<tr>
<td>First specimen</td>
<td>250</td>
<td>37 (15%)</td>
<td>19 (8%)</td>
<td>41 (16%)</td>
<td>66%</td>
</tr>
<tr>
<td>Second specimen</td>
<td>244</td>
<td>27 (11%)</td>
<td>13 (5%)</td>
<td>30 (12%)</td>
<td>79%</td>
</tr>
<tr>
<td>Third specimen</td>
<td>227</td>
<td>29 (13%)</td>
<td>11 (5%)</td>
<td>31 (14%)</td>
<td>87%</td>
</tr>
<tr>
<td><strong>Gastric lavage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>38 (15%)</td>
<td>17 (7%)</td>
<td>40 (16%)</td>
<td>64%</td>
</tr>
<tr>
<td>First specimen</td>
<td>250</td>
<td>19 (85%)</td>
<td>8 (3%)</td>
<td>20 (8%)</td>
<td>32%</td>
</tr>
<tr>
<td>Second specimen</td>
<td>244</td>
<td>22 (9%)</td>
<td>12 (5%)</td>
<td>26 (11%)</td>
<td>56%</td>
</tr>
<tr>
<td>Third specimen</td>
<td>234</td>
<td>18 (8%)</td>
<td>10 (4%)</td>
<td>22 (9%)</td>
<td>64%</td>
</tr>
</tbody>
</table>

Data are number or %.

Table: Cumulative yield of *M. tuberculosis* from repeated induced sputum or gastric lavage specimens

- Value of multiple respiratory sampling
- No decreased yield in HIV-infected children

• Sensitivity of a single Xpert MTB/RIF assay (performed at reference facilities and compared with culture) was 98.2% among 561 patients with smear-positive TB and 72.5% among 171 patients with culture-positive smear-negative TB.

• Sensitivity for detecting smear-negative TB increased to 85.1% with two assays and 90.2% when 3 Xpert MTB/RIF assays were performed.

• **Use extrapolated to children**
<table>
<thead>
<tr>
<th>Test specification</th>
<th>Minimum required value</th>
<th>Xpert MTB/RIF specifications</th>
<th>Comparison with minimum requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical decision</td>
<td>Treatment initiation</td>
<td>Treatment initiation&lt;br&gt;Selection of regimen</td>
<td>Exceeded</td>
</tr>
<tr>
<td>Sensitivity for smear-positive culture-positive PTB in adults</td>
<td>≥95%</td>
<td>99.8% (95% CI: 99.0–100; three Xpert MTB/RIF assays compared with culture)&lt;br&gt;98.2% (95% CI: 96.8–99.0; one Xpert MTB/RIF assay compared with culture)</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Sensitivity for smear-negative culture-positive PTB in adults</td>
<td>60–80%</td>
<td>90.2% (95% CI: 84.9–93.8; three Xpert MTB/RIF assays compared with culture)&lt;br&gt;72.5% (95% CI: 42.4–79.9; one Xpert MTB/RIF assay compared with culture)</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Specificity in adults without TB</td>
<td>≥95%</td>
<td>98.1% (95% CI: 96.6–98.9; three Xpert MTB/RIF assays compared with culture)&lt;br&gt;99.2% (95% CI: 98.1 – 99.6; one Xpert MTB/RIF assay compared with culture)</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Sensitivity for all types of TB in children</td>
<td>80% compared with culture&lt;br&gt;60% for probable TB</td>
<td>No data available</td>
<td>No data</td>
</tr>
<tr>
<td>Specificity in children</td>
<td>95% compared with culture&lt;br&gt;90% for probable TB</td>
<td>No data available</td>
<td>No data</td>
</tr>
<tr>
<td>Time to results</td>
<td>≤3 hours</td>
<td>2 h</td>
<td>Exceeded</td>
</tr>
<tr>
<td>Throughput</td>
<td>20 tests per day by one laboratory staff member</td>
<td>Total hands-on time of 2 min&lt;br&gt;Total daily throughput depends on the size of the instrument</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Specimen type</td>
<td>Adults: urine, oral, breath, venous blood and sputum&lt;br&gt;Children: urine, oral and capillary blood</td>
<td>Sputum&lt;br&gt;May work with other samples except for venous blood</td>
<td>Satisfied</td>
</tr>
</tbody>
</table>
AIMS

1. Assess the diagnostic accuracy and clinical utility of the GeneXpert® MTB/RIF assay for the diagnosis of intrathoracic TB in HIV-infected and uninfected children aged 0-5 years using relevant clinical samples.

2. Develop a standard protocol and approach for phase 3 paediatric TB diagnostic studies (detection organism)
PRIMARY HYPOTHESES

• The overall yield of GeneXpert in HIV-infected and uninfected children aged 0-5 years with intrathoracic TB will be lower than the yield obtained by MGIT liquid culture under optimal conditions.

• The observed severity of intrathoracic disease is correlated with the diagnostic yield of GeneXpert.

• In children with extensive intrathoracic TB, the sensitivity and specificity of GeneXpert® MTB/RIF assay will approximate that of liquid culture. In children with less severe disease, the yield of Xpert will be lower than liquid culture.
PRIMARY OBJECTIVES

In HIV-infected and uninfected children aged 0-5 with intrathoracic TB:

1. Evaluate the sensitivity and specificity of GeneXpert compared to MGIT from respiratory samples (gastric aspirate and induced sputum)
2. Correlate yield of GeneXpert, culture and smear in relation to disease severity

- The primary outcome variable, the yield from respiratory samples, will be analyzed by GeneXpert® MTB/RIF assay and by MGIT culture methods and Auramine staining in children with different spectrum of intrathoracic disease.
SECONDARY OBJECTIVES

1. Compare the yield of GeneXpert and diagnostic tests used from different respiratory samples (GA, sputum and IS) and stool.

2. Compare the prevalence of rifampin resistance detected by GeneXpert and current existing standard of care tool (LPA; Hain Lifesciences).

3. Investigate the use of Xpert, smear and culture to monitor treatment response at 1 month following initiation of anti-tuberculosis therapy.


5. Compare time to treatment initiation of treatment using culture, smear and GeneXpert.
METHODS

• **Design:** Prospective cohort study; hospital-based
• **Phase 3:** assessing test performance in clinical setting close to “real-life setting”. Representative spectrum of disease providing estimates of clinical validity in all relevant patient groups.
• **Sampling:** all consecutive routine child TB suspects investigated (Monday-Friday)
  – Sampling stratified by TB disease severity (50:50) and HIV status (20%); age strata: 0-2 vs. 2-5 y; balancing retrospectively
• All participants followed equally for at least 8 weeks
• Random selection of non-TB cases follow-up beyond 8 weeks; all probable and confirmed TB cases and selected controls followed for 6 months
STUDY SETTING

- South Africa: 4th highest number all TB cases (454,000 new)
- Prevalence 998 per 100,000; 44% HIV-infected (2009)
- Western Cape Province: Childhood TB: 620/100,000 (2009)
- Median age hospital TB cases: 2.2 years
- Universal BCG at birth (99% coverage)
- 30% of children with TB are HIV-infected
ELIGIBILITY

Children with suspected intrathoracic TB

Inclusion criteria

• Aged 0-5 years
• Weight ≥2.5kg
• Written informed consent for participation including HIV
• Intrathoracic TB with limited extrathoracic manifestations of TB (peripheral lymphadenopathy, isolated abdominal lymph nodes) will be included.
• Miliary TB evident on CXR (but without evidence of TB meningitis or other target organ involvement) will be included.
Exclusion criteria

• On anti-tuberculosis therapy for more than 7 days
• Intrathoracic TB in conjunction with disseminated forms of extrathoracic TB
• Not living in geographical area with ready access to hospitals

• *Initial enrolment, subsequent exclusion*: Children with drug-resistant TB will be withdrawn from study at the time of detection of drug resistance; these children will be referred to a specialist MDR Clinic
• **Deferral:** children with acute severe illness e.g. lower respiratory tract infection requiring supplemental oxygen, gastroenteritis with severe dehydration, acubleeding tendencies such as thrombocytopenia or disseminated intravascular coagulation, will be deferred for enrolment until clinically stable.
ENTRY CRITERIA: TB SUSPECT

Suspicion of TB based on presence of ≥ 1 of following signs/symptoms

– Any current cough > 2 weeks
– Documented failure to thrive or severe acute malnutrition without obvious cause
– Unexplained fever
– Unexplained fatigue, lethargy or reduced playfulness
– Documented recent infective TB contact in preceding 12 mo
CASE DEFINITIONS

“Confirmed intrathoracic TB”

1. Suggestive history, symptoms or signs (≥2):
   - Persistent cough >2 weeks not responding to a course of appropriate antibiotics or bronchodilators
   - Unexplained fever >1 week
   - Weight plateauing/weight loss or malnutrition documented objectively
   - Lethargy/lack of playfulness
   - Evidence of exposure to *M. tb*: reactive TST or known close TB contact, **AND**

2. CXR compatible with intrathoracic TB*, **AND**

3. Positive culture of at least 1 respiratory sample (including mediastinal lymph node aspirate) or of peripheral lymph node aspirate/biopsy confirming *M. tuberculosis*. 
2. “Probable TB”

• Criteria 1 and 2 as in “Definite TB”, but without bacteriological confirmation.
3. “Possible TB”: Children screened for TB based on suggestive symptoms but CXR not indicative of TB, have negative bacteriological investigations and have evidence of exposure to TB by history or reactive Mantoux skin test. No clear alternative diagnosis is established.

4. “Not/unlikely TB”: no evidence of TB exposure/infection and an alternative diagnoses is established. All classified as “not TB” will be assigned a final alternative diagnosis at 8 weeks.
• Systematic objective review of all cases with disease classification based on all serial data during screening period (8 weeks)
• Probable TB: based on CXR
• Inclusion of FNA + culture in presence of CXR findings
• Discrepancies noted between “research definition”, clinical decision definition and decision to treat (documented in all) Research vs. clinical care team
• *Cult+ with well defined symptoms but normal CXR at baseline= confirmed TB (also consider f/u CXR)
• Separate infant case definitions
• No response to ATT included
• Systematic data collection: evaluate different case definitions and response to therapy
STUDY MEASURES

• Standard CRFs, source docs, QA and QC
• Standard symptomatology: respiratory symptoms, changes in weight or failure to gain weight, loss of appetite, reduced playfulness, fever, night sweats and enlargement of peripheral nodes: symptom score cards, serial assessment
• TST: standardized (baseline and 8 weeks if initial negative)
• CXR: SOP, independent readers, 3rd assessment, blinded, standard definition of disease, serial assessment
• HIV testing and immune status (baseline)
• Nutritional status: anthropometrics, serial assessment
• Duration TB therapy: regimen, adherence: serial assessment
TB Disease Spectrum

• Description of CXR findings (process)
• New classification based on pathophysiology of TB in children, imaging (chest radiography, computerized tomography and bronchoscopy) and bacteriology.
• Disease classified as “Severe” or “Non-severe” after considering the extent of disease (disease containment) and the presence of complications.
• Uncontained and complicated disease is classified as “severe”. Contained disease is “non-severe”.

Wiseman, in progress
- Age, gender, ethnicity
- **Socio-economic status:** estimate of total household income, total number of individuals supported by income, estimate of monthly expenditure for foodstuffs, type of dwelling, access to basic services (water, sanitation, electricity) and parental education level.
- **Daily nutritional intake:** detailed account of typical day’s food consumption, including any supplements, calculations of total daily caloric and intake.
- **Presence of feeding difficulties** (e.g. reflux, anorexia)
- Concurrent illness
- Exposure to passive household **tobacco smoking** exposure
- Costing data
GeneXpert (index test)

- GeneXpert samples will be analyzed in a dedicated TB research lab using SOP. 2 ml of the inactivated material transferred to the test cartridge (equivalent to 0.7 ml of untreated sputum or 0.5 ml of decontaminated pellet)
- Optimization of GA, stool completed (pilot study)
- A single laboratory technician trained as operator, completed certificated proficiency testing
- Investigators will remain blinded to GeneXpert results: DR results fast-tracked, unblinded
- Sampling: 2 IS, 2 GA and 2 stools

Sample storage and future use

- Stool, serum, urine, diagnostic markers and treatment response
MICROBIOLOGY

- Standard SOPs used for sample collection, processing, culture and smear microscopy. Mycobacterial culture will be completed by a single laboratory technologist following a rigorous protocol to prevent mycobacterial cross-contamination. Smear will be completed in all children using Auramine staining with smear grading and fluorescent microscopy.

- Primary mycobacterial cultures will be established by inoculation of samples into Middlebrook 7H9 broth-based MGIT (BD), following standard protocol for decontamination. Lymph node aspirates and pleural fluid will be directly inoculated into MGIT tubes. Cultures will be incubated for 42 days or until flagged as positive.

- Isolates will be confirmed as *M. tuberculosis* through PCR.

- TTD will be documented (measure of bacterial load).

- Quality assurance will be conducted routinely; samples will be processed in an accredited biosafety level 2 (P2) TB service lab.
OUTCOME DATA

• In all TB cases (N=220)
• In subset of controls: “Not TB/unlikely TB” (N=220): randomly selected (simple randomization)
• Bacteriology, symptoms, weight, CXR: serial measures
SAMPLE SIZE ESTIMATES

• Assumptions: culture yield in HIV-negative children (primary hypothesis powered for the largest group) with severe intra-thoracic TB: 60%. Based on confirmed and probable TB

• GeneXpert® (index test) is less sensitive than culture: a single sample will detect 75% of culture positive cases (i.e. GeneXpert yield= 45%).

• Alpha level of 0.05 and power of at least 0.80 leading to an estimated sample size of 85 HIV-negative children with severe disease.

• Representative group of 40-50 HIV infected children (20%).

• Allowing for 10% loss to follow-up, we will enroll a total of 110 children with severe disease leading to a total of 220 children in a ratio of 50:50

• For SPE calculations, by enrolling a random, equal number of controls without TB (N=220), assuming GeneXpert specificity to be 98%, the width of the 95% confidence interval for specificity will be 5%, 94-99%, which is adequate.

• Given a sample size of 110 in each disease group, assuming Xpert will detect 45% of severe cases: 89% power to detect a 15% difference in yield compared to non-severe cases; 99% power to detect 20% diff.
ANALYSIS AND REPORTING

1. Primary analysis: compare yield of index GeneXpert to culture (ref) and smear from 2 consecutive respiratory samples on 2 consecutive days across all 4 strata

2. Percentage + amongst cult+ and % neg amongst culture neg across all 4 strata; test agreement

3. Sensitivity, specificity, positive and negative predictive value of the index test. SENS and SPE for the MTB/RIF test will be estimated for a single test and the combination of two tests across all 4 strata (limitations)

4. Total positive yield of *M. tb* detected by any modality will be calculated. Combinations classified as positive if at least one of the component test results was positive.

Main exposure of interest: TB disease severity

Covariates of interest: age, HIV status and degree of HIV-related immune suppression, duration of TB therapy, nutritional status.
• Systematic documentation of all technical errors, lab contamination and failed tests. Indeterminate rate will be number of tests classified as “invalid,” “error,” or “no result” divided by the total number performed. When results are indeterminate and sufficient sample remains, assay will be repeated once, 2nd result used.
• Analysis of single direct test and the combination of two tests: Wilson’s binomial method will be used to calculate 95% CI.
• Missing data excluded from analysis.
• For all intra-patient GeneXpert results, and for comparisons across subgroups and testing methods, generalized estimating equations (GEE) will be used to calculate CIs to account for clustering.
ANALYSIS (3)

- Detailed reporting of all data components and diagnostic strata including eligibility
- Systematic reporting of all excluded and included patients (flow diagram)
- Diagnostic accuracy across all groups
- Subgroup analysis in HIV-infected
- Systematic data collection to enable analysis on composite reference standard (serial symptoms, CXR, RTT)
- All lab analyses blinded to clinical data
- Clinical assessment blinded to GeneXpert data
- Comparisons across all 4 strata of diagnostic certainty
- STARD guidelines
PILOT DATA

20 GA samples processed; 3 culture+ 2 GeneXpert+ (unmodified SOP)

Patient ID: 
Sample ID: PGX_P003590_11Mar2011
Test Type: Specimen
Sample Type: 

<table>
<thead>
<tr>
<th>Assay</th>
<th>Assay Version</th>
<th>Assay Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert MTB-RIF G3</td>
<td>3</td>
<td>In Vitro Diagnostic</td>
</tr>
</tbody>
</table>

Test Result: **MTB DETECTED HIGH; Rif Resistance NOT DETECTED**

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Ct</th>
<th>EndPt</th>
<th>Analyte Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe D</td>
<td>16.4</td>
<td>243.0</td>
<td>POS</td>
</tr>
<tr>
<td>Probe C</td>
<td>14.8</td>
<td>339.0</td>
<td>POS</td>
</tr>
<tr>
<td>Probe E</td>
<td>16.2</td>
<td>166.0</td>
<td>POS</td>
</tr>
<tr>
<td>Probe B</td>
<td>16.5</td>
<td>195.0</td>
<td>POS</td>
</tr>
<tr>
<td>SPC</td>
<td>28.4</td>
<td>320.0</td>
<td>NA</td>
</tr>
<tr>
<td>Probe A</td>
<td>14.6</td>
<td>237.0</td>
<td>POS</td>
</tr>
</tbody>
</table>

User: Babalwa Mtuze
Status: Done
Start Time: 11/03/11 13:55:22
End Time: 11/03/11 15:42:00
Reagent Lot ID*: 02101
Expiration Date*: 02/10/11
Cartridge S/N*: 29486165
S/W Version: 2.1
Notes:
Error Status: OK

Errors
<None>

For In Vitro Diagnostics Use Only.
ANTIPICATED OUTPUTS

• GeneXpert performance in young children using clinically relevant samples (GA, IS, stool)
• Generic protocol and tools for wider use
• Yield in relation to TB disease severity and HIV
• Standard classification and application of TB disease spectrum
• Classification of all cases into strata of diagnostic certainty
• Standard assessment of markers of treatment response in all cases and in randomly selected controls
• Use and compilation of SOPs (clinical and lab)
• Systematic data collection: future assessment of composite reference standard and different case definitions
• Opportunity to analyze based on different case definitions
• Biorepository and clinical database, other novel markers
• Impact on clinical care; cost-effectiveness
LIMITATIONS

• Reference standard: liquid culture: SENS; application to subset of children only
• Index test: limited SENS – but rapid
• Hospital-based population (disease spectrum balanced)
• Biological samples: ideally non-respiratory (stool)
Protocol team: Elisabetta Walters, Robert Gie, Carl Lombard, Andreas Diacon, Catherine Wiseman, Simon Schaaf and Anneke Hesseling

www.ndwgchildsubgroup.org