

Implementation research... almost: *Optimising urine-based TB diagnosis*



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Outline

- Urine for diagnosis
- The need for urine-based TB diagnosis
- What to look for – TB antigens
- Urine-based TB diagnosis: optimisation before implementation
- LAM – development/initial evaluation
- LAM – reevaluation/vulnerable group targeting
- POC LAM – lateral flow assay ('a new ball game')
- Forward – answering the bottom line
- Round again – back to the 'bench'

Urine for diagnosis

Early physicians interested in the properties of urine



Urine in some cultures seen as mystical



Numerous urine-based diagnostics from simple dipsticks to antigen-detection kits for infections such as *strep. pneumoniae* and *Legionella spp.*



Why urine?

Advantages of use

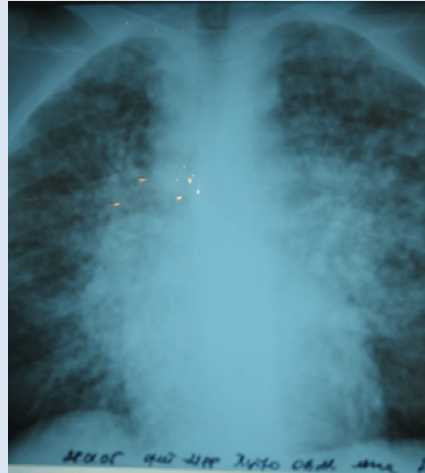
- Urine easily attainable
- Almost always available
- Low infectious risk during collection
- Ease of processing and storage
- Simple and non-viscous fluid (POC test)



Why urine for TB diagnosis?

- All previous advantages PLUS
- EPTB/disseminated TB
- Children
- Sputum scarce

Diagnostically challenging!

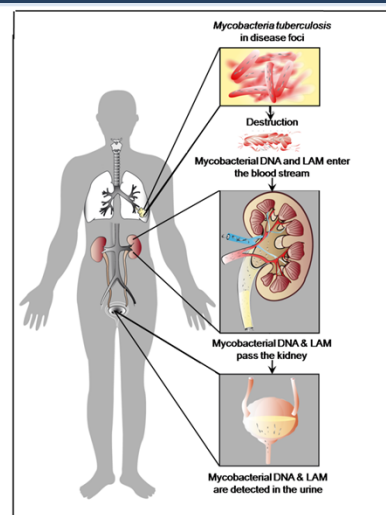


What to look for in urine?

- Presence of viable TB bacilli unusual (poor performance of urine smear and culture as renal TB uncommon for of EPTB)
- Need to look for host/pathogen filtered substances e.g. *M.tb* antigens

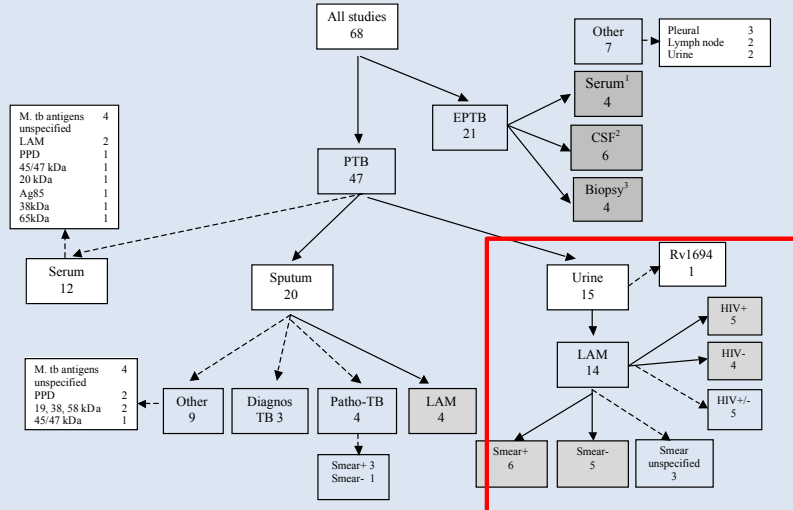


Renal TB



Peter and Dheda *COPM* 2010

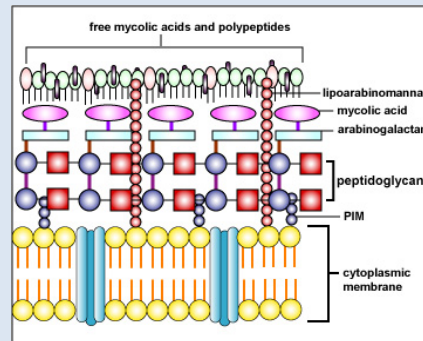
TB antigen detection



Flores et al. Clin Vaccine Immunol 2011

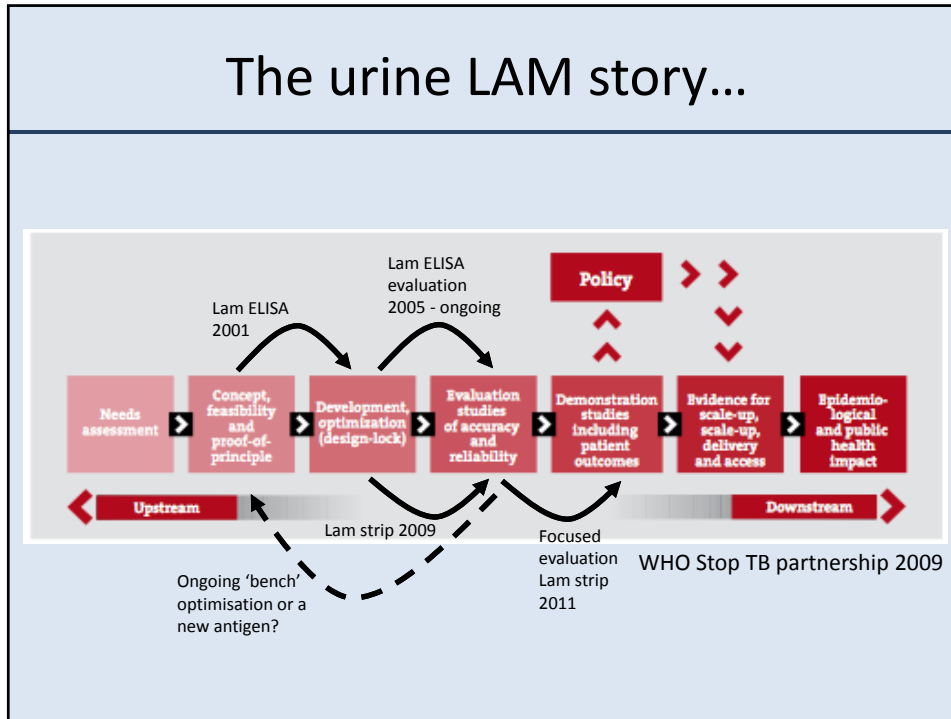
Lipoarabinomannan

- 17.5 kD glycolipid in the outer cell-wall of mycobacteria
- Released from metabolically active or degrading bacterial cells during TB infection
- Heat stable, filtered by the kidney and detectable in the urine



Peter et al. COPM 2010
Minion et al ERJ 2011

The urine LAM story...



Development and initial evaluation

- Hamasur and colleagues developed LAM ELISA with 1st generation pre-commercial prototype [MTB LAM ELISA, Chemogen, Portland, USA]
- 2005, Tanzania, Boehme *et al* 235 pts. (60% HIV-infected)

Overall sensitivity: 80% (HIV-infected: 81%)

Overall specificity: 99%

VERY PROMISING!

Subsequent evaluation

Study	Clinical subgroup and country	Evaluable pts tested/ Total number recruited	HIV (%pos.)	Overall sensitivity(95%CI), HIV pos. pts, HIV pos. pts with CD4< 200cells/ μ l	Overall specificity (95%CI), HIV pos. pts, HIV pos. pts with	Sensitivity (95%CI) In smear negative, Culture- positive, TB cases [n]
Daley et al. ^b (22)	Outpatient clinic (India)	200/200	8.5%	OVERALL 18(9-33) HIV+ 20(1-77) CD4<200 N/R	88(81-92) 83(51-97) N/R	[n=12] 25 (4-64) N/R N/R
Reither et al. ^b (24)	Outpatient clinic (Tanzania)	151/291	59.1%	OVERALL 51(28-63) HIV+ 62(47-75) CD4<200 N/R	88(79-94) 84(68-94) N/R	[n=21] 38 (N/R) 42 (N/R) N/R
Mutetwa et al. ^b (23)	Outpatient clinic (Zimbabwe)	261/397	77%	OVERALL 44(36-54) HIV+ 52(43-62) CD4<200 N/R	89(81-94) 86(73-93) N/R	[n=40] 28 (13-43) N/R N/R
Dheda et al. ^c (in preparation)	Outpatient clinic (South Africa)	427/500	31%	OVERALL 13(8-19) HIV+ 21(11-35) CD4<200 37(19-59)	99(97-100) 100(91-100) 100(84-100)	[n=70] 9 (3-20) 18 (7-39) 29 (8-64)
Lawn et al. ^b (26)	ARV clinic, asymptomatic HIV patients (South Africa)	235/235	100%	OVERALL 38(27-51) HIV + 38(27-51) CD4 50-100 41(22-64) CD4< 50 6(7(44-84)	100(N/R) 100(N/R) N/R N/R	[n=50] 36 [†] (N/R) 36 [†] (N/R) 35 [†] (N/R) 56 [†] (N/R)
Shah et al. ^c (25)	In-patients (South Africa)	315/499	85%	OVERALL 59(52-66) HIV+ 67(59-74) CD4 50-100 74(51-87) CD4 <50 85(73-93)	96(91-99) 94(87-98) N/R N/R	[n=111] 56 (N/R) N/R N/R N/R

^b Used a second version (different polyclonal antibody) of the MTB LAM ELISA prototype (Chemogen, Portland, USA).

^c Used the commercially available Clearview[®] TB ELISA assay (Inverness Medical Innovations

Peter et al. COPM 2010

Where to from there?

Targeting/potential utility

- Potential use in vulnerable group – HIV-infected with advanced immunosuppression
- Development of point-of-care urine LAM lateral flow test

Specificity concerns

- Concern about specificity in countries north of SA (?NTMs higher rates)
- Need to investigate further with additional studies and back to the 'bench'



LAM strip test evaluations

2 published studies to date

1) Outpatients, TB screening (active case finding) in HIV-infected patients pre-ARV initiation

TB: 17.4% (94/542); median(IQR) CD4 cell count: 169.5 (100-233)

Lawn et al. 2011 *LID*

2) HIV, TB suspects with illness severity to require hospitalisation

TB: 48% (116/242); median(IQR) CD4 cell count: 90 (47-197)

Peter et al. 2012 *ERJ*

TB Screening in ARV-clinic

Manufacturer's grade 1 visual cut-point

- Overall LAM strip sensitivity: 28% (19-39)
 - Overall LAM strip specificity: 99% (97-100)
- (2 NTM false positives)

CD4<100 cells/ml:

- Overall LAM strip sensitivity: 52% (33-71)
- Sputum smear + LAM: 66% (46-82)
- Single sputum MTB/RIF: 76% (57-90)
- LAM strip equivalent to LAM ELISA

Lawn et al. 2011 *LID*

TB diagnosis in hospitalised HIV-infected

Diagnostic test(s)	Group 1 patients				Group 2 non-TB control patients (N=88)
	Microbiological reference (<i>M.tb</i> culture positive vs. negative) (N=242)		Composite reference standard (Definite- & Prob.-TB vs. non-TB) (N=214)		
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	
Rapid smear microscopy [†]	56 ^{*1#2} (47-65) 65/116	90 ^{#4} (84-95) 114/126	44 ^{*1#6} (37-51) 82/187	96 (89-100) 26/27	N/R
LAM ELISA [‡]	59 (50-68) 68/115	80 ^{*2*3#4#5} (72-86) 98/123	51 (44-58) 93/183	96 ^{*2} (89-100) 24/25	99 ^{*3} (94-100) 87/88
LAM strip test [‡] (grade 1 cut-point)	66 ^{*2} (57-74) 77/116	66 ^{*5#5#5} (57-73) 82/125	60 ^{*1} (53-67) 112/187	96 ^{*1} (89-100) 26/27	90 ^{*#5} (82-95) 79/88
LAM strip test [‡] (grade 2 cut-point)	50 ^{*#3} (41-59) 58/116	75 ^{*7} (67-82) 94/125	45 ^{*#7} (39-53) 85/187	96 ^{*1} (89-100) 26/27	99 ^{*#5} (94-100) 87/88
LAM strip test (grade 2 cut-point) in smear-negative/sputum scarce <i>M.tb</i> culture positive patients	39 (28-52) 22/56	N/A	38 (29-48) 36/95	N/A	N/A
Combined sputum smear microscopy and LAM strip test (grade 2 cut-point)	71 ^{*#3} (62-78) 82/116	73 ^{*8*9} (65-80) 92/126	63 ^{*#6#7} (56-70) 118/187	93 ^{*8} (83-100) 25/27	99 ^{*9} (94-100) 87/88

Peter et al. 2012 *ERJ*

TB diagnosis in hospitalised HIV-infected

HIV-infected patients with CD4 count ≤200 cells/ml				
Group 1 patients				Group 2 patients (N=84)
Microbiological reference (<i>M.tb</i> culture positive vs. negative) (N=173)		Composite reference standard (Definite- & Prob.-TB vs. non-TB) (N=147)		
Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	
56 ^{*2} (45-66) 45/81	91 ^{#4#5} (86-97) 84/92	44 ^{*6} (36-53) 58/131	100 (79-100) 16/16	N/R
70 ^{*1} (59-79) 56/80	76 ^{#2*4} (67-85) 69/91	59 ^{*3} (50-67) 76/129	94 (70-100) 15/16	97 ^{*4} (90-100) 28/29
72 (61-80) 58/81	61 ^{*5} (51-71) 56/92	64 (56-72) 84/131	94 (70-100) 15/16	87 ^{*5} (75-99) 26/30
58 ^{*#3} (47-68) 47/81	72 ^{*9} (63-81) 66/92	52 ^{*7#7} (43-60) 68/131	94 (70-100) 15/16	97 ^{*9} (90-100) 29/30
51 (36-66) 21/41	N/A	43 (32-54) 31/73	N/A	N/A
75 ^{*2#3} (65-83) 61/81	70 ^{*11#5} (60-79) 64/92	68 ^{*10#6#7} (60-75) 89/131	100 ^{*11} (79-100) 16/16	N/R

- Sensitivity improved with decreasing CD4 cell count
- Grade-2 cut-point superior specificity in group-2 patients
- Specificity differences between analysis 1 and 2 likely reflect suboptimal culture performance in hospitalised TB HIV co-infected patients with advanced immunosuppression

CONCERNS:
Specificity may be suboptimal (already raised)
The lateral flow assay (human element)

Peter et al. 2012 *ERJ*

Agreement... disagreement

What could account for this difference in agreement?

Problem with the interpretation of faint bands (grade 0/1)

- Different study populations (hospitalisation, lower median CD4)
?low-level cross-reactants in urine of hospitalised patients
- LAM ELISA optical density high in 24 positive patients in the outpatient study (more likely to have higher LAM strip grade)
- Batch variability
- Reader experience
- Storage conditions

Where to from here?

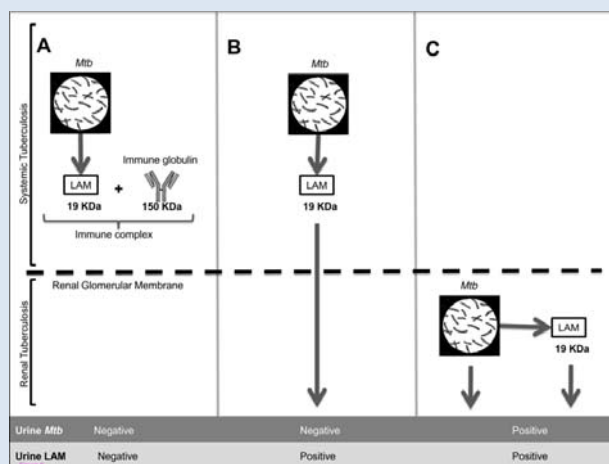
Current/ongoing studies

- Liz Talbot's data (also showing modest performance)
- CDC study (Dorman/Nicol) – Ugandan
- Nicol – children data

Results consistent with published data

Similar difficulties reading strips at faint bands in different light conditions

Mechanisms for LAM positivity in HIV-infected



Host/Pathogen factors:

- Total bacillary load (Shah et al. 2010 JCM)
- Low CD4
- Mycobacteriuria (non-viable)

Not

- RFLP TB strain
- TB treatment outcome
- ?proteinuria

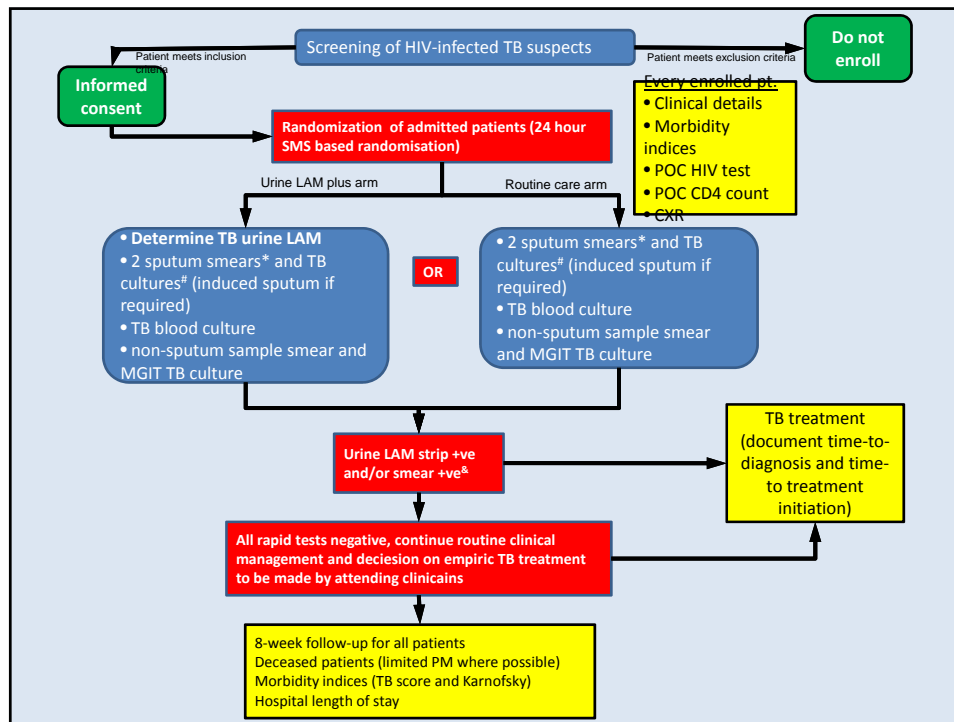
Wood et al. 2012 *BMC inf dis*

Targeting to sputum scarce

- Sputum-based diagnostic unhelpful
- 10-20% HIV-infected patients
- Urine-based LAM and Xpert MTB/RIF
- 48% urine Xpert MTB/RIF positive
- 48% urine LAM strip test positive
- 64% combined Xpert + LAM
- Urine centrifugation and pelleting improved performance

Patient outcomes - RCT

- Testing the bottom-line with a suboptimal test...
- Given the advantages, and using it as a diagnostic adjunct, will urine LAM strip test impact patient care
- Given empiric TB treatment trials in this group and the impact of treatment algorithms on mortality (Holtz et al. 2011 *LID*)



Back to the 'bench'

- Improved/different detection platforms
e.g. Aptamers, SERS
- Mass spec approach to look for low conc. LAM in non HIV-infected patients
- Monoclonal *m.tb*-specific anti-LAM antibodies
- Different antigens/biomarkers (proteomic discovery work)

Summary

- Urine-based diagnostics for TB offer important advantages
- Urine-LAM (ELISA and strip) performs best in HIV-infected patients with advanced immunosuppression
- New problems of lateral flow format
- Can a suboptimal test have patient-important impact?
- Parallel processes in test optimisation, evaluation and implementation required with
 - test evaluations (old and new formats)
 - investigation of host-pathogen factors affecting test performance
 - test optimisation to improve appropriate performance ('rule-in')
 - ongoing discovery work for better antigens and biomarkers

Funding Agencies:



EFP7



Discovery



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EDCTP



South African
MRC