Systematic Review Case Study: Lipoarabinomannan

Advanced TB Diagnostic Research Course
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Outline

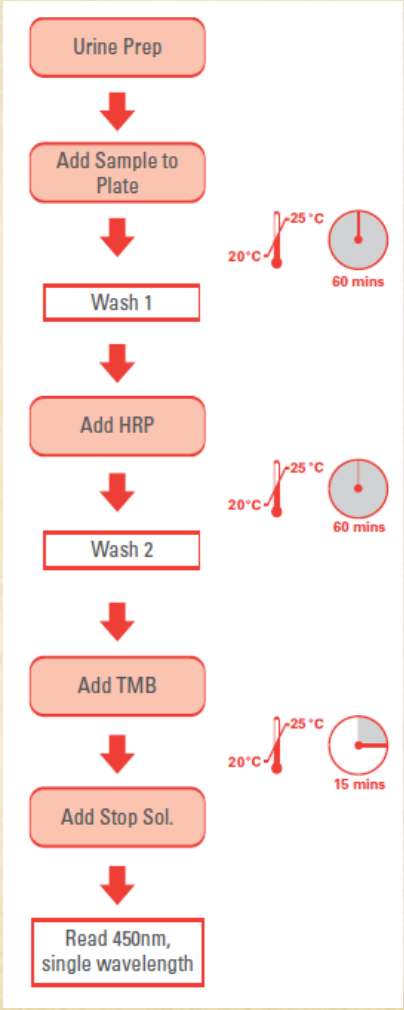
- Introduction: Why we were doing this?
- Methods: How did we do it?
- Results: What did we find?
- Discussion: What did we learn?

Lipoarabinomannan

- Structurally important 17.5kD heat-stable glycolipid in cell wall of *Mycobacterium tuberculosis*
- Constitutes up to 15% of total bacterial weight
- Immunogenic virulence factor that is released from metabolically active or degrading bacteria during infection
- Can be detected in urine

Antigen Detection

- Urine
  - Non-invasive, simple to collect, process, store
  - Fewer infection control concerns
  - Particularly in children
- 2.5 hour turnaround time
- Point of Care capability
  - Lateral flow dipstick format
- Initially very positive evaluations of non-commercial prototype
  - Subsequent studies less conclusive
  - But licensed use is *only* for screening of HIV+ TB suspects, to be followed up by confirmatory testing



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Diagnosing tuberculosis with urine lipoarabinomannan: systematic review and meta-analysis

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Systematic Review

- Search:
  - 3 databases: PubMed, EMBASE, Web of Science
  - No language restrictions on search
  - Hand search of reference lists of included studies, contacted industry reps and experts in field
- Eligibility:
  - Any LAM detection assay
  - Patients suspected or known to have TB
  - Acceptable reference standards: culture, AFB smear/histopathology, NAAT
  - … clinical information frequently used to subgroup primary study results (argh!!)
- Selection:
  - Titles/Abstracts screen by 1 reviewer
  - Full text review and selection by 2 independent reviewers
- Extraction:
  - Piloted before finalized
  - 2 independent reviewers, disagreements resolved by consensus
- Quality:
  - QUADAS

APPENDIX I

LAM antigen detection for the diagnosis of active TB
EXTRACTION FORM

Study #: _____ Reviewer: _____
Author: _____ Year: _____ Language: _____
Published: Y / N Country: _____ Sponsor: _____

Index Test: _____ Commercial: Y / N If Yes, Manufacturer: _____	Urine Specimens: □fresh □previously frozen □ NR □processed □unprocessed □ NR Specify: _____
Reference: □ culture: _____ (□solid □liquid) □ NAAT: _____ □pathology □ other: _____	Quality Control Reported: Index: Y / N Reference: Y / N Positive Controls Used (Index): Y / N Negative Controls Used (Index): Y / N
Patient Population: □ adults only □ paediatric only □ both □ HIV excluded □ HIV included: _____% □ smear – only □ smear + included: _____% Setting: □ research setting □ reference setting □ periphery □ NR	Study Design: □prospective □retrospective □NR □case control □xsectional □NR Selection: □ consecutive/random □ other_____ □ NR Blinding reported: □index □reference □NR Validation: □complete □partial/differential □NR
Time to Result Reported:	Indeterminate Results Reported:

Data:

Strata	Index	Reference	IND	TP	FP	FN	TN	Total N

Reference Standards

- Many studies defined "Clinical Cases" where microbiological results were negative (smear and culture), but patients were diagnosed on the basis of clinical and radiological features and/or response to treatment

- Instead of excluding these studies, 3 analyses were done:
  - Analysis A = Microbiologic reference standard, with "clinical cases" excluded
  - Analysis B = Strict microbiologic reference standard, with "clinical cases" considered Negative for TB
  - Analysis C = Microbiologic + Clinical reference standard, with "clinical cases" considered Positive for TB

Example

10 Reference Positive

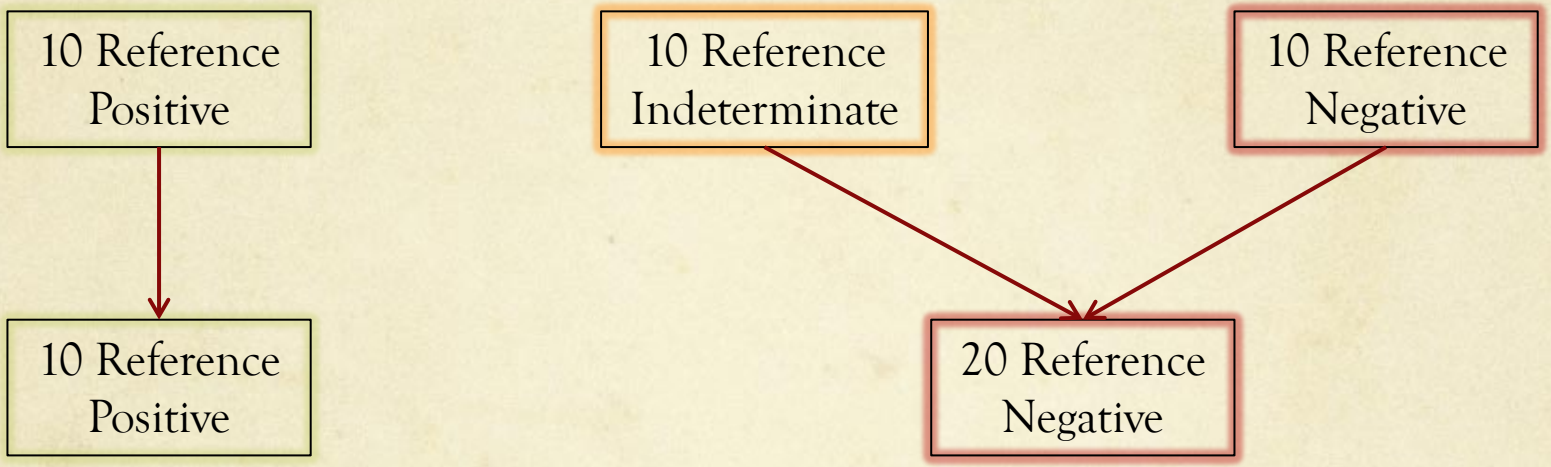
10 Reference Indeterminate

10 Reference Negative

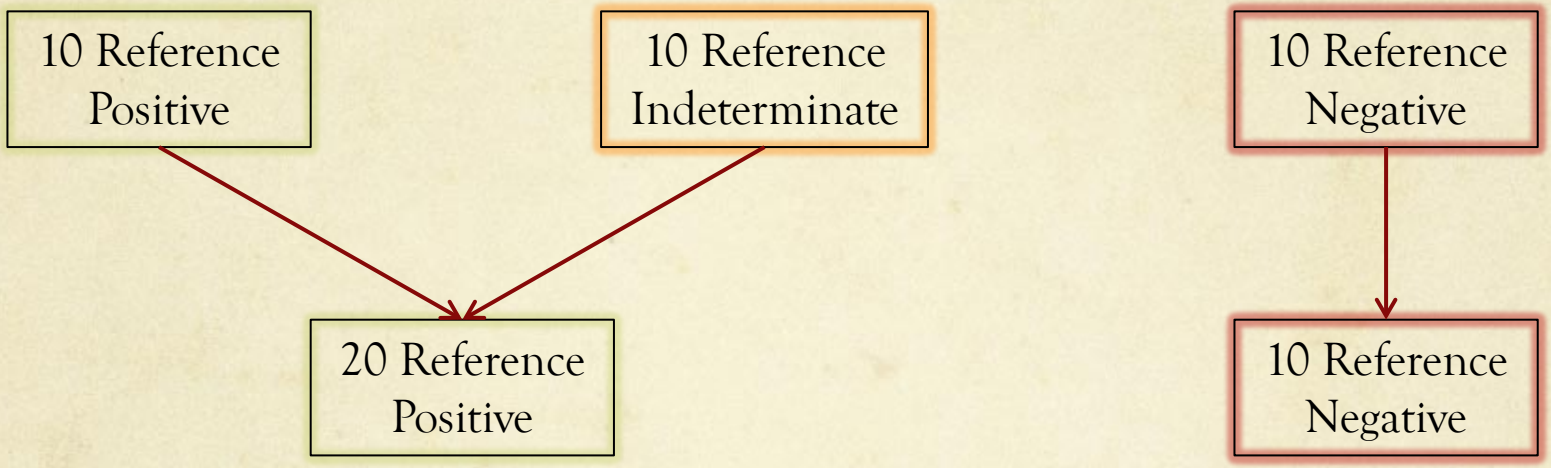
ANALYSIS “A”


10 Reference Positive
10 Reference Positive
10 Reference Indeterminate
10 Reference Negative
10 Reference Negative

ANALYSIS "B"


10 Reference Positive
10 Reference Positive
10 Reference Indeterminate
10 Reference Negative
20 Reference Negative

ANALYSIS "C"

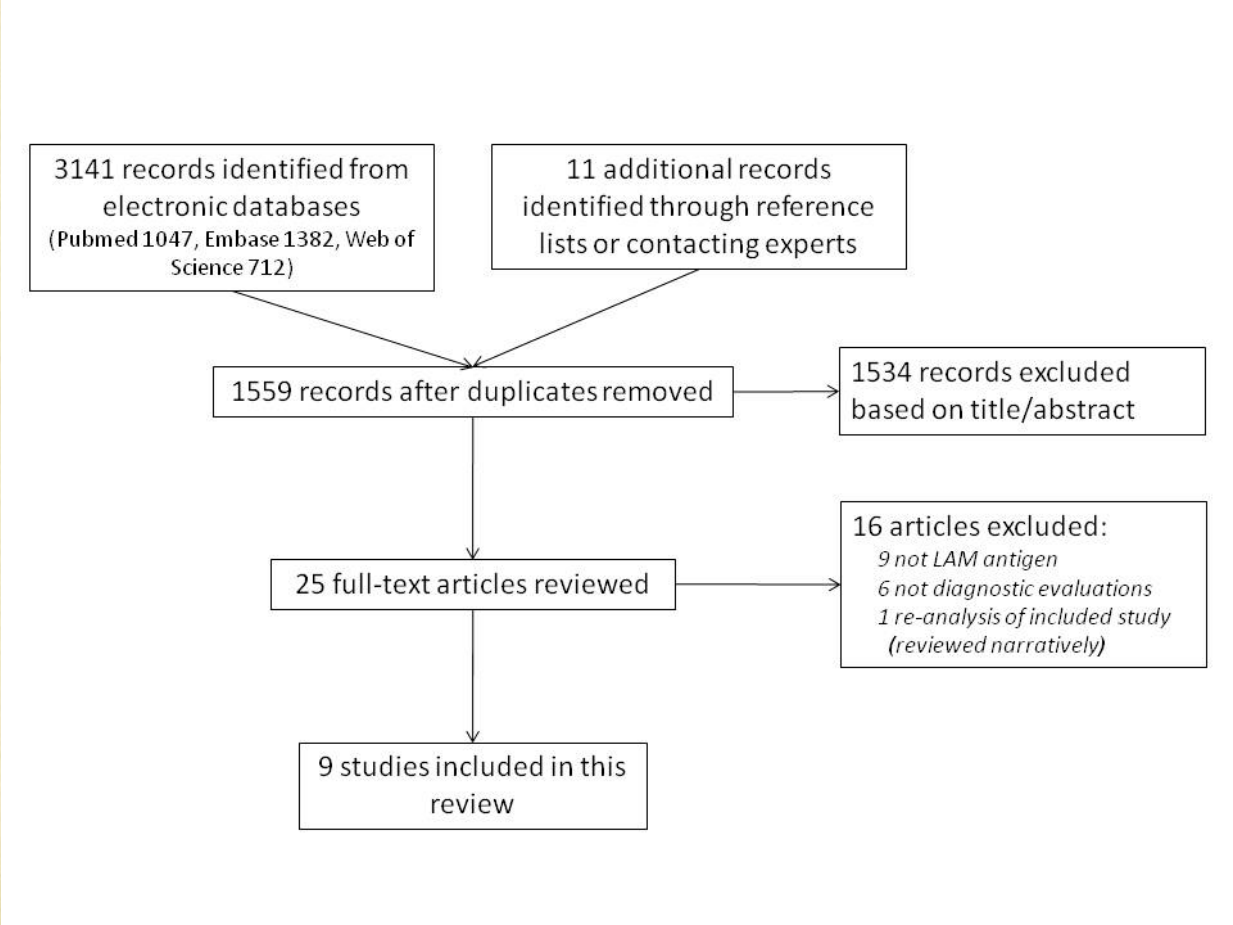

10 Reference Positive
10 Reference Indeterminate
10 Reference Negative
20 Reference Positive
10 Reference Negative

Analysis

- Predefined Subgroups:
  - HIV status
  - Urine specimen fresh vs. frozen
  - Assay version
  - CD4 count within HIV + [planned]

- Pooling done with bivariate random effects regression models

Search & Selection



Study Characteristics

Reference	Author (Year)	Country	Total N	Manufacturer	Urine	% Smear +	% HIV +	HIV Stratified	Reference[a]	Type of Analyses Possible[b]		
										A	B	C
(17)	Hamasur (2001)	SWEDEN	54	NON-COMMERCIAL	FROZEN	11.1	NR	No	Culture + AFB + Clinical[c]	+	-	-
(18)	Tessema (2001)	ETHIOPIA	1000	NON-COMMERCIAL	FROZEN	13.9	NR	No	AFB + Clinical	+	+	-
(21)	Boehme (2005)	TANZANIA	231	CHEMOGEN	FRESH	35	69	No	LJ + CXR	+	+	+
(27)	Reither (2009)	TANZANIA	207	CHEMOGEN	FRESH	16.5	59.1	Yes	LJ + MGIT + Clinical	+	+	+
(28)	Mutetwa (2009)	ZIMBABWE	261	CHEMOGEN	FRESH	30.5	77	Yes	LJ + Clinical	+	-	-
(29)	Lawn (2009)	SOUTH AFRICA	235	CHEMOGEN	FROZEN	3.4	100	Yes	MGIT	-	+	-
(20)	Shah (2009)	SOUTH AFRICA	404	INVERNESS	FROZEN	16	85	Yes	MGIT + Pathology + Clinical	+	+	+
(30)	Daley (2009)	INDIA	200	CHEMOGEN	FROZEN	22.5	8.5	Yes	LJ + MGIT + Clinical	-	+	+
(25)	Dheda (2010)	SOUTH AFRICA	306	INVERNESS	FROZEN	20.9	27.3	Yes	MGIT + Clinical	+	+	+

[a]diagnostic information available to contribute to reference standard; AFB, LJ, MGIT and Pathology considered microbiologic.

[b]Types of Analyses:

A – Analysis excludes clinical cases (patients that were microbiologically-negative, but had strong clinical/radiologic suspicion for TB)

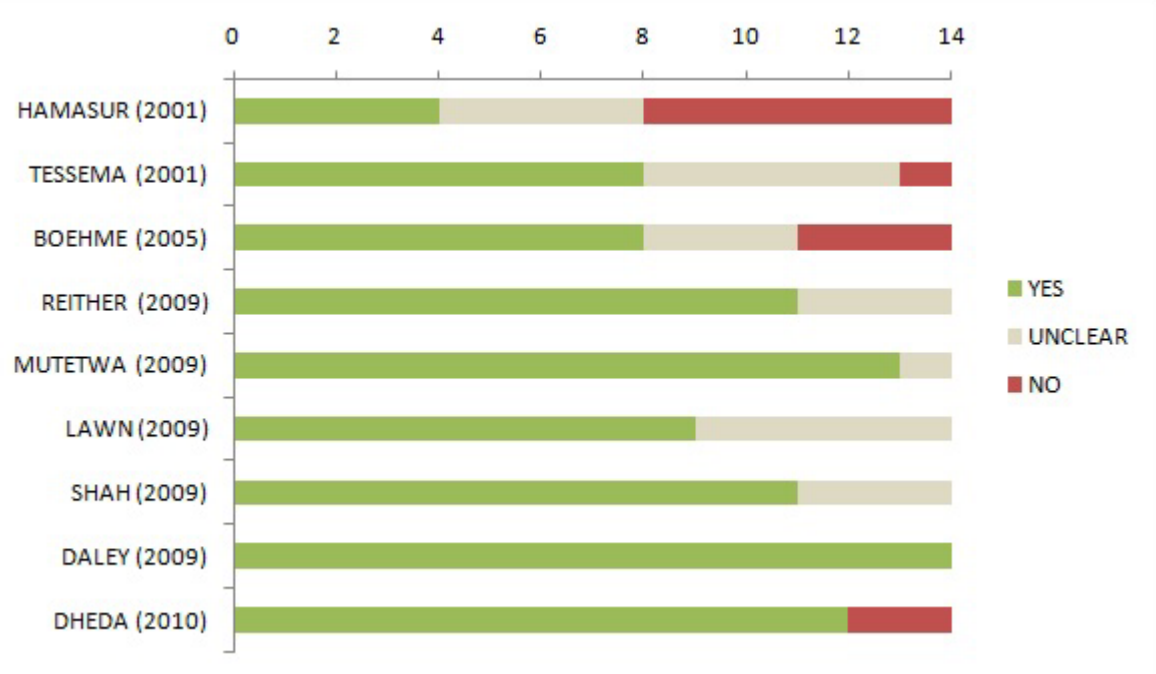
B – Analysis groups clinical cases with all other patients that were microbiologically-negative to be reference negative.

C – Analysis groups clinical cases with patients that were microbiologically-positive as reference positive

[c]Culture confirmation (not otherwise specified) in 9 cases; AFB smear and clinical diagnosis of controls

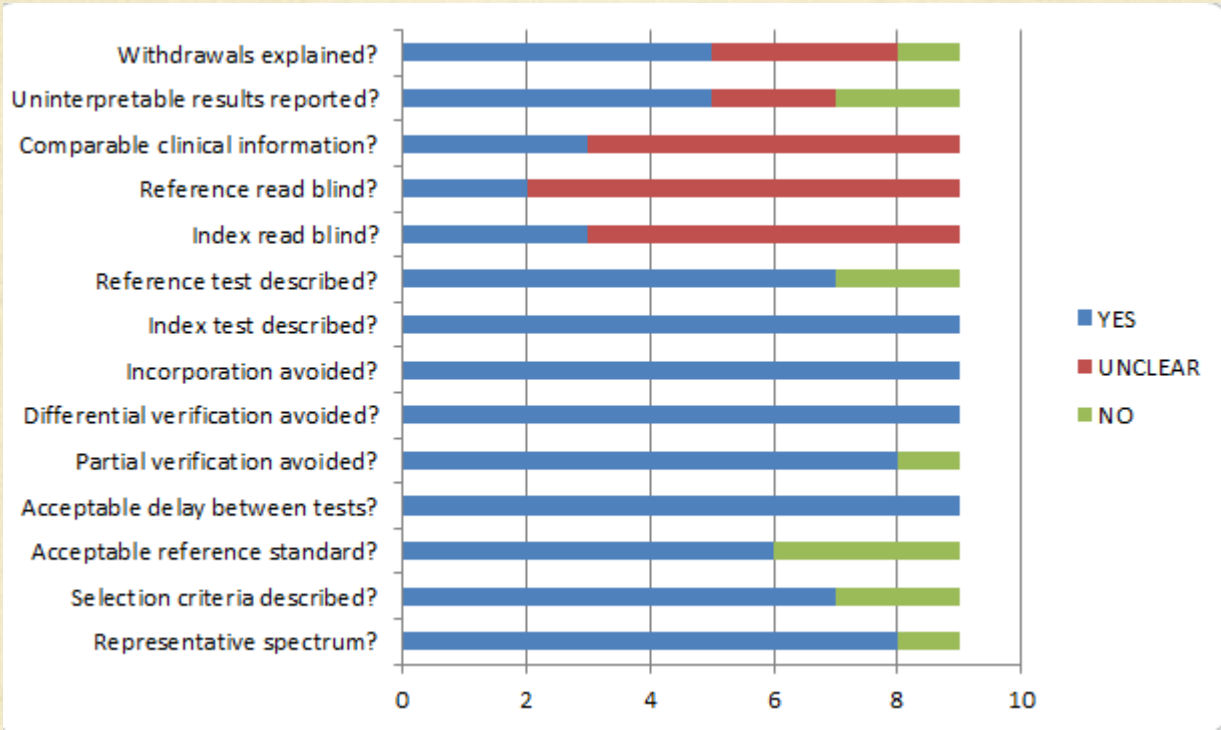
NR = not reported; AFB = acid fast bacilli present in microscopic smear; LJ = Lowenstein-Jensen culture; MGIT = Mycobacterial Growth Indicator Tube (BACTEC, 460 or 960)

QUADAS



QUADAS criteria judged on data used for meta-analysis, not necessarily all data presented in studies

QUADAS



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not necessarily all data presented in studies

Pooled Meta-Analysis Estimates

	Sensitivity[a] (95% CI)	I^2 (p-value)	Specificity[a] (95% CI)	I^2 (p-value)
Analysis (A) – excluding clinical cases (n=7)	60% (38, 79)	97.0% (p<0.0001)	93% (88, 96)	84.5% (p<0.0001)
Analysis B – clinical cases considered NOT active TB (n=7)	47% (26, 69)	97.3% (p<0.0001)	93% (83, 97)	94.8% (p<0.0001)
Analysis C – clinical cases considered active TB (n=5)	34% (14, 62)	98.4% (p<0.0001)	94% (87, 98)	85.5% (p<0.0001)

- Sensitivity highest when clinical cases were excluded; lowest when considered active TB
  - Individual studies range from 13 – 99%
- Specificity less variable
  - Individual studies 79 – 100%

HIV status

		Sensitivity[a] (95% CI)	I^2 (p-value)	Specificity[a] (95% CI)	I^2 (p-value)
Analysis (A) – excluding clinical cases	HIV +ve (n=4)	51% (32. 69)	90.7% (p<0.0001)	94% (79, 98)	77.6% (p=0.004)
	HIV -ve (n=4)	14% (7, 24)	53.3% (p=0.09)	97% (90, 99)	63.1% (p=0.04)
Analysis B – clinical cases considered NOT active TB	HIV +ve (n=4)	56% (40, 71)	24.1% (p<0.0001)	95% (77, 99)	92.0% (p<0.0001)
	HIV -ve (n=3)[b]	18% (10, 29)	0.0% (p=0.88)	90% (85, 93)	69.3% (p=0.04)
Analysis C – clinical cases considered active TB	HIV +ve (n=2)[b]	49% (43, 54)	54.4% (p=0.14)	91% (85, 96)	69.5% (p=0.07)
	HIV -ve (n=2)[b]	16% (8, 28)	0.0% (p=0.4)	94% (86, 98)	70.8% (p=0.06)

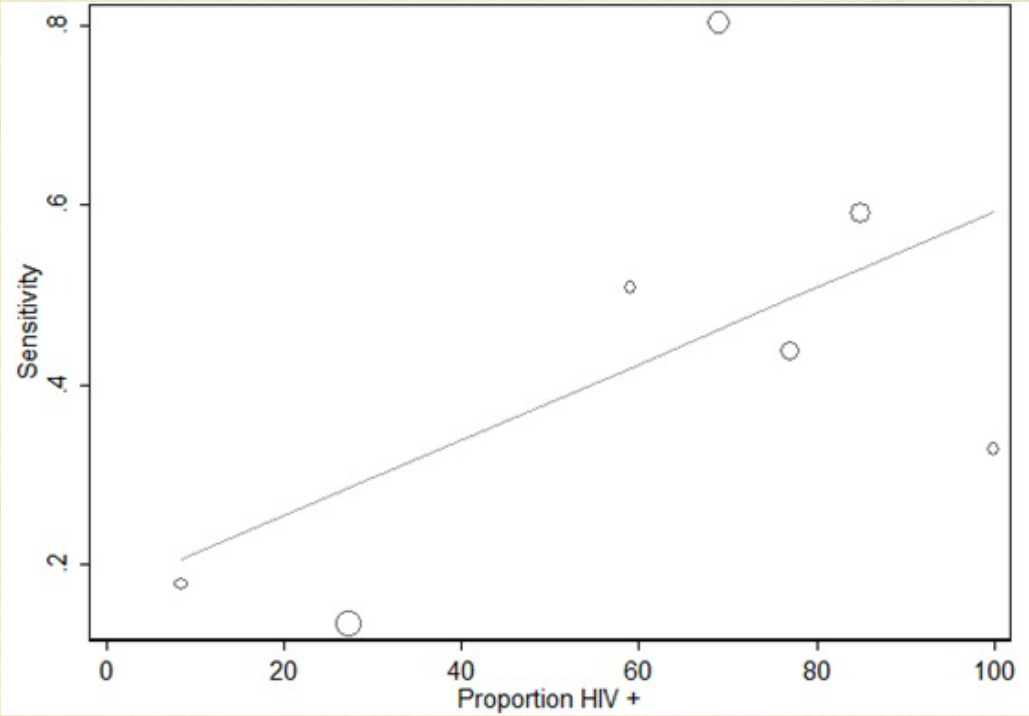
- 5 studies compared accuracy in HIV+/-
- All found improved sensitivity in HIV+ (range 2.5 – 52.8%)
- 4/5 found decreased specificity (range 4.9 – 7.3%)

Stratified by CD4 count

Author (Year)	Sensitivity (95% CI)	Specificity (95% CI)
Lawn (2009)[a]		
CD4 <50	67% (41, 87)	100% (94, 100)
CD4 50-100	35% (14, 62)	100% (0.94, 1.00)
CD4 >100	4% (0, 22)	100% (94, 100)
Dheda (2010)[b]		
CD4 <200	37% (16, 62)	100% (81, 100)
CD4 ≥200	0% (0, 18)	100% (17, 100)
Shah (2010)[c]		
CD4 <50	85% (73, 93)	NA
CD4 50-100	71% (51, 87)	NA
CD4 101-150	56% (30, 80)	NA
CD4 >150	51% (38, 64)	NA

- Not able to pool due to heterogeneous methods and cut-offs used
- All 3 studies found improved sensitivity with lower CD4 counts

Meta-Regression for HIV status



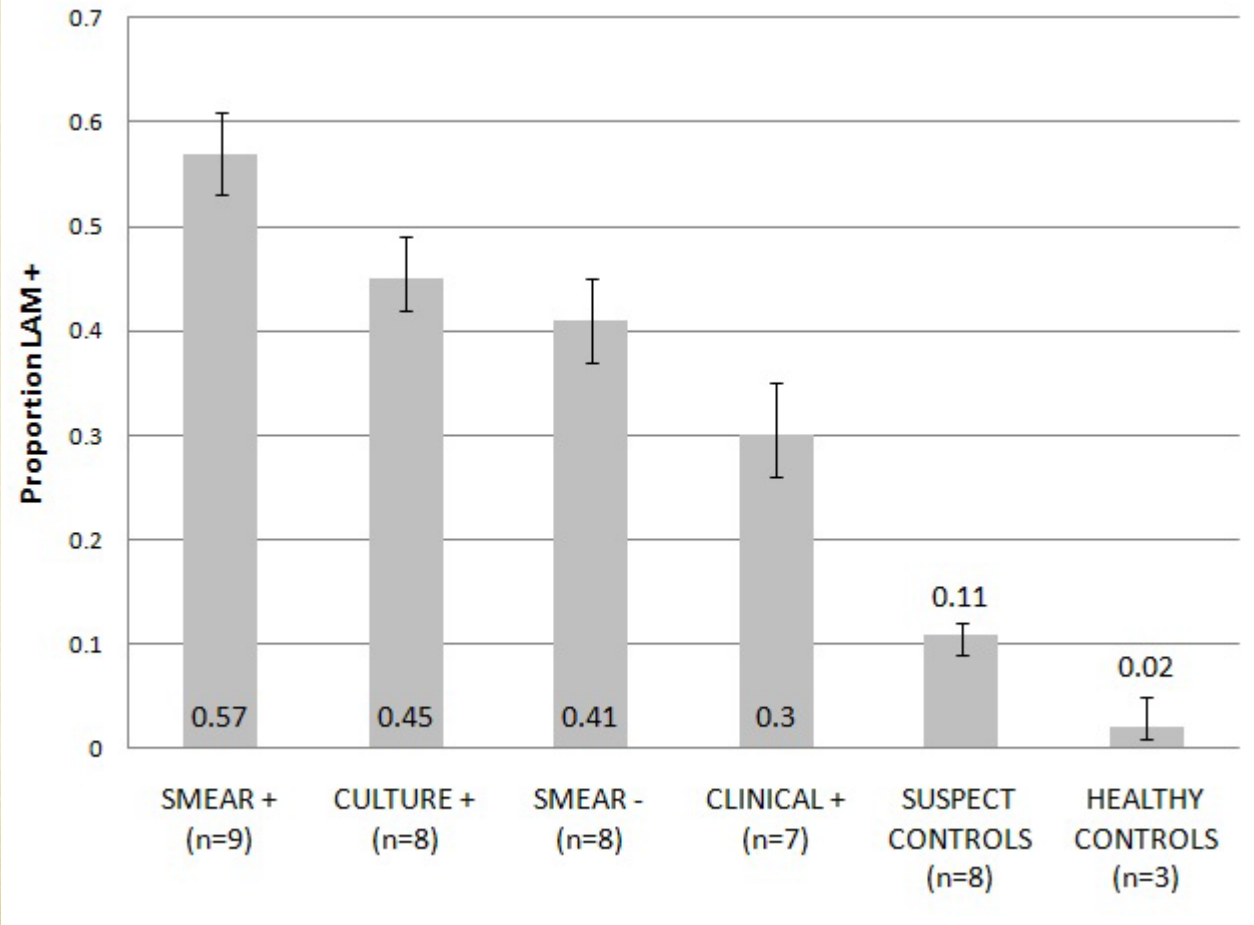
- Logistic model, weighted inversely by SE of sensitivity, combined using random effects
- Overall proportion of HIV+ TB suspects in each study as an independent predictor of estimated sensitivity
- Not statistically significant, but visual trend

Subgroup Analysis

	Sensitivity (95% CI)	I^2 (p-value)	Specificity (95% CI)	I^2 (p-value)
Urine Preparation (n studies)[b]				
Fresh (n=3)[c]	59% (53, 64)	95.4% (p<0.0001)	89% (85, 92)	0.0% (p=0.9)
Frozen (n=6)	49% (22, 77)	97.3% (p<0.0001)	97% (89, 99)	93.3% (p<0.0001)
Test Version (n studies)				
Non-commercial (n=2)[c]	82% (76, 88)	39.8% (p=0.2)	87% (85, 89)	62.3% (p=0.1)
Chemogen (n=5)	45% (26, 66)	95.1% (p<0.0001)	94% (82, 98)	88.9% (p<0.0001)
Inverness (n=2)[c]	40% (35, 46)	98.7% (p<0.0001)	98% (95, 99)	61.6% (p=0.1)

- Urine
  - No statistically significant difference between studies using fresh vs. frozen urine
- Assay Version
  - Studies evaluating the early non-commercial prototype found significantly higher sensitivity than subsequent studies

Proportion of Specimens LAM+ by Clinical Status



Conclusions

- Despite appealing test characteristics, LAM urinary assays have suboptimal sensitivity in routine TB diagnosis
- Sensitivity is improved in HIV+ patients, particularly with low CD4 counts

Residual Questions

- Role for LAM assay in screening HIV+ patients?
  - Incremental yield: we found LAM was + in 41% of smear-negative, culture-positive specimens
- Is specificity good enough to serve a "rule-in" function?
  - Individual studies vary considerably
  - Evidence for false-positives due to NTM, Candida spp, oral microbiota
- No evidence available for use in pediatric TB

Last Notes

- When performing primary diagnostic studies – please define your reference standard appropriately!
  - Pretending that reference indeterminates don't exist doesn't make them go away
  - Clear reporting will allow readers to analyze results according to their needs
- QUADAS now improved with QUADAS2
  - Still need to make sure all reviewers are very clear on the interpretation of each item as it relates to each review you do
- Lumpers and Splitters (you know who you are)
  - Be self-aware and double check your assumptions when making decisions about pooling estimates


THE
END