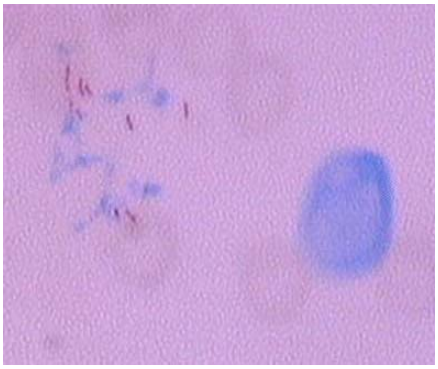
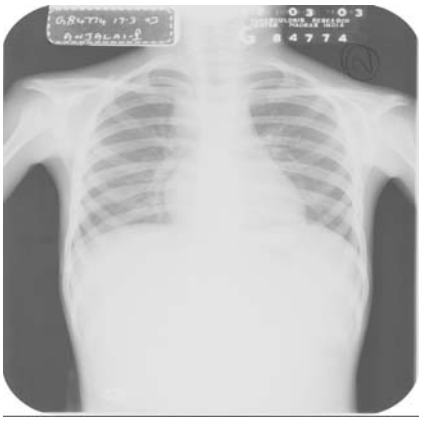
Diagnosis of TB in HIV-infected persons and children: Challenges and Solutions

Soumya Swaminathan, MD
Coordinator, WHO/TDR, Geneva





Global TB Burden - 2009

- **9.4 million new TB cases**, **1.7 million deaths**
  Children?
- Estimated 10%–15% of cases
- 900,000-1.4 million cases
- But
  - Less specific symptoms, less likely to expectorate
  - Considered less infectious - receive lower priority
  - Specimens other than sputum – fewer bacilli and may include test inhibitors
  - Diagnostic tests developed for adult TB perform poorly in children

Global TB Report 2010

Tuberculosis in India - 2009

- Total TB cases notified 1.53 million
- Mortality rate 23/100,000
- 17% of TB patients knew their HIV status
- 12% of tested TB patients were HIV+
- 13,500 children with TB notified – no data on HIV co-infection
- In south Africa, active screening detected childhood TB at 400/100,000
- Burden higher than reported

www.tbcindia.org, Marais et al Infect Dis Clin NA 2010

Risk of Progression from Infection to Disease

- Natural history (1920-1950)

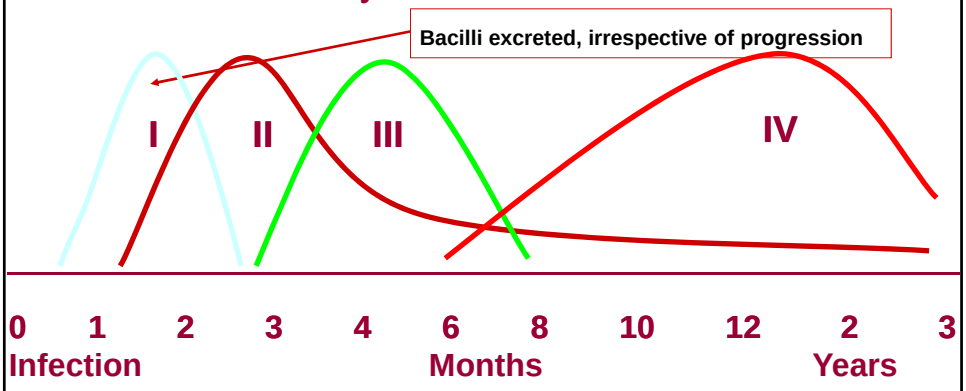
Exposure
↓
Infection
↓
Disease

Risk Factors

- Age (<2-3 years)
- Nutritional status
- Timing of infection
- Immune status (HIV)
- ? Others (exposure to indoor air pollution, cigarette smoke, infectious dose)

IJTLD 2004; 8: 392-402

Wallgren's timetable: 90% of disease occurs in 1st year after infection



I Hypersensitivity

II Miliary TB and TBM

III Lymph node disease / Pleural effusion

IV Adult-type disease

Age and risk – modified by HIV

Pathogens in children failing antibiotics

McNally L . Lancet 2007;369: 1 440

	Younger than 1 year				1 year or older		
	Total (n=90)	Infected (n=74)	Exposed uninfected (n=9)	Uninfected (n=7)	Total (n=20)	Infected (n=13)	Uninfected (n=7)
Pneumocystis jirovecii	29 (32%)	26 (35%)	3 (33%)	0	0	0	0
Mycobacterium tuberculosis	15 (17%)	13 (18%)	0	2 (29%)	9 (45%)	5 (39%)	4 (57%)
Cytomegalovirus	40 (45%)	37 (51%)	2 (22%)	1 (14%)	4 (20%)	3 (23%)	1 (14%)
Streptococcus pneumoniae	9 (10%)	7 (9%)	0	2 (29%)	3 (15%)	3 (23%)	0
Staphylococcus aureus	13 (14%)	11 (15%)	2 (22%)	1 (14%)	6 (30%)	4 (31%)	2 (29%)
Other gram positive	6 (7%)	5 (7%)	0	1 (14%)	3 (15%)	3 (23%)	0
Haemophilus influenzae	5 (6%)	3 (4%)	1 (11%)	1 (14%)	4 (20%)	2 (15%)	2 (29%)
Klebsiella pneumoniae	9 (10%)	8 (11%)	1 (11%)	0	0	0	0
Escherichia coli	8 (9%)	7 (9%)	1 (11%)	0	0	0	0
Salmonella spp	1 (1%)	1 (1%)	0	0	0	1 (8%)	0
Legionella spp	1 (1%)	1 (1%)	0	0	0	0	0
Other gram negative	10 (11%)	8 (11%)	1 (11%)	1 (14%)	3 (15%)	2 (15%)	1 (15%)
Adenovirus	6 (7%)	4 (5%)	0	2 (28%)	3 (15%)	2 (15%)	1 (14%)
Respiratory syncytial virus	11 (12%)	8 (11%)	3 (33%)	0	2 (10%)	0	2 (29%)
Other virus	8 (9%)	6 (8%)	1 (11%)	1 (14%)	3 (15%)	3 (23%)	0
Aspergillus spp	0	0	0	0	1 (5%)	1 (8%)	0
Streptomyces spp	1 (<1%)*	1 (<1%)	0	0	0	0	0
Saccharomyces spp	0	0	0	0	1 (5%)	1 (8%)	0

All data are number (%). *Only children who had all study investigations and failed therapy are included (admission and non-responder blood culture; admission nasopharyngeal aspirate and NB-BAL or lung aspirate for viral immunofluorescence; and culture, induced sputum, and NB-BAL or lung aspirate for *P jirovecii* pneumonia and tuberculosis; gastric washings for tuberculosis; NB-BAL, lung aspirate, or pleural aspirate for bacteria). Bacteria isolated from nasopharyngeal swabs or induced sputa are not regarded as significant and therefore not included.

Table 5: Organisms isolated from children who were investigated for failing to respond by HIV status and age

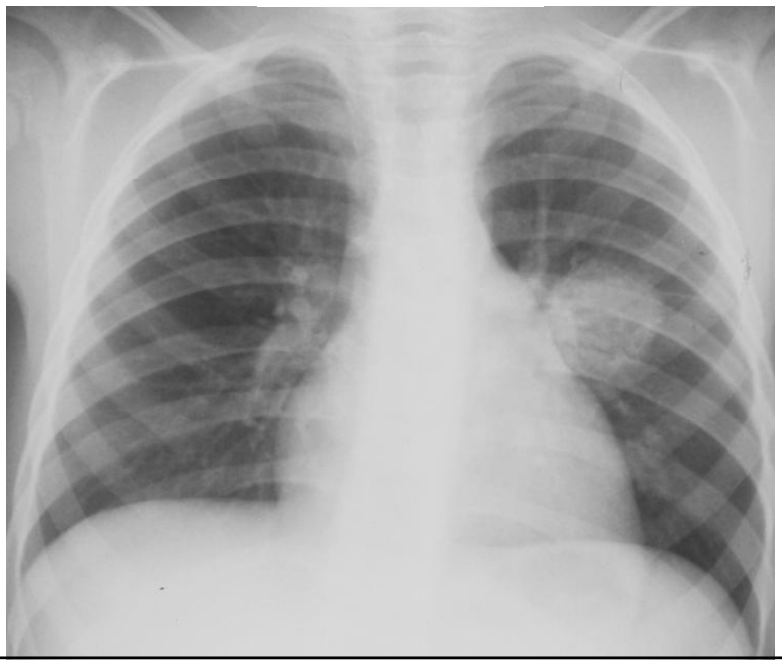
Lung diseases identified at necropsy

TB third most common

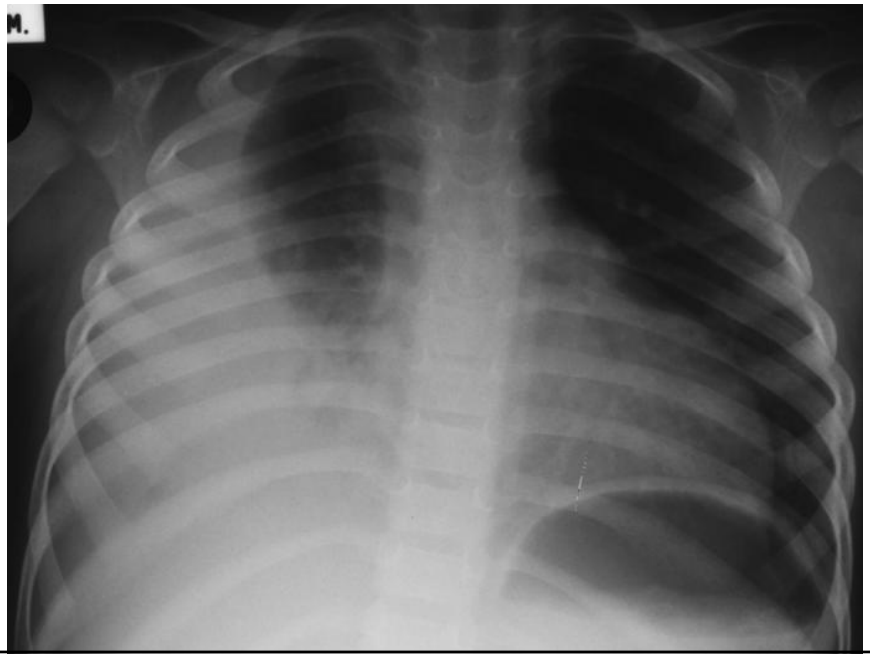
	Total*	Adjusted % (SE)†	HIV-positive (n=180)	HIV-negative (n=84)
Diagnosis				
Acute pyogenic pneumonia	116 (44%)	39·1% (3·2)	74 (41%)	42 (50%)
PCP	58 (22%)	27·5 % (3·1)	52 (29%)	6 (7%)
Tuberculosis	54 (20%)	18·8% (2·5)	32 (18%)	22 (26%)
CMV	43 (16%)	20·2% (2·8)	40 (22%)	3 (4%)
Interstitial pneumonitis	30 (11%)	11·8% (2·1)	15 (8%)	15 (18%)
Shock lung	27 (10%)	11·5% (2·2)	24 (13%)	3 (4%)
Pulmonary oedema	19 (7%)	6·4% (1·6)	10 (6%)	9 (11%)
Lymphocytic interstitial pneumonitis	10 (4%)	3·8% (1·2)	9 (5%)	1 (1%)

Chintu C et al Lancet 2002; 360: 985-90

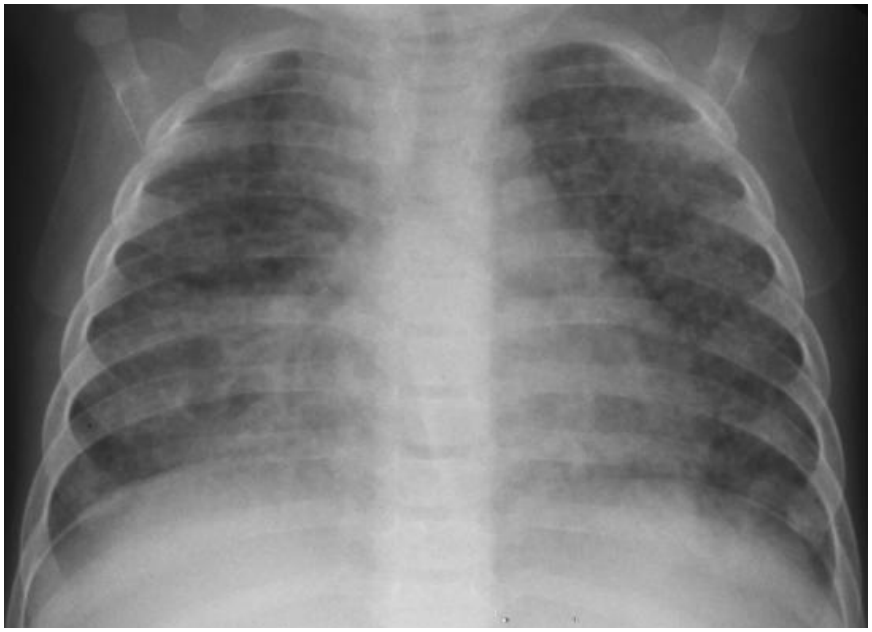
Ghon focus



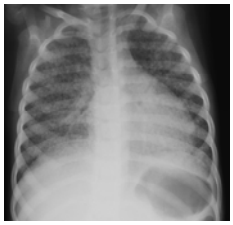
Pleural effusion

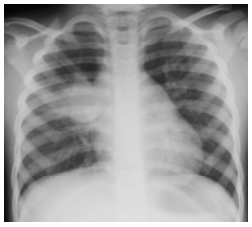


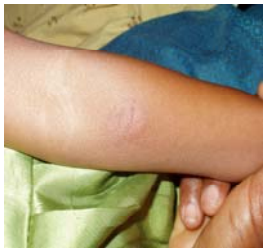
Disseminated (miliary) disease

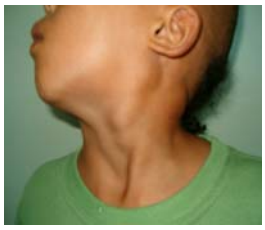


Clinical symptoms
Nutritional status
Contact history
Chest radiograph
Tuberculin skin test









16 diagnostic algorithms

The Keith Edwards (Papua New Guinea) TB score

Feature	0	1	2	3	4	Score
Duration of illness (in weeks)	<2	2–4		>4		
Nutrition status (% of wt for age)	>80	60–80		<60		
Family history of TB	None	Family history of TB, but not sputum-positive		Family member with sputum-positive TB		
Significant Mantoux*				Positive*		
Lymph nodes: large, painless, firm; sinus in neck/axilla				Yes		
Night sweats or unexplained fever for >2 wks			Yes			
Angle deformity of spine					Yes	
Malnutrition not improving after 4 wks of treatment				Yes		
Joint swelling, firm, non-fluid, non-traumatic				Yes		
Unexplained ascites				Yes		
Coma for >48 h (with or without convulsions)				Yes		
					TOTAL	

INT J TUBERC LUNG DIS 6(12):1038–1045

REVIEW ARTICLE

A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis

A. C. Hesseling,* H. S. Schaaf,* R. P. Gie,* J. R. Starke,† N. Beyers*

- High sensitivity and very low specificity
- High specificity and very low sensitivity
  - Screen most obvious cases
  - Performs worse in most difficult cases
  - Setting and stage at presentation
  - Depend on available investigations

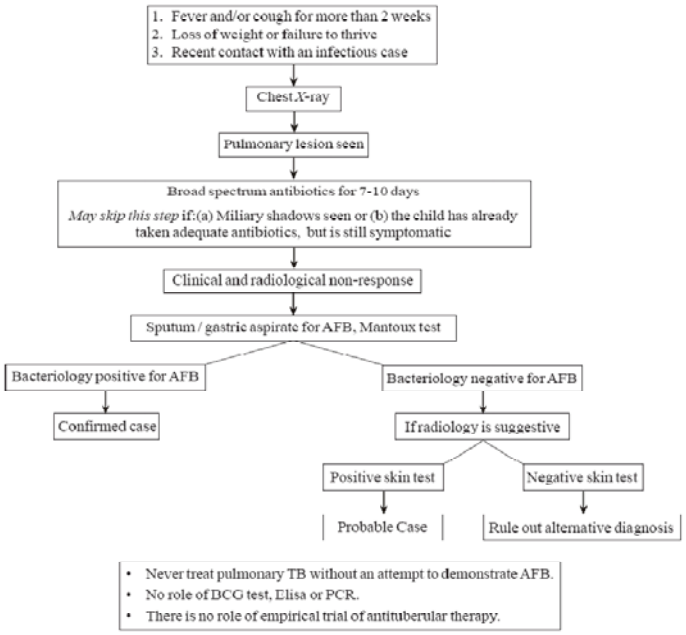
Table 4 Review of previous studies of scoring systems

Scoring system	Type of study	Country	Sensitivity %	Specificity %	HIV?
Edwards[13]	Prospective	Zambia	88	25	Yes
Edwards[13]	Retrospective	Papua New Guinea	62	95	No
Edwards[13]	Prospective	India	91	88	No (excluded)
Edwards[13]	Prospective	Brazil	84	97	No
Jones (Stegen)[17]	Prospective	Brazil	56	94	No
Brazil MOH[20]	Retrospective	Brazil	89	87	No (excluded)
Fourie/The Union[14]	Retrospective	Multiple	41 (0–4 years) 62 (5–14 years)	44 (0–4 years) 50 (5–14 years)	No

Poor agreement between scores

Hesseling et al IJTLD 2007:11:245

Algorithm for diagnosis of tuberculosis in children



IAP 2010

Differential diagnosis of pulmonary TB in HIV infected children

	Age ranges	Clinical features	Radiological features
TB	All ages	Subacute onset[a] persistent cough weight loss or failure to thrive, persistent fever	Lymph node enlargement, miliary Parenchymal infiltration, primary complex
Bacterial pneumonia	All ages	Rapid onset, high fever, elevated leukocyte count, tachypnoea	Bronchopneumonia or lobar consolidation
Viral pneumonia	More common in infants	Air trapping with wheezing, tachypnoea	Diffuse interstitial infiltration, hyperinflation

[a] Onset can occasionally be acute, especially in immunocompromised infants.

(WHO/HTM/TB/2006.362)

Differential diagnosis of pulmonary TB in HIV infected children

	Age ranges	Clinical features	Radiological features
Lymphoid interstitial pneumonitis	Older children (> 2 years)	Gradual onset, cough, mild hypoxia, generalized lymphadenopathy, parotid enlargement, finger clubbing	Diffuse reticulonodular (miliary) pattern, lymph node enlargement
Pneumocytis jiroveci pneumonia	Infants	Abrupt onset, tachypnoea, cough, severe hypoxia, fever ±	Diffuse interstitial infiltration, hyperinflation
Bronchiectasis/ chronic lung disease	Older children	Gradual onset, cough productive of copious sputum (purulent, occasionally blood stained), halitosis, finger clubbing	Honeycombing, usually of lower lobes

(WHO/HTM/TB/2006.362)

Specimen Collection in Children

- Sputum Induction: outpatient setting, can be performed in young children and infants, no SAE, yield from one IS = 3 GL
- Nasopharyngeal aspiration: minimal facilities and training, yield similar to GA
- Stool: stringent decontamination procedures required, less sensitive
- String test: suitable for older children, time of string in stomach can be ~ 1 hour
- Lymph node aspiration: safe outpatient procedure, yield higher than respiratory specimens, should be done if palpable peripheral LNs
- Combined yield of multiple specimens (sputum, NPA, SI, GA) collected in 1 day similar to yield of specimens collected over consecutive days

Zar etal Arch Dis Child 2000;82:305, Owens et al Arch Dis Child 2007;92:693, Oberhelman et al Lancet ID 2010; 10:612, Al-Aghbari et al Plos One 2009; 4:e5140, Franchi LM Lancet 1999; 21:1681

Yield of *Mycobacterium tuberculosis* in culture using various specimen collection methods

Type of specimen	Yield of *M.tb* in culture	Remarks
Gastric lavage	**40% -92%**	Difficult, invasive procedure, increased yield in infants and extensive disease, 3 consecutive specimens required after overnight fasting. Can be done by trained nurses
Broncho-alveolar lavage	**4% - 43%**	Extremely invasive, requires tertiary care facilities. Useful if performed with diagnostic bronchoscopy
Naso-pharyngeal aspiration	**24% - 30%**	Less invasive. Appropriate for low income countries with limited facilities
Laryngeal swab	**27% - 63%**	Useful in older children who are unable to expectorate
Induced sputum	**20% - 30%**	Yield comparable with gastric lavage and naso-pharyngeal aspiration. Requires training, can be done by nurses. Useful in hospital setting. Infection control procedures needed.
String test	**Yet to be determined**	Patients as young as 4 years tolerated the procedure well. Peak discomfort at the time of swallowing and mild during string retrieval. Further studies required.

Differential Performance of Diagnostic Tests

Test	Adults (%) Sputum	Children (%) Gastric aspirate
Sensitivity		
Smear positive	60-75	10-20
Culture positive	90	10-60
TST positive	80 (<50% HIV)	50-80
NAAT positive	65-90	25-85
Specificity		
Smear positive	>95	Low, variable
Culture	98-99	>90
TST positive	10-20	~50
NAAT positive	98	85-98

Factors affecting yield of culture

- Patient population (primary care vs referral hospital)
- Severity of illness
- Type of specimen
- Collection procedure and expertise of staff
- Transportation and decontamination procedures
- Lab quality

Test	Publications		Performance in children
	Adults	Children	
Fine needle aspiration	> 6000	140	Potentially good. Most promising when combined with culture or NAAT
Fluorescence Microscopy (FM)	299	1	Sens/Spec 58%/95% against culture
LED-FM	33	0	No data
MODS	31	2	more sensitive than Lowenstein-Jensen culture. Collection of duplicate gastric-aspirate specimens for MODS culture was the best available diagnostic test in one study
BACTEC 960	49	0	Anecdotic data suggest performance in children similar to adults
Fully automated BACTEC	13	0	
Line Probe assays	113	1	
Commercial NAAT)			
loop-mediated isothermal amplification (LAMP) - PCR	13	0	No data
Automated NAAT (Xpert)	4	0	

Detection of Mtb nucleic acid

- Commercial NAAT: Performance similar to smear neg TB (~60% sensitivity), high specificity, not well evaluated in children
- In house NAATs: heterogeneity in performance
- Nucleic acid in non-respiratory specimens:
  - Stool (poor sensitivity ~35%)
  - Transrenal DNA: needs evaluation
  - Blood: 26% of microbiologically confirmed children positive compared tp 26% children with LTBI and 7% without TB

Fully automated NAAT

- Xpert
- Recommended by WHO 2010
- Funding through Global Fund
- Results in 2 hours
- Multiple pathogens (individual cartridges)
- 98% sensitive in SM+
- 75% sensitive in SM-
- Equal results in HIV+ patients

Other Tests

- LAM assay: ELISA-based test in urine for mycobacterial glycolipid. Sensitivity in adults varies from 44-67%, higher in HIV+. Needs evaluation in HIV+ children
- Volatile organic compounds in breath: will be simple and non invasive. Recent study in adults – 85% sens and 65% specificity. Needs improvement

Newer Diagnostic tests

- Liquid culture (MGIT): more sensitive than solid media
- MODS (microscopic observation drug susceptibility assay): needs validation
- Xpert TB (multiplex PCR, fully automated): 75% sensitive for smear neg TB in adults, studies required using different specimens from children
- Serological tests: no role
- IGRA (interferon gamma release assay): not recommended for diagnosis of active or latent infection

Need for Reference Standard

- Different from clinical case definition (used for management of patients)
- In research settings, accepted reference standard is culture (preferably with species identification)
- For initial proof of principle studies, important to use "gold standard"
- More pragmatic approaches for diagnostic test evaluation in operational settings

When there is no gold standard....

- Different approaches to creating a reference standard
  - Imputation of missing values
  - Discrepant analysis
  - Use multiple tests to create a standard
    - Rule based approach (use multiple tests to define subjects according to the certainty of diagnosis)
    - Panel based approach
    - Statistical methods eg latent class analysis
  - Overall goal is to decide whether test is providing true information (analytical validity), which is meaningful (clinical valididty) and useful (clinical utility)

Latent Class Analysis

- Allows unbiased estimate of validity of diagnostic test in absence of reference std
  - Assumes variable of interest not observable (latent)
  - Subjects belong to mutually exclusive classes of latent variable
  - Observable variables measure the latent var
  - Latent variable determines association between observable variables
  - Also assumes conditional independence of latent classes
  - Has been used to estimate validity of serologic test for Chagas, DAT for visceral leishmaniasis etc
  - Needs to be applied to data set in children and tested

Other Needs for TB Diagnostics Research in Children

- Standardized specimen collection, handling and processing (SOPs)
- Standard application of test techniques, study-related investigations, interpretation (CXR) and reporting
- Methods of sampling and participant selection
- Standard ascertainment of TB disease
- Systematic characterization of TB disease and comparison groups
- Cross-sectional studies preferable to case-control and minimum set of variables should be collected

Way Forward...

- TDR Diagnostic Expert Evaluation Panel (DEEP) guide on pediatric TB: Nat Rev Microbiol series
- Consensus standard SOPs to be developed for public domain website, explore LCA
- Engage test developers (FIND, academia, NGOs)
- Engage donors
- Increase visibility of childhood TB
- Integrate childhood TB in MDGs initiatives
- Conduct multi-centric evaluations

TB in HIV-infected

- Depends on stage of immunodeficiency
  - At high CD4 counts, typical clinical and radiographic presentation, positive smears
  - At low CD4 counts (<200 cells/mm), more smear negative and extra-pulmonary disease
- Paradox: high bacillary burden in tissues, very little in sputum
- Patients prone to many other infections: wide differential diagnosis
- Patients often very ill, diagnosis is urgent
- Urgent need: POC test

Rule in or Rule out TB

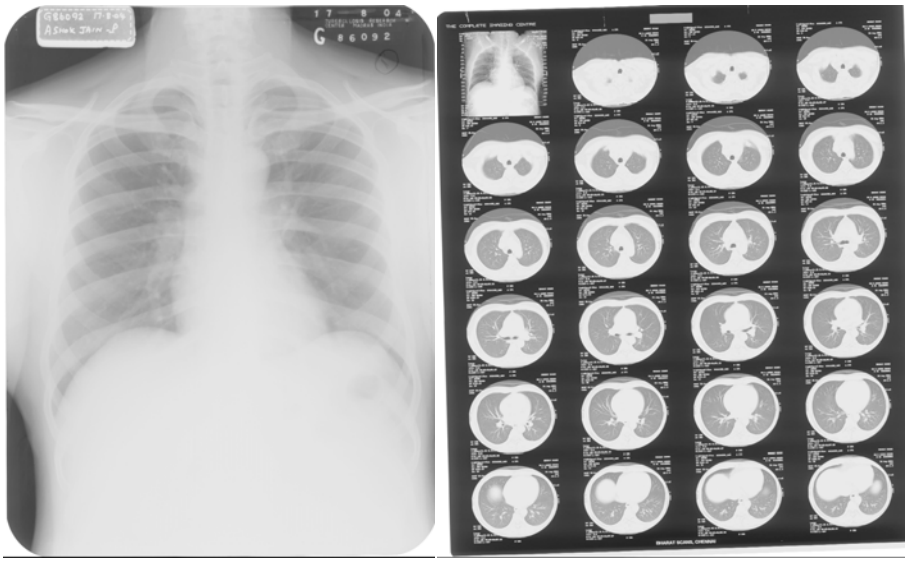
- Quality care of HIV-infected persons includes treatment AND prevention of TB
- Newly diagnosed and those on follow-up need periodic screening for TB
  - If TB diagnosed, start treatment
  - If no TB and no contra-indications, start IPT

Approaches to Diagnosis

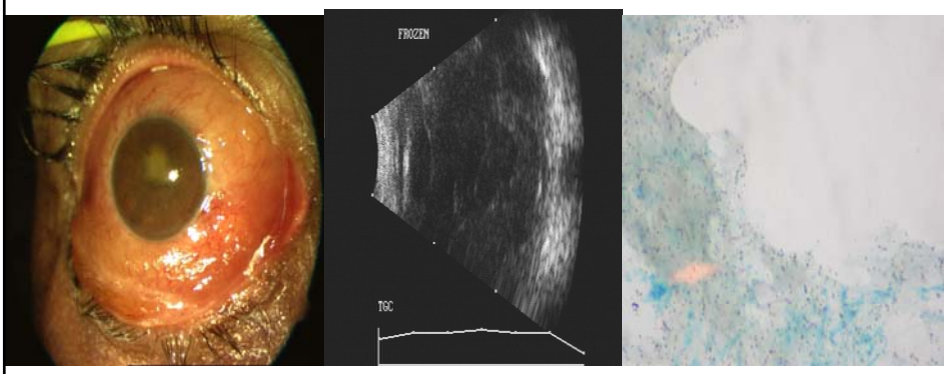
- Clinical algorithm to rule out TB (cough or fever any duration or night sweats >3 weeks: 93% sensitivity, 36% specificity)
- Chest xRay: atypical, normal in ~15-20%
- Tuberculin skin test: <50% positive
- IGRA: ~70% sensitive
- Products of M.tb: transrenal DNA, urinary LAM – maybe more sensitive than in HIV-
- Screening with culture yields 5-25% in unselected patients with newly diagnosed HIV
- Culture: not sensitive in extrapulmonary disease, takes time

Cain KP et al NEJM 2010; 362:707, Kranzer K Lancet ID 2010; 10: 93, Golub J IJTLD 2005; 9:1183, Shah S J AIDS 2009; 50:537

HIV+ Man with Fever, Painful Red Eye: Chest X-ray normal, 3 smears neg, CD4 86

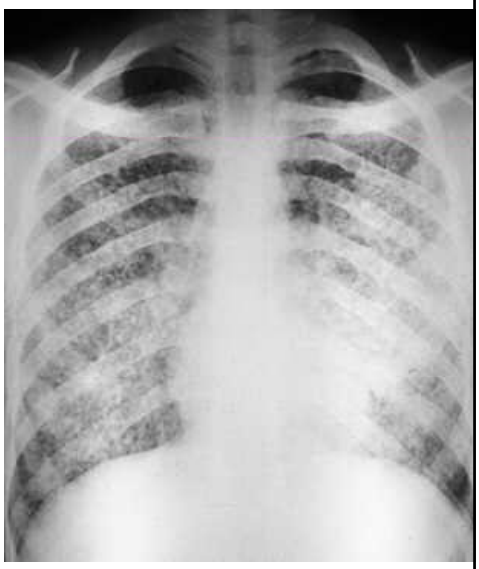


Panopthalmitis due to TB in a patient with advanced AIDS and pulmonary TB



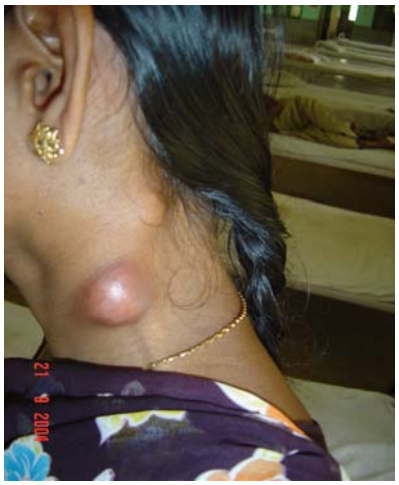
Diagnostic Issues

- More extra-pulmonary, atypical forms
- 4% asymptomatic HIV+ patients with normal chest x-ray and negative sputum smears have pos. sputum cultures for *M.tuberculosis* (Swaminathan et al IJTLD 2004)
- Active case-finding among antenatal women detected TB cases (AIDS 2003;17(9):1398-400)
- Normal x-ray does not rule out TB
- "Smear neg. TB" could be other OI's



Immune Reconstitution Syndrome

- Occurs in 20-30% of patients starting ART
- TB commonest form of IRS: enlarging LNs, worsening chest x-ray, fever
- Crypto meningitis, herpes zoster and simplex also seen
- Due to exuberant cytokine response
- Risk factors: ARV within 6 wks, dissem dis, low baseline CD4, rise in CD4%, fall in VL, ? High bacillary burden



Predictive value of combinations of symptoms

Symptoms	Sensitivity	Specificity	PPV	NPV
Cough+Fever	54	84	61	79
Cough+wt.loss	60	77	55	81
Cough+ abn X-ray	31	69	65	80
Cough+Fever +wt.loss	46	78	64	88
Cough+Fever + abn X-ray	43	98	88	79
Cough+wt.loss + abn X-ray	49	96	84	81
Cough+fever+wt. loss+ abn x-ray	37	98	89	78

Cough, fever, weight loss, x-ray

Combination	Sensitivity (%)	Specificity (%)
Any 1	94	33
Any 2	83	64
Any 3	66	84
All 4	37	98

NPV of all 3 symptoms being absent plus normal CXR 97%

TRC unpublished observations

Radiographic Findings in Tuberculosis with and without HIV Infection

	HIVTB (n = 86) %	TB (n = 99) %
Normal	13.5	0
Parenchymal Opacities	56	90
Cavity	14	39
Pleural Effusion	2	0
Miliary TB	13.5	1

Swaminathan et al Ind J Chest Dis Allied Sci 2007

Tuberculin skin test results in patients with active tuberculosis

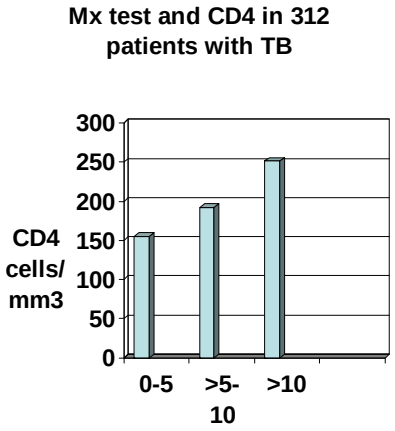
CD4 cell count cells/μl	Number of Patients per Strata	Positive TST > 5 mm	Induration size Median (IQR)
< 200	205	41%	0.0 (0.0-15.0)
> 200	107	70%	15.0 (0.0-20.0)
p-value		p<0.001	p<0.001

Overall, 51% of HIVTB patients had pos. TST

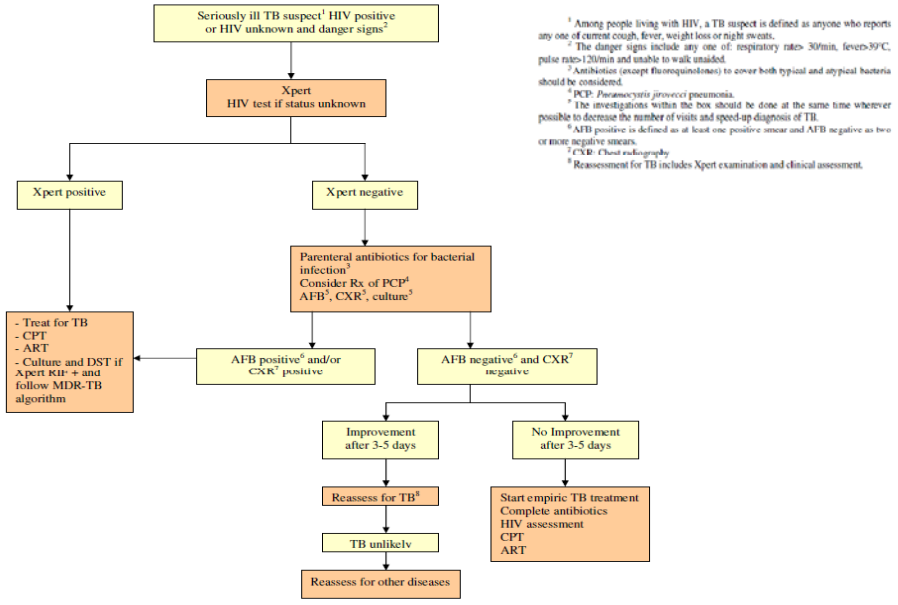
Swaminathan et al IJTLD 2008

Value of tuberculin skin test

- Positive correlation between Mantoux test and CD4 count
- Not much increase in sensitivity by using 5mm cut-off
- Mantoux poor diagnostic test for TB
- Misses 30-40% latent TB infection also

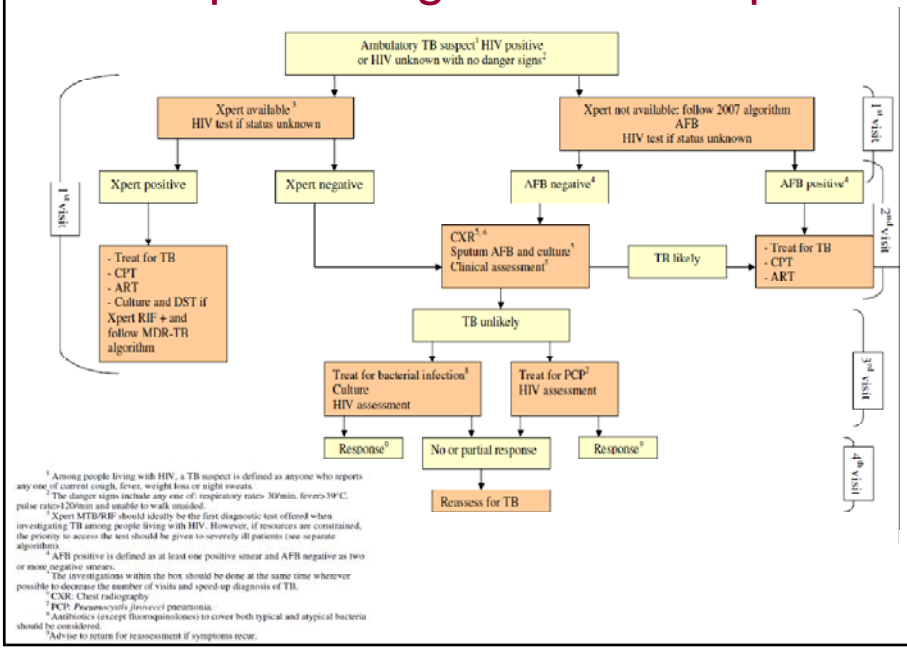


Proposed Algorithms for Xpert



[1] Among people living with HIV, a TB suspect is defined as anyone who reports any one of current cough, fever, weight loss or night sweats.
[2] The danger signs include any one of: respiratory rate> 30/min, fever>39°C, pulse rate>120/min and unable to walk unaided.
[3] Antibiotics (except fluoroquinolones) to cover both typical and atypical bacteria should be considered.
[4] PCP: *Pneumocystis jirovecci* pneumonia.
[5] The investigations within the box should be done at the same time wherever possible to decrease the number of visits and speed-up diagnosis of TB.
[6] AFB positive is defined as at least one positive smear and AFB negative as two or more negative smears.
[7] CXR: Chest radiography
[8] Reassessment for TB includes Xpert examination and clinical assessment.

Proposed Algorithms for Xpert



[1] Among people living with HIV, a TB suspect is defined as anyone who reports any one of current cough, fever, weight loss or night sweats.
[2] The danger signs include any one of: respiratory rate> 30/min, fever>39°C, pulse rate>120/min and unable to walk unaided.
[3] Xpert MTB/RIF should ideally be the first diagnostic test offered when investigating TB among people living with HIV. However, if resources are constrained, the priority to access the test should be given to severely ill patients (see separate algorithm).
[4] AFB positive is defined as at least one positive smear and AFB negative as two or more negative smears.
[5] The investigations within the box should be done at the same time wherever possible to decrease the number of visits and speed-up diagnosis of TB.
[6] CXR: Chest radiography
[7] PCP: *Pneumocystis jirovecci* pneumonia.
[8] Antibiotics (except fluoroquinolones) to cover both typical and atypical bacteria should be considered.
[9] Advise to return for reassessment if symptoms recur.

Implementation issues

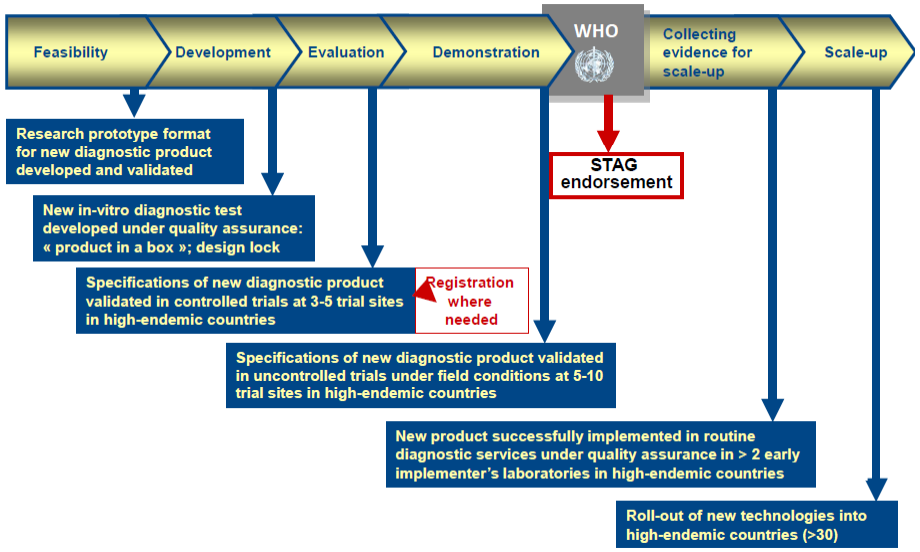
For TB and HIV/AIDS national programmes:

- Where is the appropriate place of Xpert in health system?
- How will we make Xpert work in tiered health/laboratory services?
- What are the capacity strengthening needs for Xpert?
- Where are the gaps and needs for scale-up?

Research needs

- Do we need validation protocol that should be used in all sites?
- What is the programmatic impact of Xpert TB for rapid diagnosis of TB and DR TB among PLHIV?
- What are best operational laboratory models that include Xpert for HIV services?

WHO process (newly revised)



Source: Dr Karin Weyer, WHO

TB REACH

- Based in Stop TB Partnership
- 120 million dollar fund
- Aim: Fund innovative ways to improve TB case finding
- Details available at website – deadline Feb 28 2011
- Good opportunity to team up and test new strategies especially in hard to reach populations, children, HIV-infected etc

Feeling Overwhelmed?

TIME AND OPTIONS FOR COLLABORATIVE INNOVATIVE ACTIONS

THANKYOU