Improving TB Diagnosis in Children

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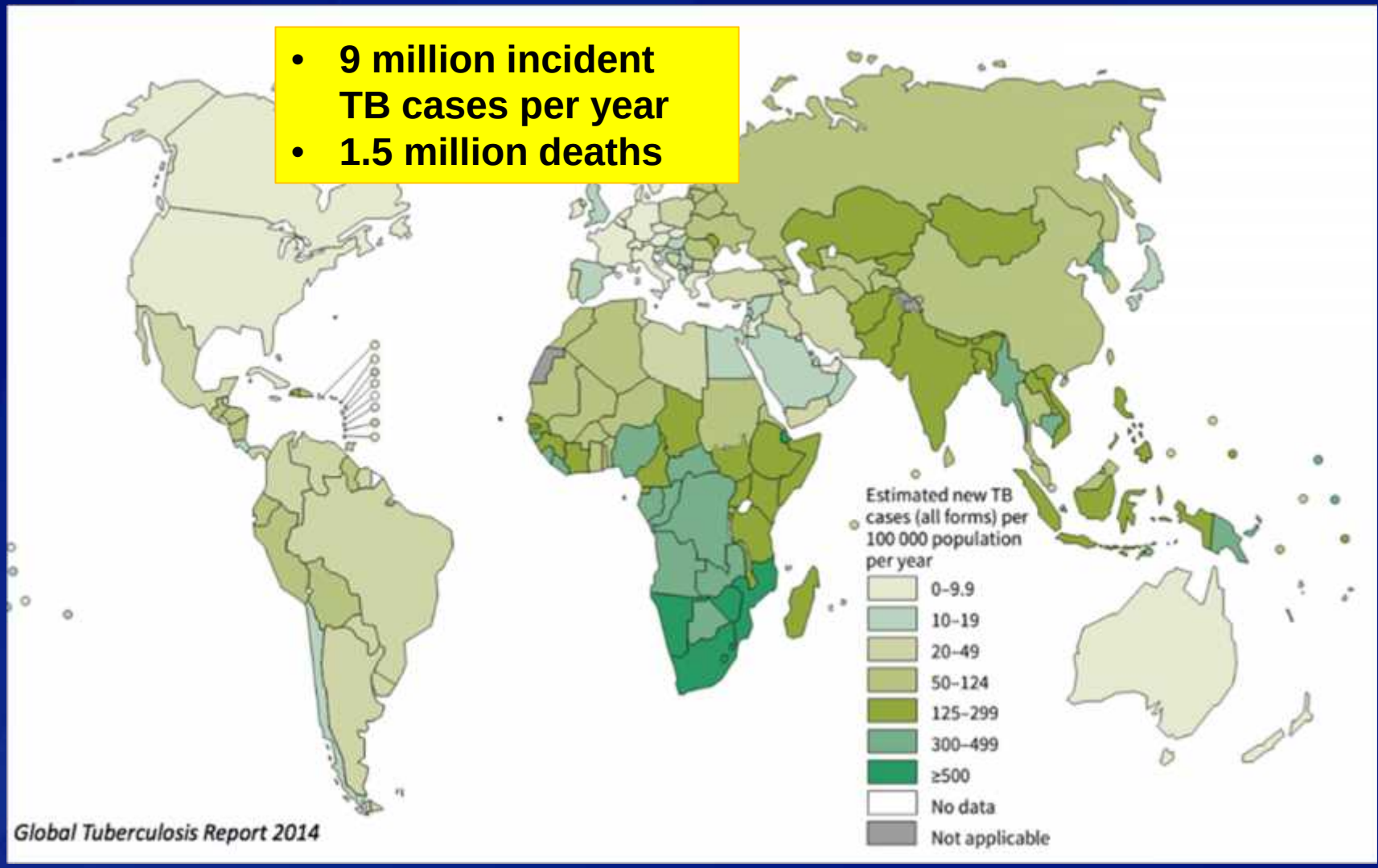
Center for Global Health
Division of Global HIV/AIDS

Global TB Incidence Rates



WHO 2014

Pediatric TB: What is burden?

- No global estimates until 2012
- First WHO estimate:
  - 530,000 incident cases per year
  - "At least" 74,000 childhood TB deaths
- Major challenges due to
  - Insufficient diagnostic accuracy
  - Poor case detection

New Global Pediatric TB Estimates

Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates

Helen E Jenkins, Arielle W Tolman, Courtney M Yuen, Jonathan B Parr, Salmaan Keshavjee, Carlos M Pérez-Vélez, Marcello Pagano, Mercedes C Becerra, Ted Cohen**

- Most rigorous analytic effort to date
- Based on systematic literature and logistic regression models
- Estimated proportion of pediatric cases among all reported cases: 8-12%
- 1 million pediatric TB cases and 31,000 children with multidrug-resistant TB per year

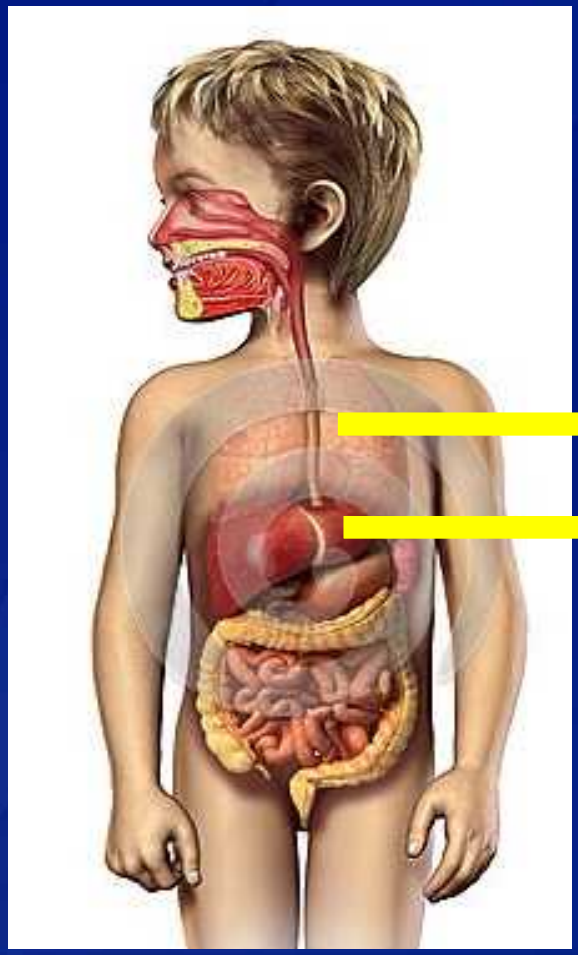
Clinical TB in Children

- Children highly susceptible to TB
- Often progress quickly to disease or death
- Clinical disease differs from adult-type disease
  - Paucibacillary disease
  - Extrapulmonary disease relatively more common
  - Subtle chest X-ray findings (cavitation rare)
  - Non-specific symptoms

Challenges of Diagnosis

- Young children unable to expectorate sputum
- Alternative specimens difficult to obtain
- Microscopy rarely diagnostic
- “Gold standard” of culture insensitive
- Imprecise clinical case definition

Recommended Specimens: Children <5



- Induced sputum
- Gastric aspirate

Picture credit: CanStock Photo

Recommended Specimens

- Gastric aspirate: Insertion of tube through nose to stomach, aspiration of fluid
- Induced sputum: Inhalation of hypertonic saline to induce coughing, nasopharyngeal suctioning of fluid

Gastric Aspirate and Induced Sputum

- Generally require hospitalization
- Require skilled medical staff and equipment
- Invasive, require restraint
- Rarely used in high-burden settings

Alternate Specimens and Tests

- Other tests have been studied
- Many studies of 1 test vs. another
  - IS vs. GA
  - Stool vs. IS and/or GA
  - Blood culture
- Limited to no evidence looking at broad range of specimens / tests, determining which combination best

Peds TB Study Main Objective

Identify the best possible combination of specimen types and diagnostic tests for TB diagnosis

Study Overview

- Design: Prospective cohort study
- Population: Children <5 years of age
- Sick cohort: prolonged symptomatology despite treatment
  - Cough ≥ 4 weeks, malnutrition ≥ 3 weeks, cervical lymphadenopathy ≥ 4 weeks, fever ≥ 1 week
  - Parenchymal abnormalities on CXR
- Cohort of healthy controls enrolled (selected non-invasive testing only)

Study Overview

- Sick cohort
  - Admission for digital X-ray, physical examination, medical history, specimen collection
  - Follow-up at 2 weeks, 2 months, 6 months
- TB treatment and IPT as indicated
- Location: Kisumu, Kenya
- Collaboration between CDC, KEMRI and Harvard
- Funding mostly from USAID, also CDC, and others

Specimen Collection (sick cohort)

Procedure	Test
2 nasopharygeal aspirates	Cx + Xpert
2 induced sputum	Cx + Xpert
2 gastric aspirates	Cx + Xpert
2 string tests	Cx + Xpert
2 stool specimens	Cx + Xpert
2 urine specimens	Cx + Xpert
1 lymph node FNA	Cx + Xpert
1 blood specimen	Cx

Clinical Case Definitions

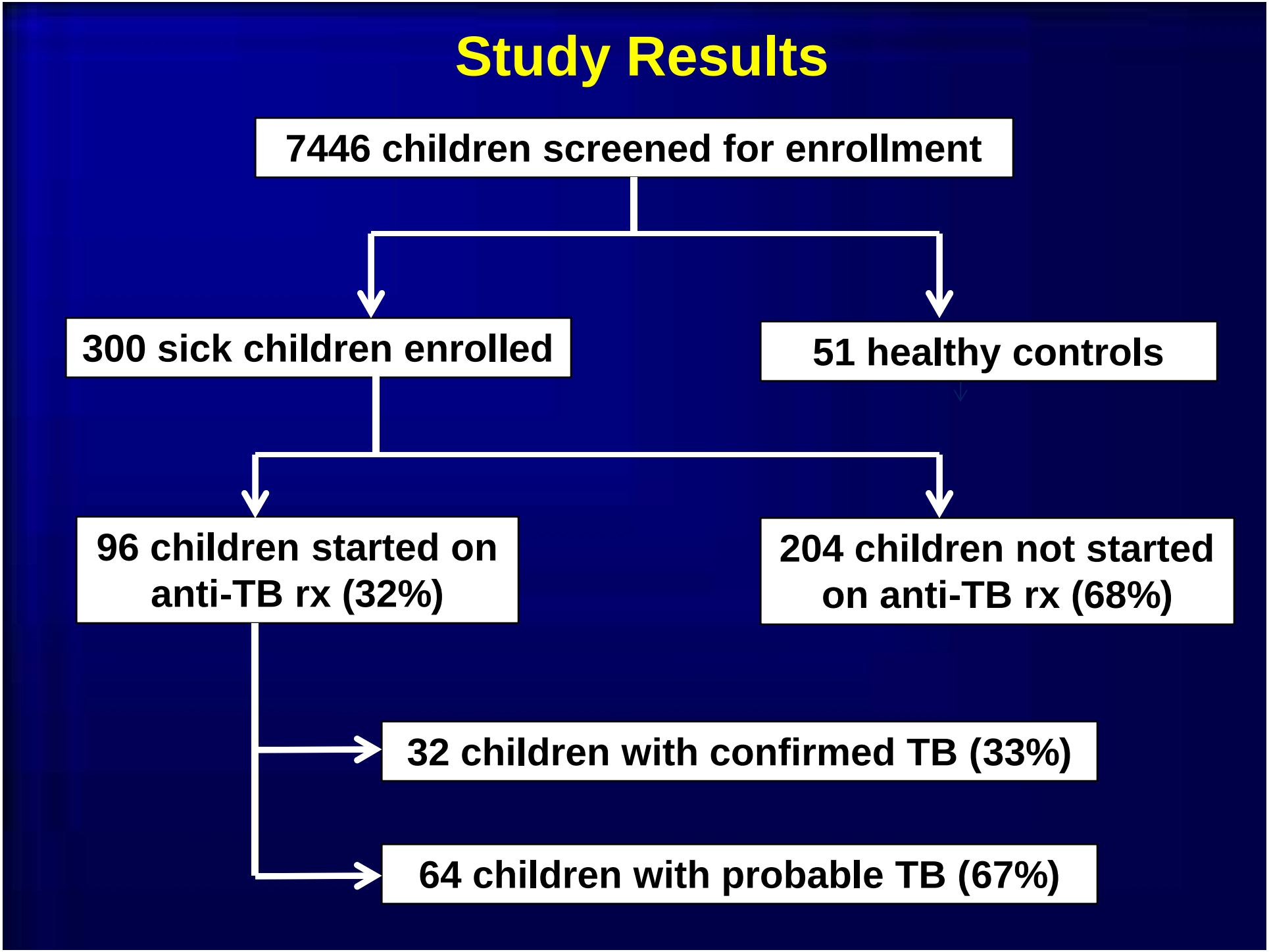
SUPPLEMENT ARTICLE

Evaluation of Tuberculosis Diagnostics in Children: 1. Proposed Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease. Consensus From an Expert Panel

SUPPLEMENT ARTICLE

Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update

Study Results



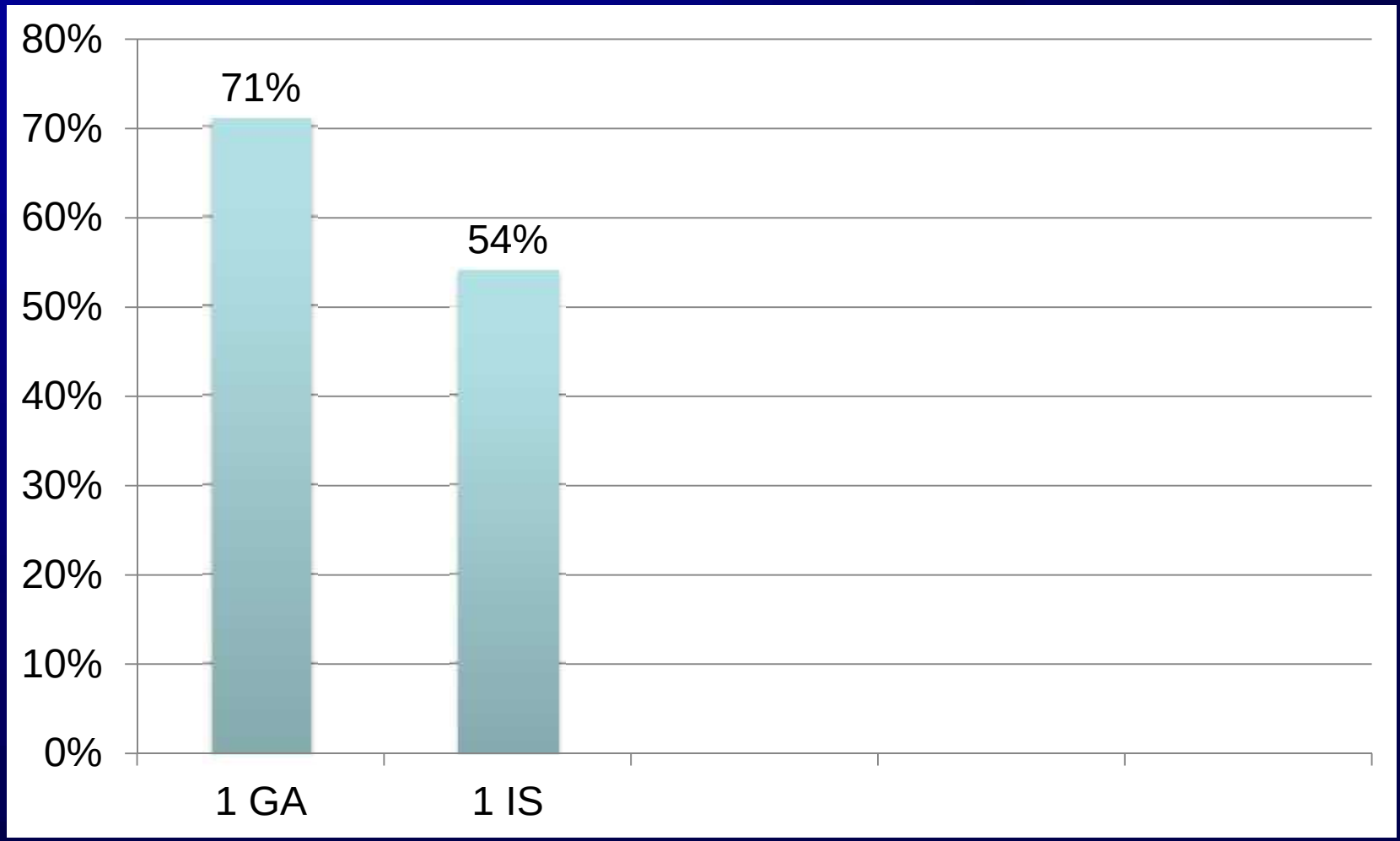
Cohort Characteristics

Characteristic	n	%
Median age in months (IQR)	25	(13-43)
Female gender	151	50
HIV infected	79	26
Severe malnutrition (< -3 WAZ)	66	22
Moderate malnutrition (>-3 to ≤2 WAZ)	27	9
Mild malnutrition (>-2 to ≤1 WAZ)	40	13
BCG vaccinated	291	97
Positive TST	39	15
Household TB exposure	96	32

Diagnostic Yield of Specimen Types (n=32)

Specimen	Diagnostic Test		
	FM+	Cx +	Xpert +
Gastric Aspirate	20%	73%	53%
Nasopharyngeal Aspirate	19%	69%	53%
Induced Sputum	14%	66%	48%
String Test	10%	40%	37%
Stool	13%	23%	43%
Urine	3%	7%	14%
Blood	NA	7%	NA
LNA FNA	0%	21%	14%

Comparative Specimen Yield (n=28)



*** Cx or Xpert positive; among children with available specimens**

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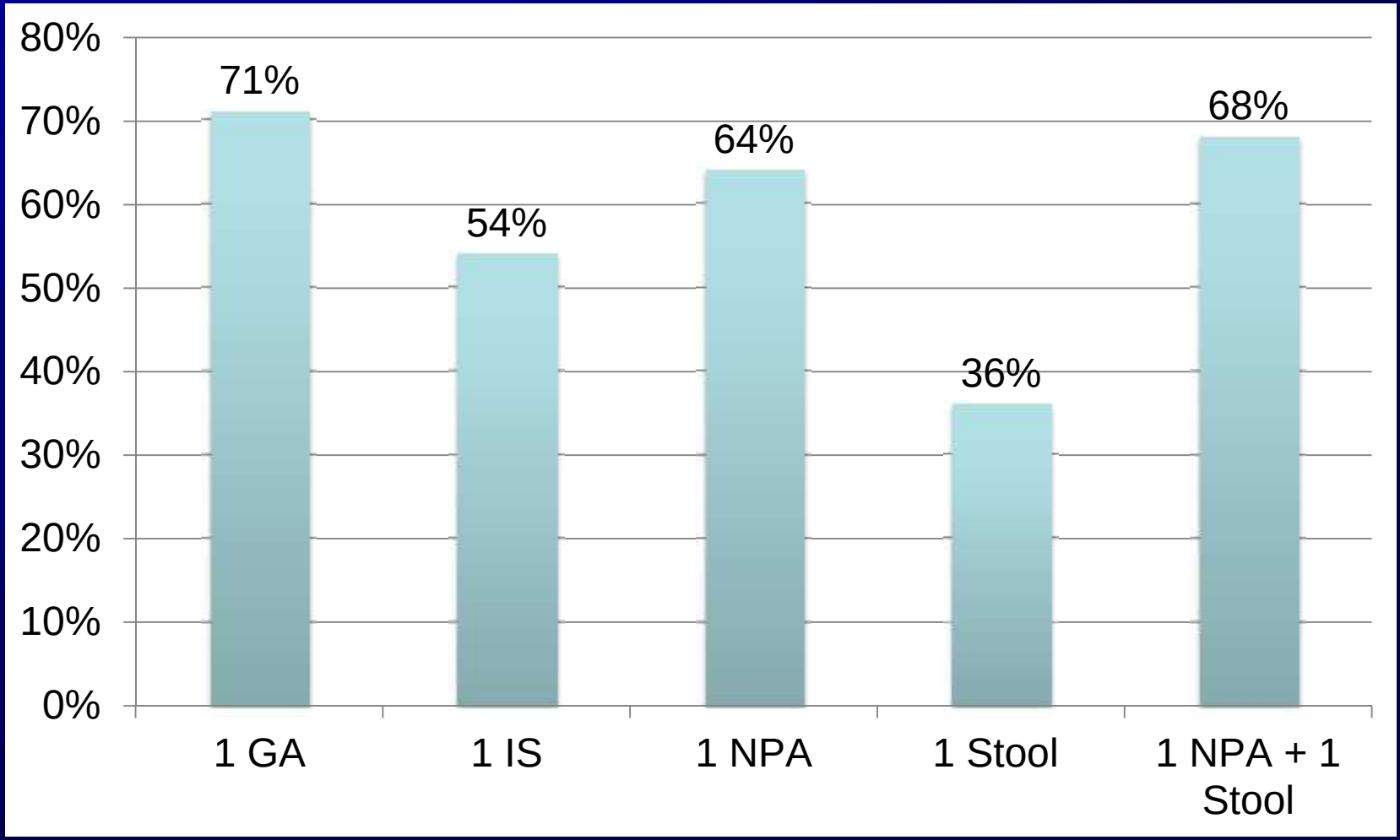


*** Cx or Xpert positive; among children with available specimens**

Additional study aims / elements

- Ancillary tests: evaluating TST, QFT (multiple versions), nutritional tests
- Co-infections: malaria, HIV, helminths, viral and bacterial respiratory pathogens
- Emerging diagnostics / biomarkers
  - Evaluating breath test / electronic nose
  - Repository: extensive storage of specimens for biomarker-related testing
- All of above useful because study has very robust gold-standard, collected non-invasive specimens on healthy controls also

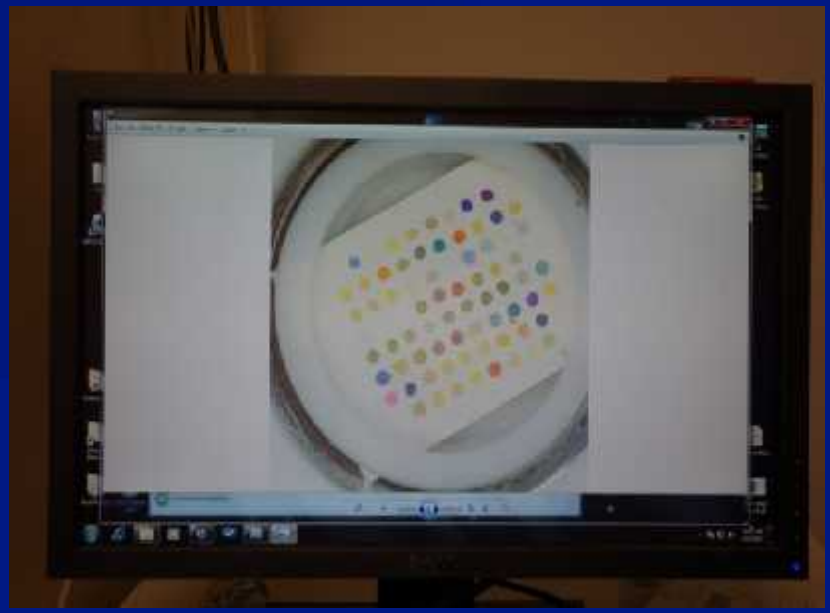
Breath-based diagnosis of TB

- Hippocrates: patients with TB have “distinct smell of breath”
- Volatile organic compounds emitted
- Rats trained to detect TB in sputum
- “Electronic nose” technology used for medical applications (e.g. cancer detection)

Aeonose

- First validation study in adults in Bangladesh
  - 85% sensitivity, 95% specificity
- No data on children

Electronic Nose: Urine Testing



- Detection of volatile organic compounds (VOCs) in urine
- VOCs interact with chemical compounds affixed to filter paper
- Specific pattern developed corresponding to MTB

Conclusions, next steps, program implications

- TB culture on gastric aspirate with the highest diagnostic yield
- Identified combination of more easily obtainable specimens with yield comparable to commonly accepted gold standard (NPA + stool)
  - Kenya may pilot this approach more broadly
- Evidence also can support determining which tests to use in clinical trials
- Evaluations of emerging diagnostics could help to advance field of TB diagnostics in children

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For more information please contact Centers for Disease Control and Prevention

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Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: http://www.cdc.gov

Other Tests

Test Type	Test	Sick cohort	Healthy controls
Ancillary	TST	Yes	Yes
	Quantiferon G-IT Assay	Yes	Yes
	Micronutrients assessment (sub-study)	Yes	Yes
	T Spot. TB (sub-study)	Yes	No
Emerging	Immunmodulated Quantiferon	Yes	Yes
	QFT Plus	Yes	Yes
	Breath testing (electronic nose)	Yes	Yes
Co-Infection	HIV, malaria, helminths	Yes	Yes
	Respiratory viral and bacterial infections	Yes	No

Biomarker Diagnostics

Specimen	Host-based	Pathogen-based
Breath	Electronic nose for volatile organic compounds (VOCs)	
Urine	Electronic nose for VOC detection	
Urine	Proteomics	ELISA-based peptide detection
Urine	Cytokine/Chemokine profiling	
Blood	Proteomics	
Blood	Transcriptomics	
Blood	Novel IFN-γ assays	
Blood	Cytokine/Chemokine profiling	
OP swab		Multiplex PCR, Metagenomics
NP swab		Multiplex PCR, Metagenomics
Gastric aspirate		Multiplex PCR, Metagenomics

Repository Specimens

Specimen	# of Aliquots	Sick Cohort	Healthy Controls
Plasma	4	Yes	Yes
QFT supernatant (TB Ag/Nil)	4	Yes	Yes
Serum	4	Yes	Yes
Whole blood (Paxgene)	1	Yes	Yes
Urine (standard collection)	7	Yes	Yes
Urine (nucleic acid storage)	1	Yes	No
Stool (standard collection)	2	Yes	Yes
Stool (nucleic acid storage)	2	Yes	Yes
NP swab (nucleic acid storage)	1	Yes	No
OP swab (nucleic acid storage)	1	Yes	No
Gastric aspirate (nucleic acid storage)	1	Yes	No
PBMCs (sub-study)	1	Yes	No

Global Pediatric TB

- Deadly
- Vastly under-diagnosed, true burden unknown
- No good diagnostic test
  - *"Gold standard" culture positive in <10-30% cases*
- Preventable and treatable

Emerging Diagnostic Tests

- Detect biomarkers of disease or infection
  - Host-specific
  - Bacteria-specific
  - Both host and bacteria
- Evaluate performance characteristics

SUPPLEMENT ARTICLE

A Blueprint to Address Research Gaps in the Development of Biomarkers for Pediatric Tuberculosis

Mark Patrick Nicol, Devasena Gnanashanmugam, Renee Browning, Eleanor S. Click, Luis E. Cuevas, Anne Detjen, Steve M. Graham, Michael Levin, Mamodikoe Makhene, Payam Nahid, Carlos M. Perez-Velez, Klaus Reither, Rinn Song, Hans M. L. Spiegel, Carol Worrell, Heather J. Zar, and Gerhard Walzl

Childhood tuberculosis contributes significantly to the global tuberculosis disease burden but remains challenging to diagnose due to inadequate methods of pathogen detection in paucibacillary pediatric samples and lack of a child-specific host biomarker to identify disease. Accurately diagnosing tuberculosis in children is required to improve case detection, surveillance, healthcare delivery, and effective advocacy. In May 2014, the National Institutes of Health convened a workshop including researchers in the field to delineate priorities to address this research gap. This blueprint describes the consensus from the workshop, identifies critical research steps to advance this field, and aims to catalyze efforts toward harmonization and collaboration in this area.

Keywords. tuberculosis; children; diagnosis; biomarker; blueprint

Childhood tuberculosis is estimated to account for 6% of the tuberculosis caseload globally, and for 4%–21% of the caseload in the 22 high-incidence countries that account for 80% of global tuberculosis cases [1]. Mathematical modeling suggests that only 35% of tuberculosis cases in children are detected [2]. Improving the accuracy of tuberculosis diagnosis in children is required to improve case detection and outcomes, surveillance, efficiency of healthcare delivery, future research, and effective advocacy.

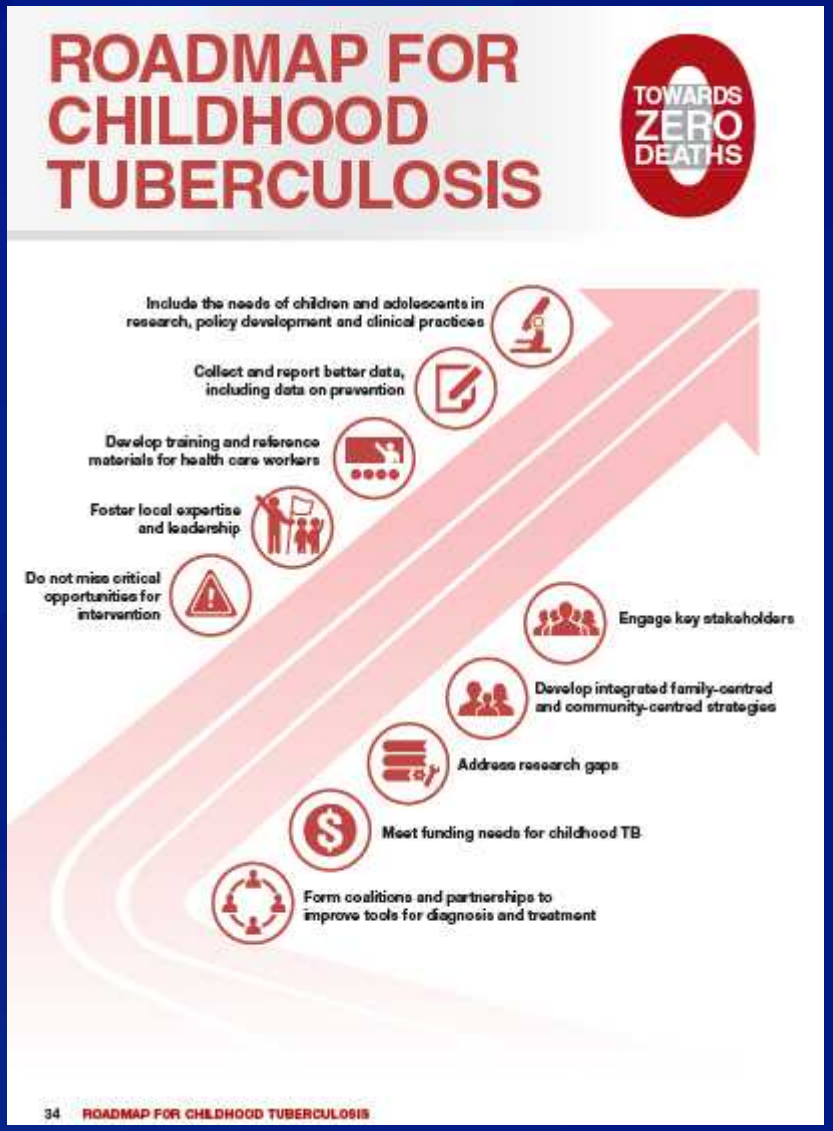
However, attaining an accurate diagnosis in children in tuberculosis endemic settings remains challenging. There is overlap of the clinical presentation of tuberculosis with other common childhood diseases such as pneumonia, human immunodeficiency virus (HIV)–associated lung disease, and severe malnutrition [3]. Clinical and chest radiographic features are often nonspecific and subject to variable interpretation [4]. Structured diagnostic scoring systems based on clinical and radiological findings and tuberculin skin testing show high variability in case yield, with poor agreement between scoring systems [5]. Microbiological confirmation is possible in children of all ages, but is rarely attempted due to perceived difficulties in obtaining respiratory specimens

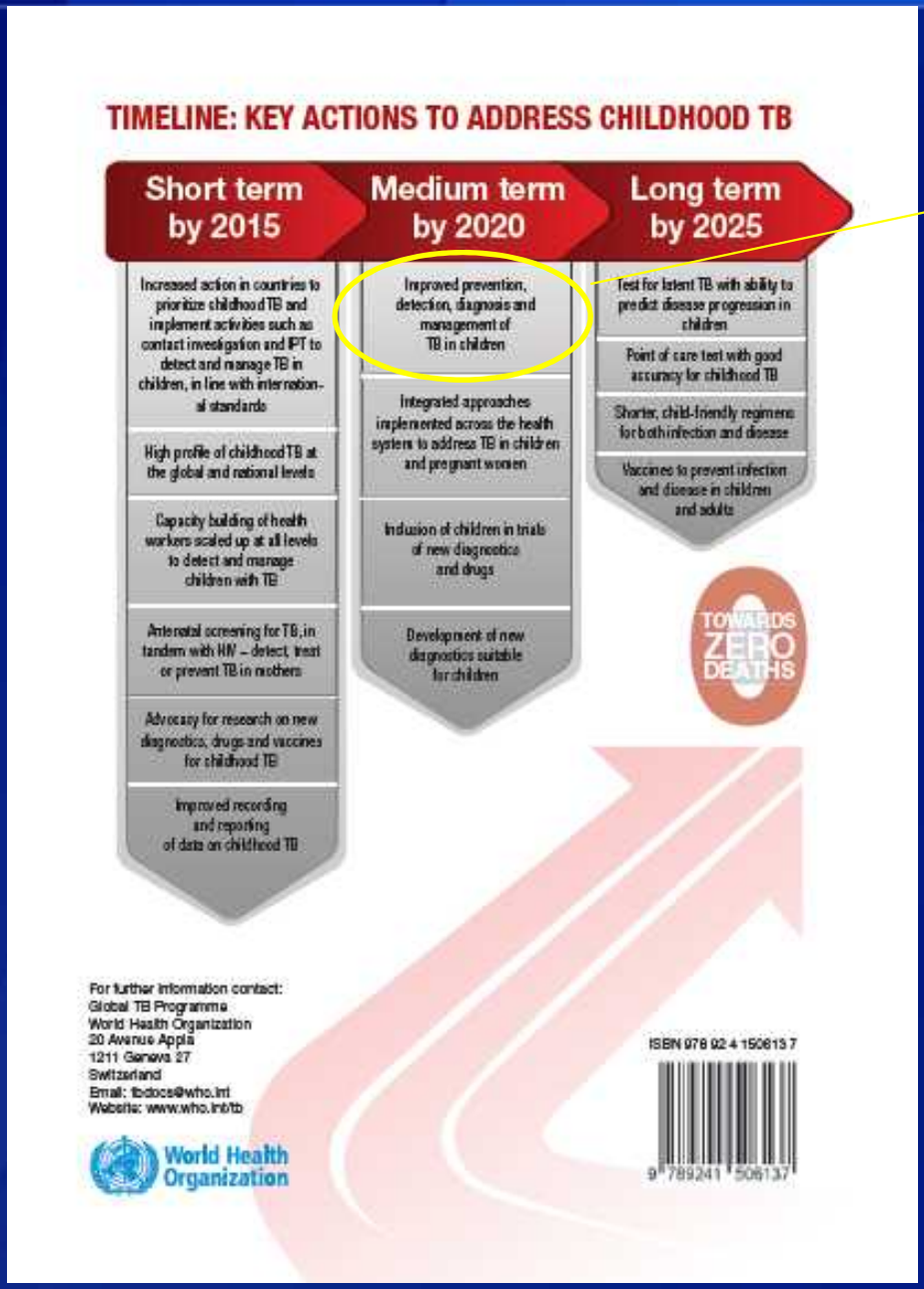
Child TB Research

- Identified as priority area of focus by WHO, International Union of TB and Lung Disease, Stop TB Partnership
- No previous comprehensive evaluation of specimens and tests for diagnosis
- Improved diagnostics urgently needed
  - Clinical care
  - Clinical and vaccine trials

ROADMAP FOR CHILDHOOD TUBERCULOSIS

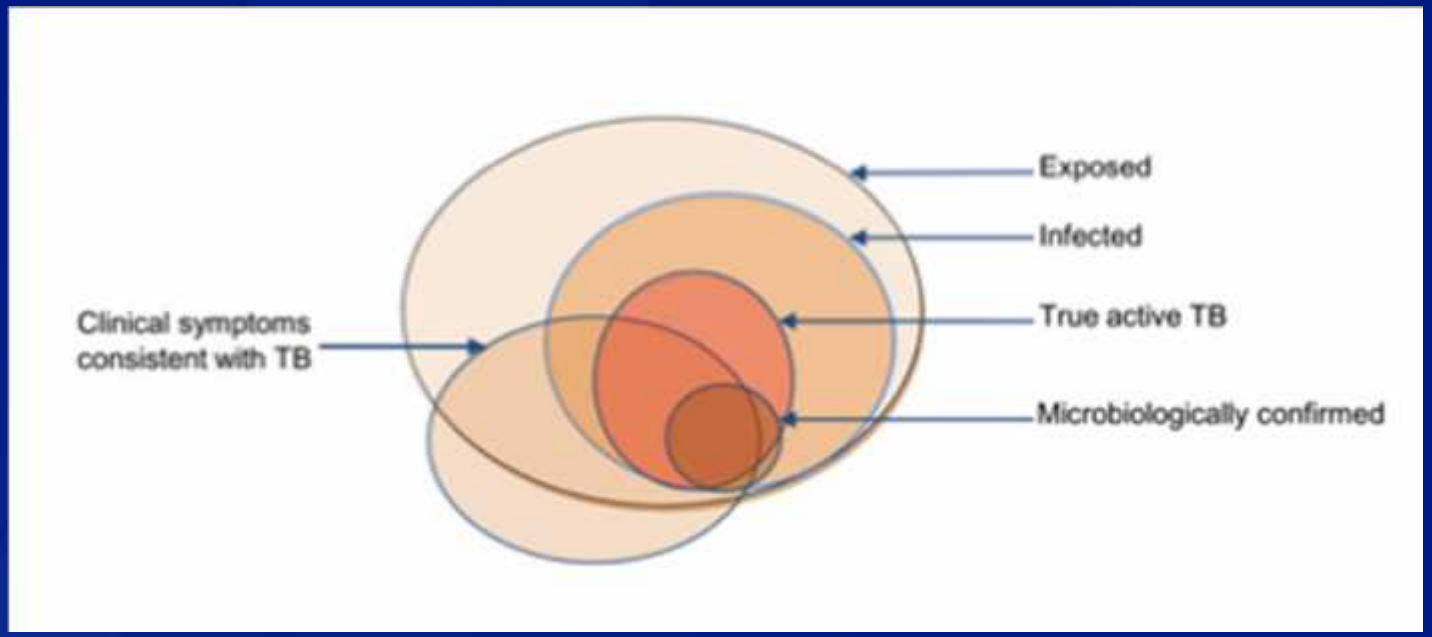
TOWARDS ZERO DEATHS





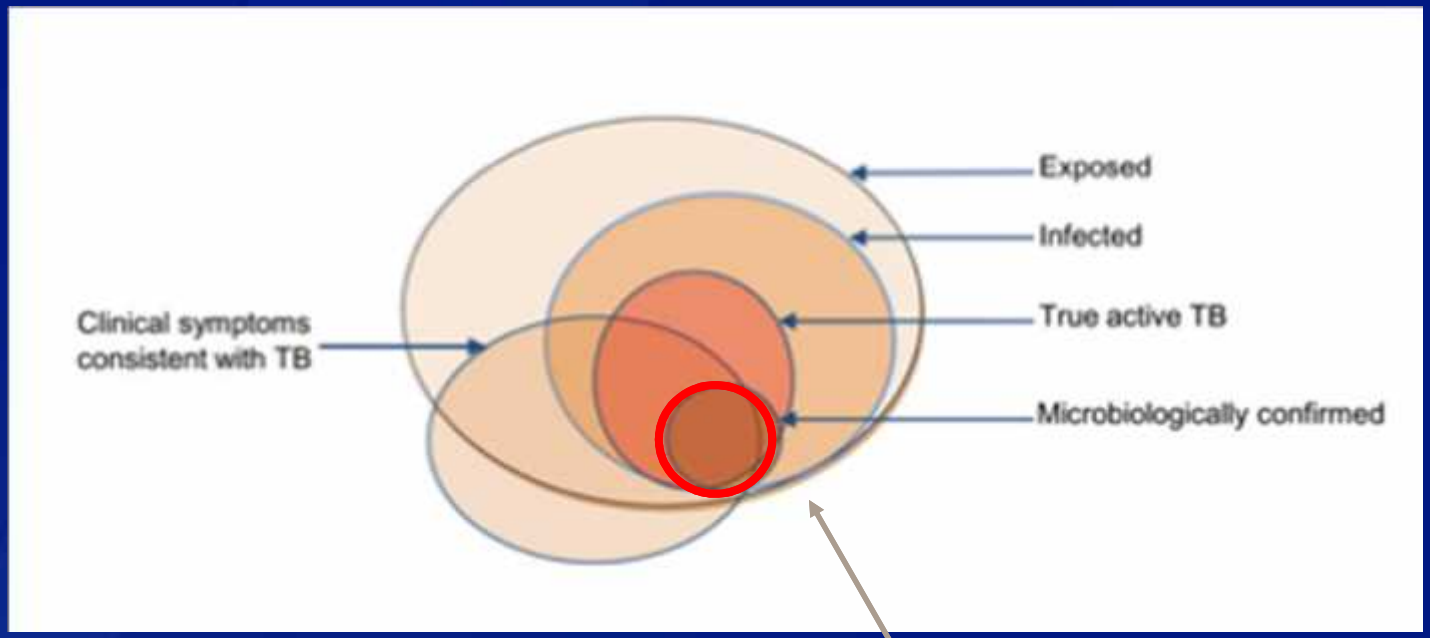
Improved Diagnostics

TB in Children: The Challenge



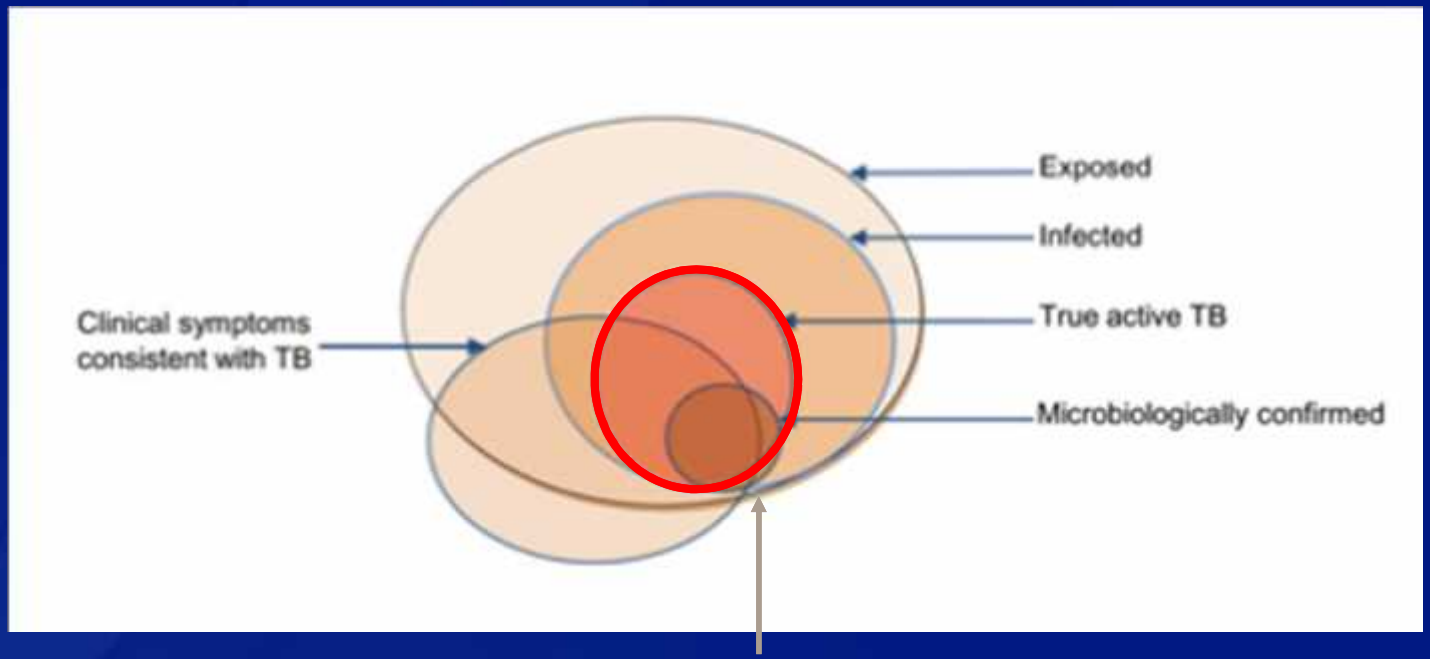
Cuevas LE, et. al., J Infect Dis (2012) 205 Suppl 2:S209-15.

Goals for Improved Diagnostics



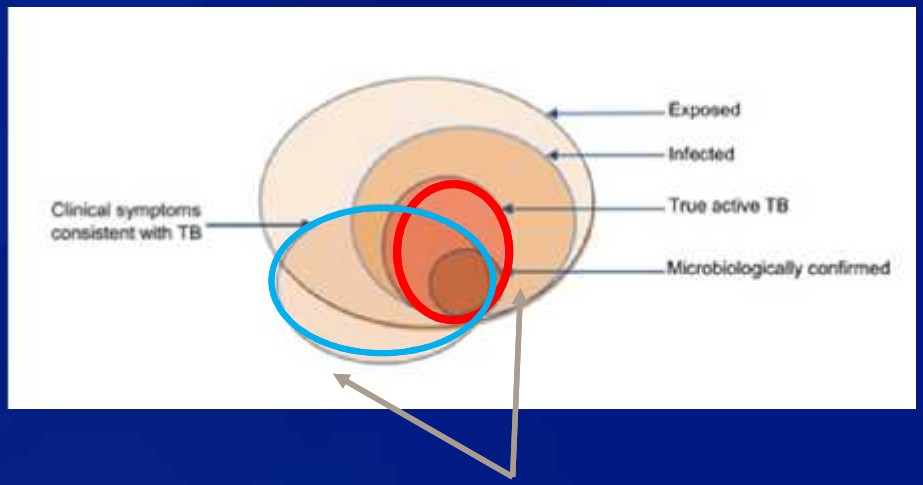
More feasible specimens and tests for bacteriologic diagnosis

Goals for Improved Diagnostics



New diagnostic tests to increase proportion of children with confirmed disease

Goals for Improved Diagnostics



Screening tests to rule out TB

Kenya TB Study Objectives

- Identify the best possible combination of specimen types and diagnostic tests for TB diagnosis
- Determine a feasible and sensitive combination of specimen types and tests
- Characterize the diagnostic performance of biomarkers for TB
- Determine impact of co-infections, immunologic status and micronutrient deficiencies on performance of biomarkers

Study Overview

- Design: Prospective cohort study
- Population: Children <5 years of age
  - HIV infected, uninfected, exposed
  - High suspicion for TB and high likelihood of culture-confirmed TB (*sick cohort*)
  - Asymptomatic children (*healthy controls*)
- Location: Kisumu, Kenya
- Collaboration: KEMRI, CDC, Harvard
- Funded by USAID + CDC

Enrolment Arms

- TB clinic
- Outpatient
- Inpatient
- TB contacts
- Healthy controls

Enrolment Criteria

Enlarged cervical LN (>1cmx1cm) not responding to standard therapy

OR

Cough >28 days including 2 weeks after antibiotics OR

(inpatient only) pneumonia not responding to 5 days antibiotics

OR

Moderate or severe malnutrition not resolving with 3 weeks standard treatment

OR

Fever > 7 days not responding after 5 days with standard antibiotic or antimalarial treatment

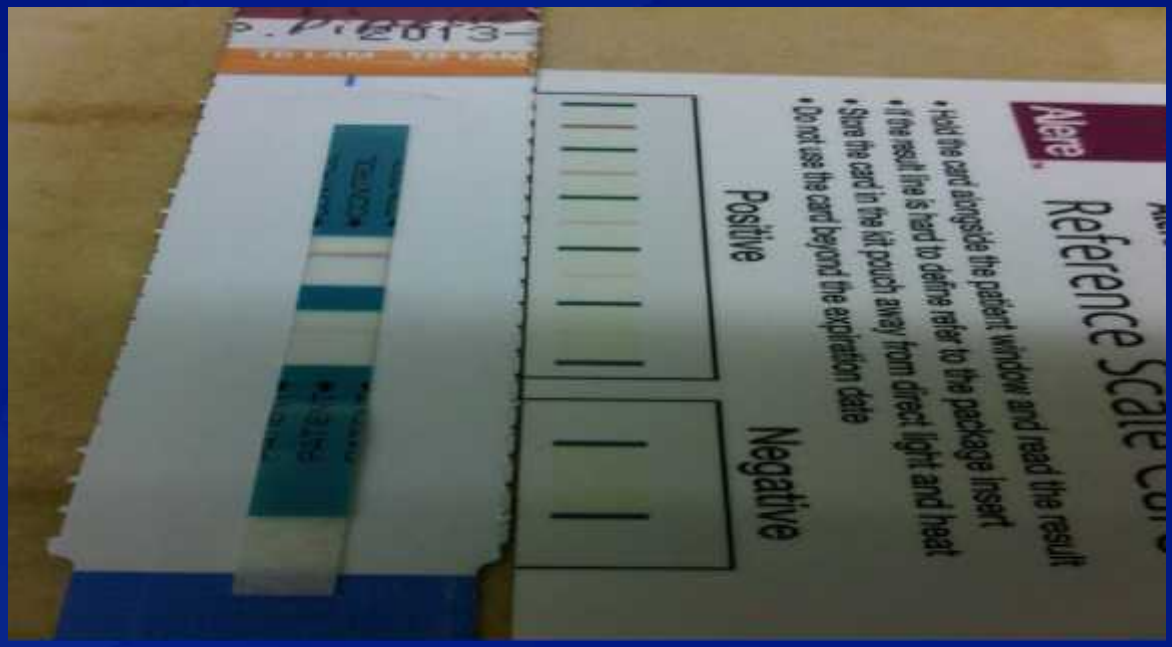
AND

Abnormal chest X-ray with parenchymal disease

Study Procedures

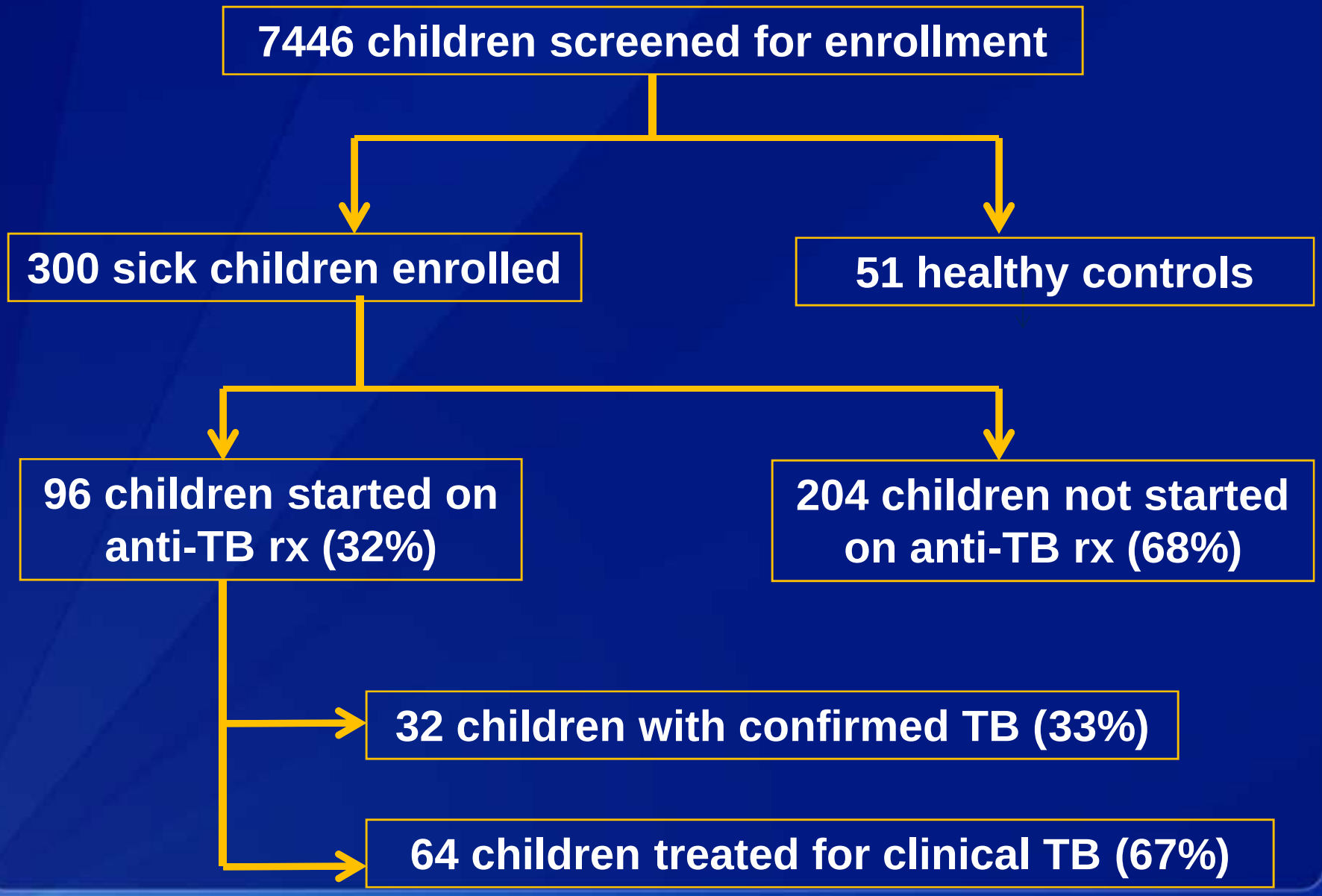
- Sick cohort
  - Admission for digital X-ray, physical examination, medical history, specimen collection
  - Follow-up at 2 weeks, 2 months, 6 months
- TB treatment and IPT as indicated
- Standard research clinical case definitions for anlalysis

TB LAM Antigen Assay



- Detects lipoarabinomannan (LAM) antigen in human urine
- Relatively sensitive (~67%) test in adults with TB/HIV (CD4<50)
- Limited data in children

Study Results



Case Classification

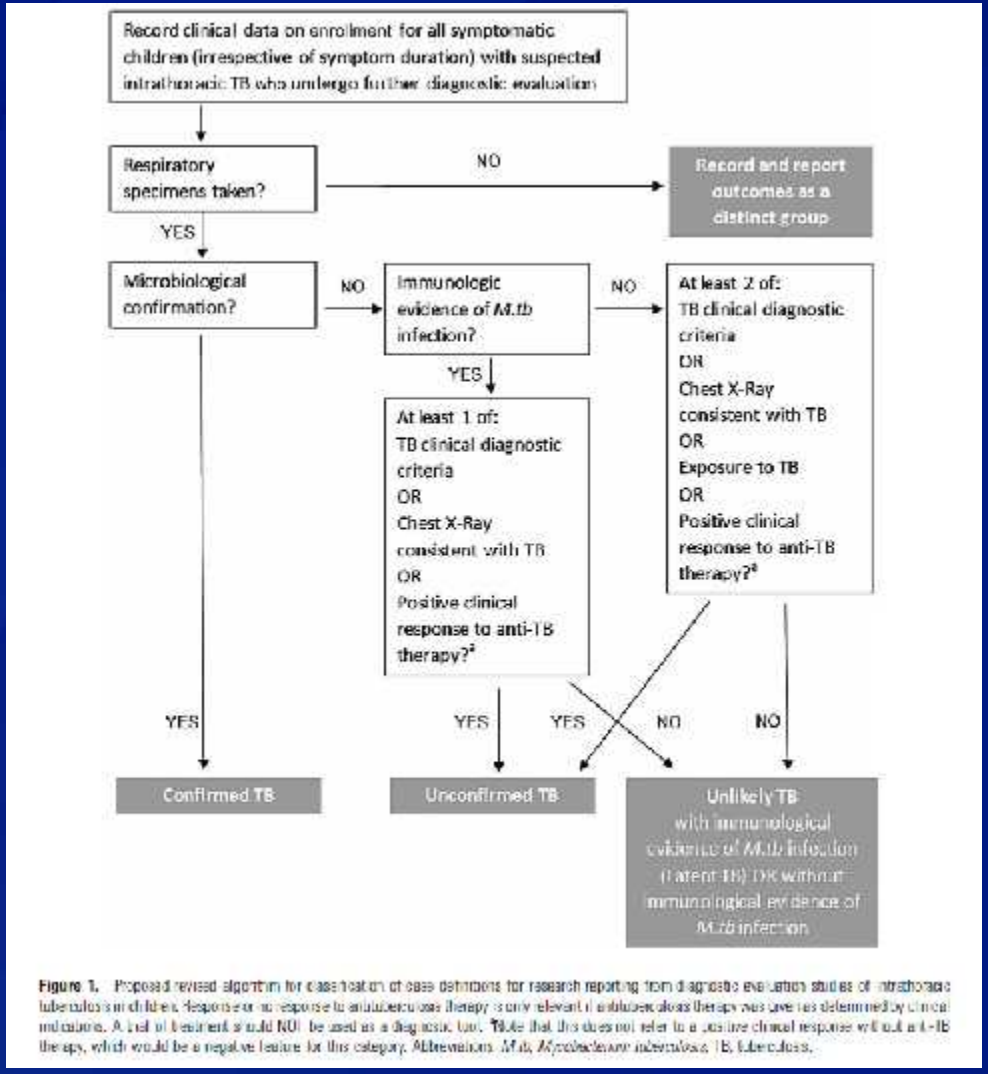
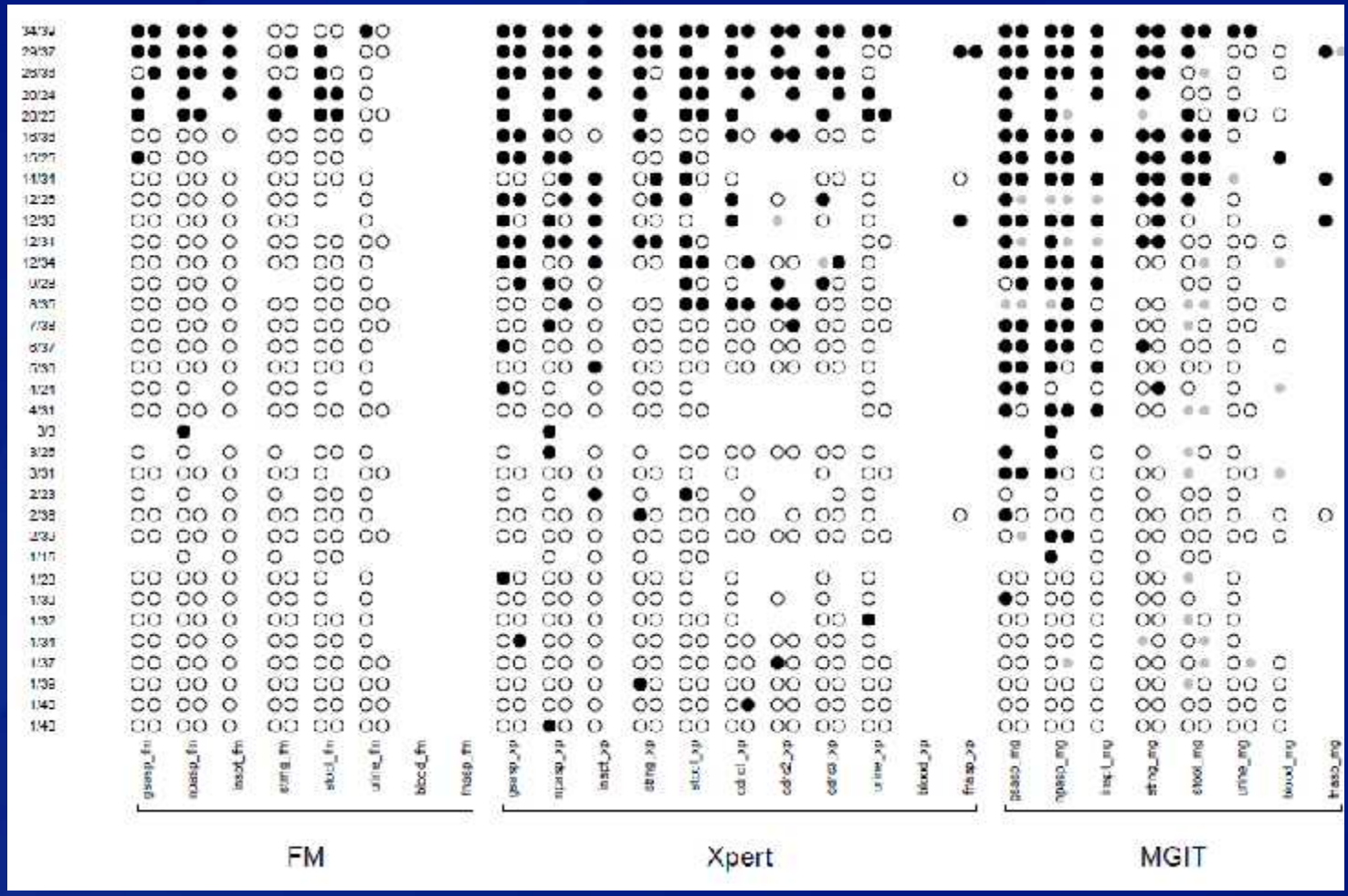


Figure 1. Proposed revised algorithm for classification of case definitions for research reporting from diagnostic evaluation studies of intrathoracic tuberculosis in children. Response or no response to antituberculosis therapy is only relevant if antituberculosis therapy was given as determined by clinical indications. A trial of treatment should NOT be used as a diagnostic tool. Note that this does not refer to a positive clinical response without anti-TB therapy, which would be a negative feature for this category. Abbreviations: *M.tb*, *Mycobacterium tuberculosis*; TB, tuberculosis.

Specimens and Bacteriologic Tests (Sick Cohort)

Procedure	Test
2 nasopharygeal aspirates	Cx + Xpert
2 induced sputum	Cx + Xpert
2 gastric aspirates	Cx + Xpert
2 string tests	Cx + Xpert
2 stool specimens (3 additional)	Cx + Xpert (Xpert)
2 urine specimens	Cx + Xpert
1 lymph node FNA	Cx + Xpert
1 blood	Cx

Bacteriologic Test Results among Confirmed TB Cases



Diagnostic Yield of Specimen Types (n=32)

Specimen	Diagnostic Test		
	FM+	Cx +	Xpert +
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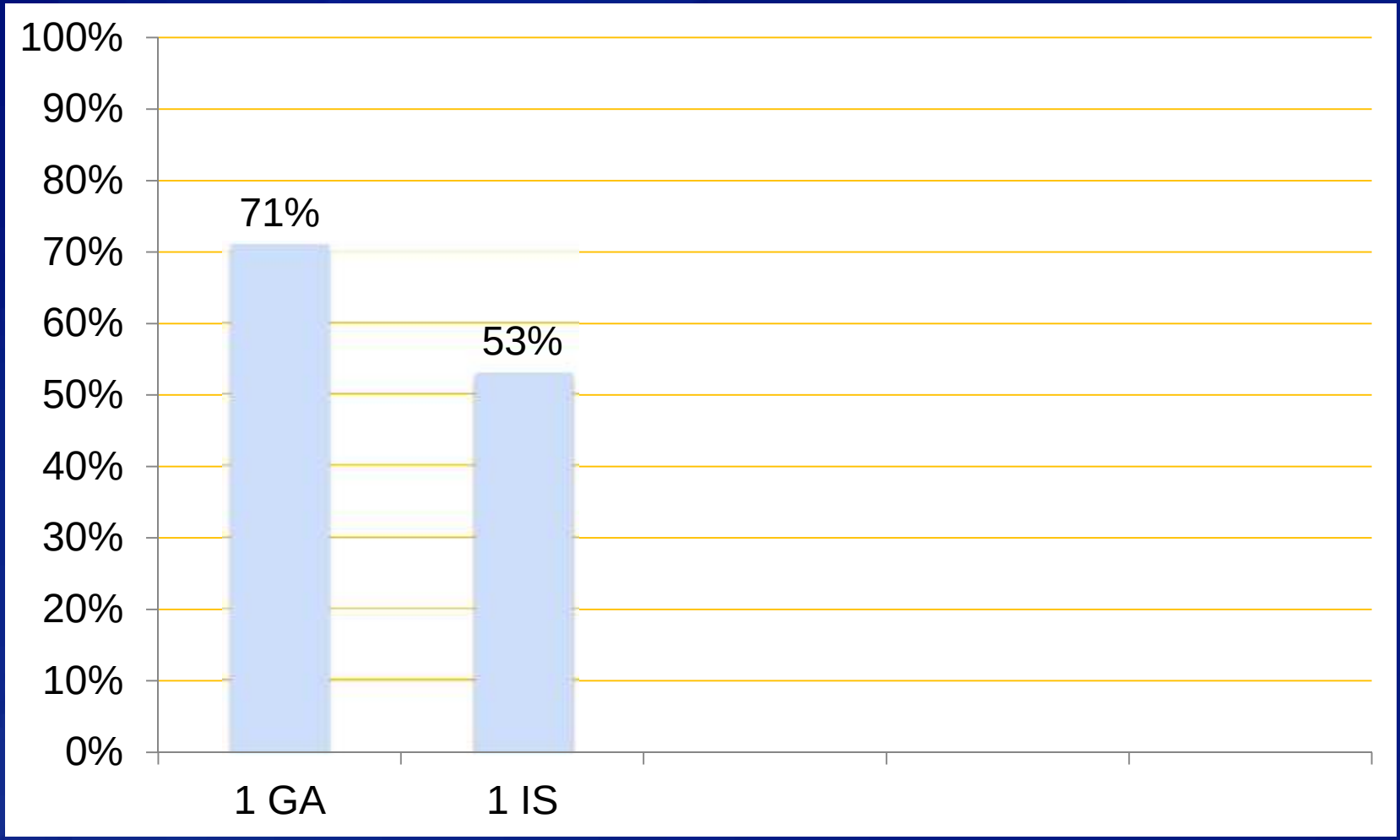
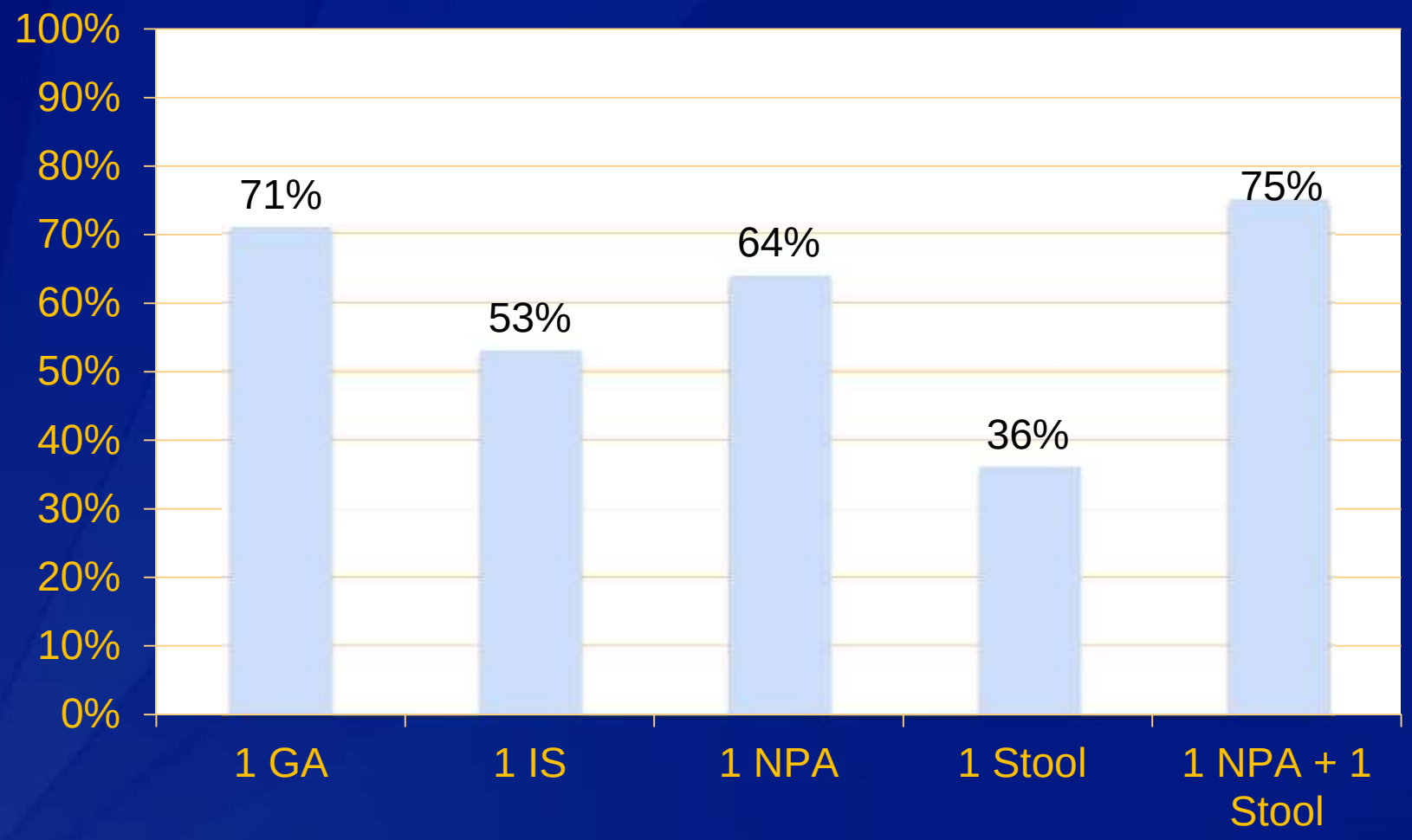
Nasopharyngeal Aspirate	19%	69%	53%
Induced Sputum	14%	66%	48%
String Test	10%	40%	37%
Stool	13%	23%	43%
Urine	3%	7%	14%
Blood	NA	7%	NA
LNA FNA	0%	21%	14%

Gold Standard Specimen Yield (n=28)



*** Cx or Xpert positive; among children with available specimens**

Comparative Specimen Yield (n=28)



*** Cx or Xpert positive; among children with available specimens**

Conclusions

- TB culture on gastric aspirate has single highest diagnostic yield but difficult to obtain sample
- Combination of more easily obtainable specimens (stool and NPA) has yield comparable to gastric aspirate
- Next steps:
  - Ongoing analysis to determine best possible combination for research and clinical purposes
  - Ongoing work on evaluation of biomarkers