Potential Diagnostic Biomarkers

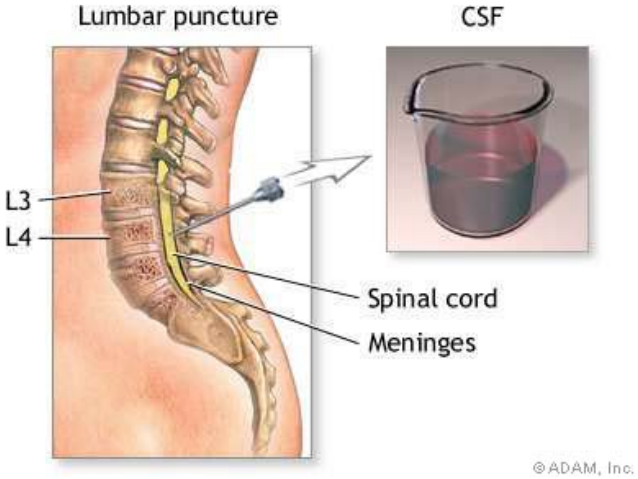
NIH, Rockville, May 2017

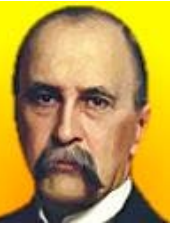
Keertan Dheda, MB.BCh (Wits), FCP(SA), PhD (Lond), FRCP (Lond)

Professor and Head:
Division of Pulmonology, Department of Medicine,
University of Cape Town

Overview

- TB-specific biomarkers (LAM)
- TB non-specific biomarkers (T cell assays, unstimulated Th1 cytokines)



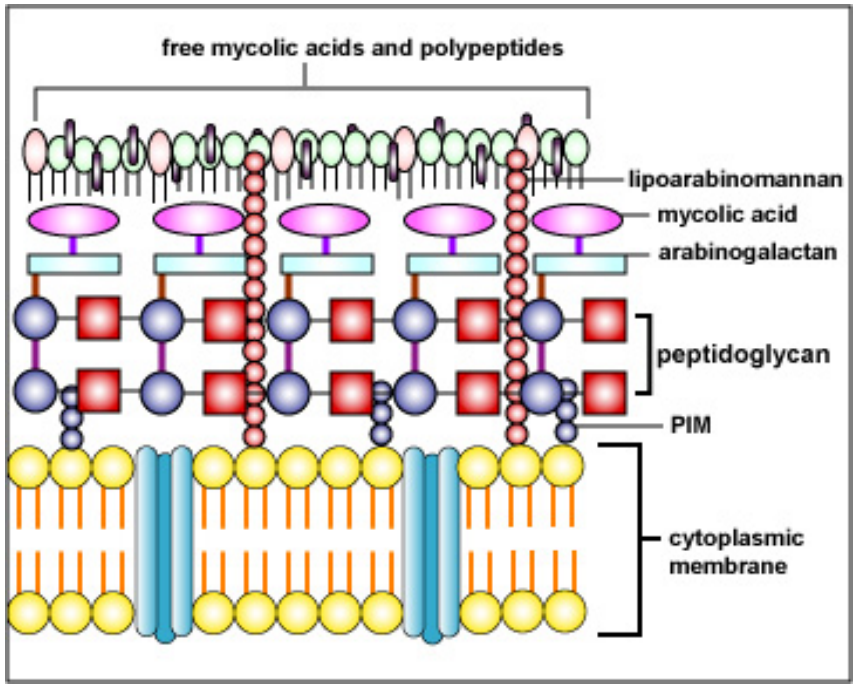


‘In the Mortality Bills, pneumonia is an easy second to tuberculosis; indeed in many cities the death-rate is now higher and it has become, to use the phrase of Bunyan 'the captain of the men of death.’

Sir William Osler

Medicine in the Nineteenth Century' (1904). In Aequanimitas with Other Addresses to Medical Students, Nurses and Practitioners of Medicine (1904),

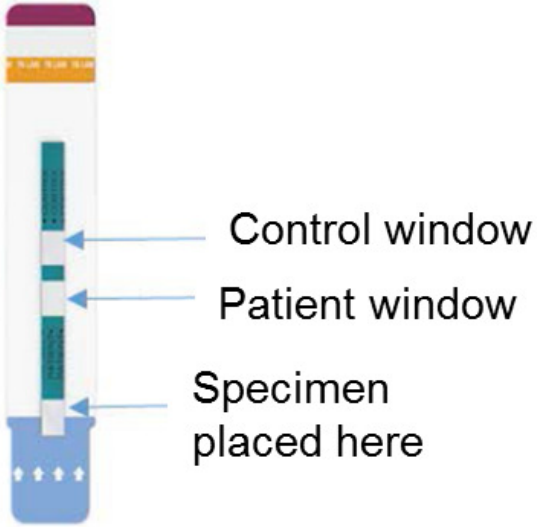
Urine + LAM= TB LFA



Advantages:

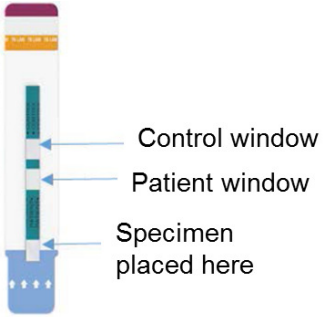
- Almost always obtainable
- Low infectious collection risk
- Simple and non-viscous fluid – easy processing

Chan JXD , Infect Immun,1991
Bricken V, Mol Microbiol, 2004
Hamasur B, Microbiol Methods, 2001

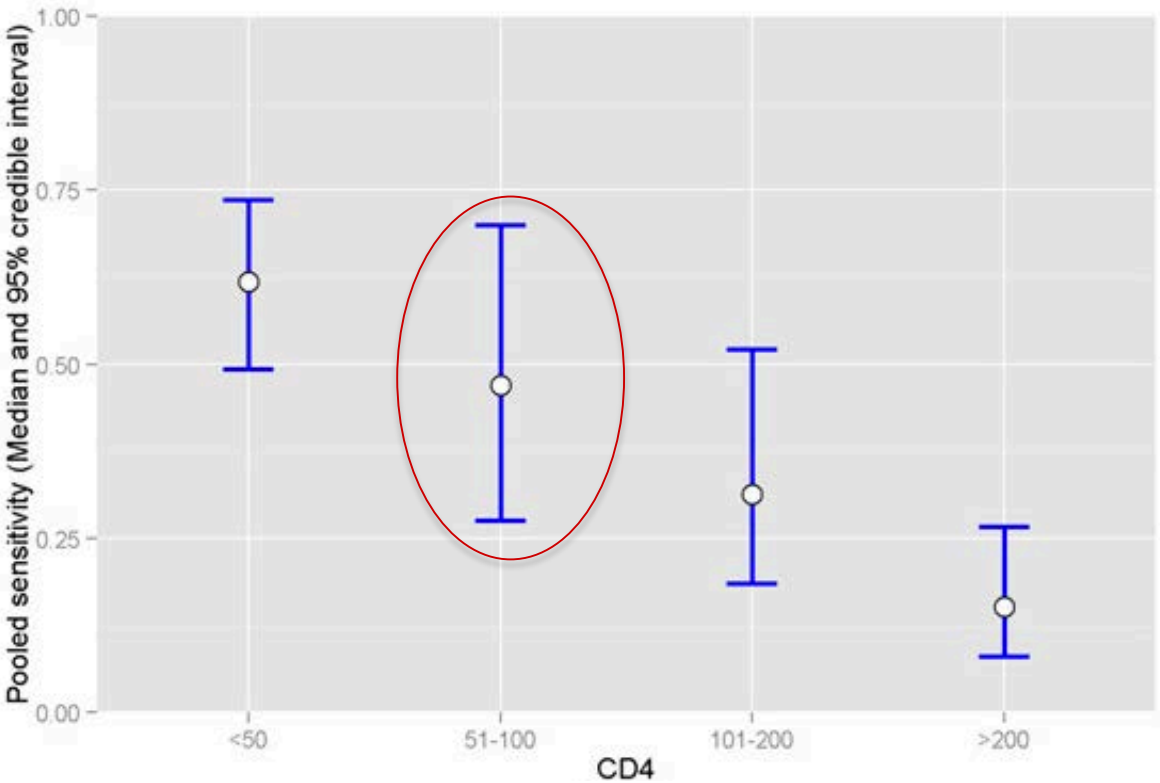


- 17.5 kD glycolipid found in the outer cell-wall of mycobacterial species

URINE LAM

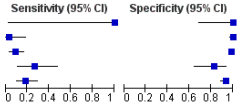


A. Sensitivity



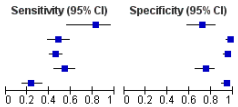
Diagnosis of TB against microbiological reference at Grade 2, CD4 > 200

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Andrews 2014	1	0	0	10	1.00 [0.03, 1.00]	1.00 [0.69, 1.00]
Lawn 2014a	1	0	27	136	0.04 [0.00, 0.18]	1.00 [0.97, 1.00]
Nakiyingi 2014	8	5	80	330	0.09 [0.04, 0.17]	0.99 [0.97, 1.00]
Peter 2012a	7	5	19	24	0.27 [0.12, 0.48]	0.83 [0.64, 0.94]
Peter 2015	14	13	61	184	0.19 [0.11, 0.29]	0.93 [0.89, 0.96]



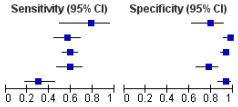
Diagnosis of TB against microbiological reference at Grade 2, CD4 < 200

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Andrews 2014	14	14	3	36	0.82 [0.57, 0.96]	0.72 [0.58, 0.84]
Lawn 2014a	52	3	55	136	0.49 [0.39, 0.58]	0.98 [0.94, 1.00]
Nakiyingi 2014	128	15	149	275	0.46 [0.40, 0.52]	0.95 [0.92, 0.97]
Peter 2012a	58	26	49	78	0.54 [0.44, 0.64]	0.75 [0.66, 0.83]
Peter 2015	23	9	74	147	0.24 [0.16, 0.33]	0.94 [0.89, 0.97]



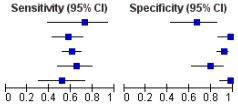
Diagnosis of TB against microbiological reference at Grade 1, CD4 < 100

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Andrews 2014	11	7	3	27	0.79 [0.49, 0.95]	0.79 [0.62, 0.91]
Lawn 2014a	41	2	31	81	0.57 [0.45, 0.69]	0.98 [0.92, 1.00]
Nakiyingi 2014	116	12	80	176	0.59 [0.52, 0.66]	0.94 [0.89, 0.97]
Peter 2012a	44	16	30	55	0.59 [0.47, 0.71]	0.77 [0.66, 0.87]
Peter 2015	14	5	32	76	0.30 [0.18, 0.46]	0.94 [0.86, 0.98]



Diagnosis of TB against microbiological reference at Grade 1, CD4 < 50

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Andrews 2014	8	7	3	14	0.73 [0.39, 0.94]	0.67 [0.43, 0.85]
Lawn 2014a	30	1	22	38	0.58 [0.43, 0.71]	0.97 [0.87, 1.00]
Nakiyingi 2014	83	12	53	130	0.61 [0.52, 0.69]	0.92 [0.86, 0.96]
Peter 2012a	26	7	14	27	0.65 [0.48, 0.79]	0.79 [0.62, 0.91]
Peter 2015	12	1	11	44	0.52 [0.31, 0.73]	0.98 [0.88, 1.00]



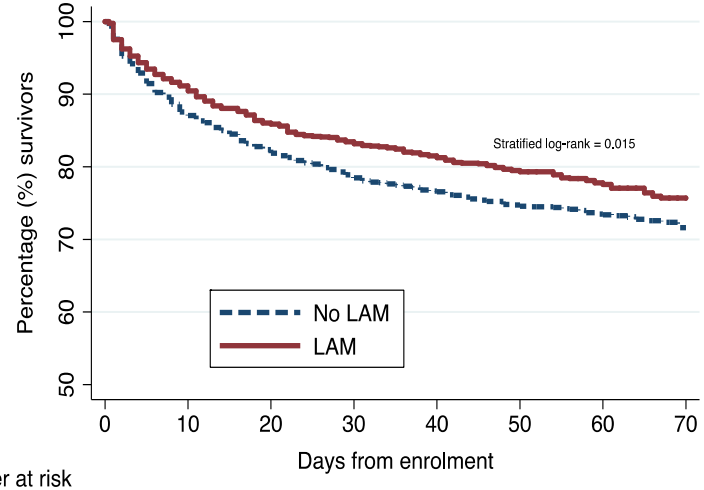
Shah M, Cochrane Library, 2016

THE LANCET
Volume 376 · Number 9734 · Pages 1-68 · July 3-9, 2010 www.thelancet.com

URINE LAM

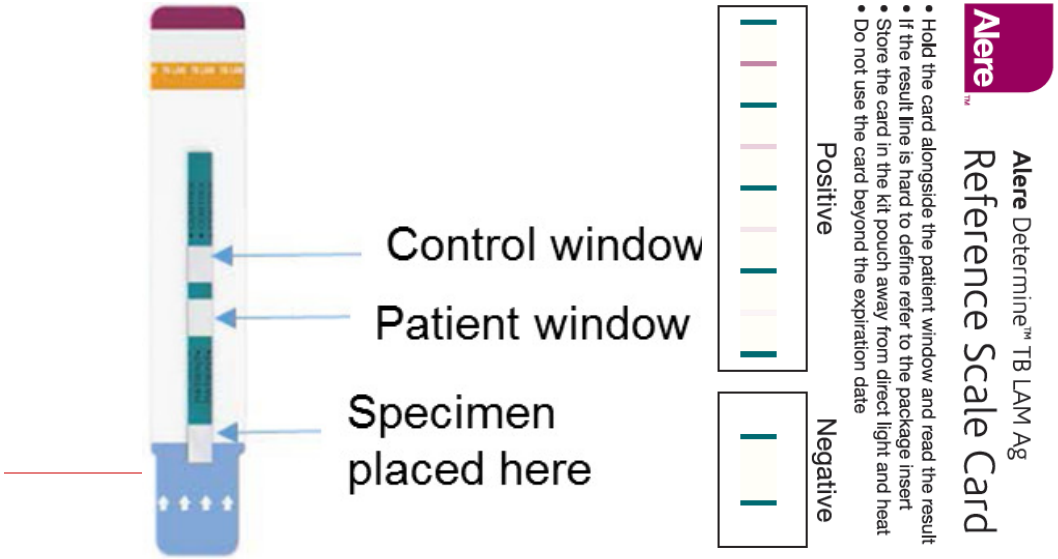
The mortality impact of point-of-care urine lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-infected hospitalised patients: a multi-country randomised controlled trial

Jonny G. Peter (PhD)*[1, 10,11,12], Lynn S. Zijenah (PhD)*[2], Duncan Chanda (MD)*[1,3,13], Petra Clowes (MD)*[4, 5], Maia Lesosky (PhD)[1], Phindile Gina (MD)[1], Nirja Mehta (MSc)[1], Greg Calligaro (MD)[1], Carl J. Lombard (PhD)[6], Gerard Kadzirange (MD)[7], Tsitsi Bandason (PhD)[8], Abidan Chansa (MD)[3, 13], Namakando Liusha (MD)[3, 13], Chacha Mangu (MD)[4], Bariki Mtafya (MD)[4, 8], Henry Msila (MD)[4], Andrea Rachow (PhD)[4, 5,9], Michael Hoelscher (PhD)[4, 5,9], Peter Mwaba (MD)[3], Grant Theron (PhD)[1, 14], Keertan Dheda (PhD)[1, 10,11, 12]



Number at risk

No LAM	1271	1069	997	953	923	896	690	263
LAM	1257	1093	1028	994	970	941	696	246



Compartment-specific LAM

- Not useful in pleural space (LAM ELISA): 8% sensitivity

  Dheda K, PloS One, 2010

- Pericardial TB: 80% HIV+ve; 33% sensitivity; 50% in CD4<100

  Pandie, Dheda & Mayosi, BMC Med, 2015

Cerebrospinal Fluid Research

BioMed Central

Short paper

Open Access

Utility of a novel lipoarabinomannan assay for the diagnosis of tuberculous meningitis in a resource-poor high-HIV prevalence setting

Vinod B Patel[1], Ahmed I Bhigjee[1], Hoosain F Paruk[1], Ravesh Singh[2], Richard Meldau[3], Cathy Connolly[6], Thumbi Ndung'u[2] and Keertan Dheda*[3,4,5]

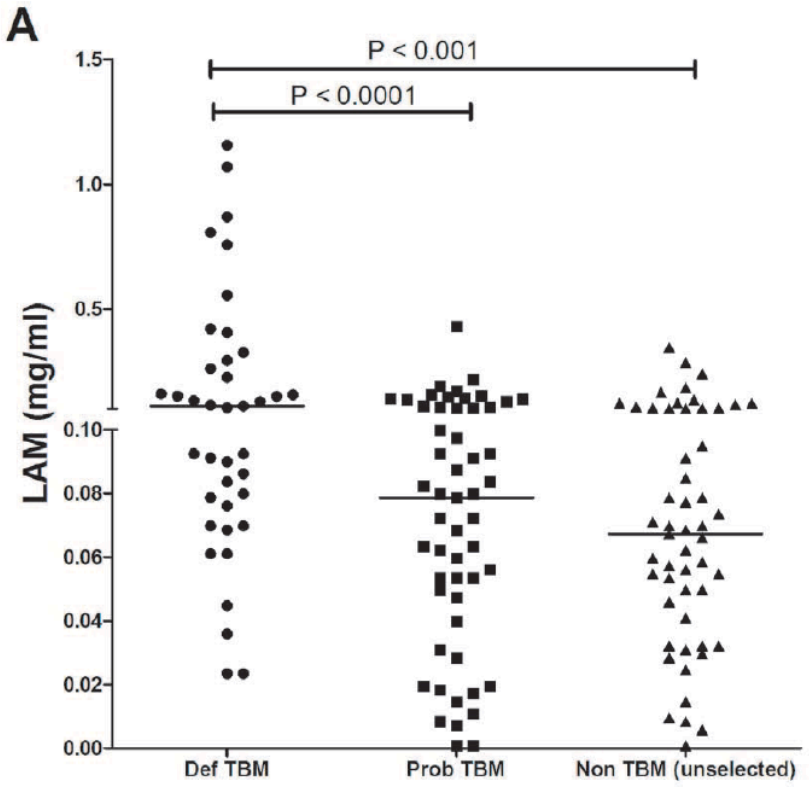
- LAM ELISA sensitivity 64% (n=14) and specificity 69% (n= 13)

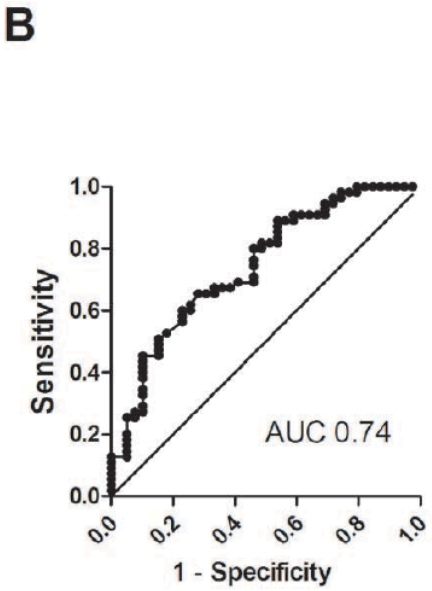
OPEN ACCESS Freely available online

PLoS one

Comparison of a Clinical Prediction Rule and a LAM Antigen-Detection Assay for the Rapid Diagnosis of TBM in a High HIV Prevalence Setting

Vinod B. Patel[1], Ravesh Singh[2], Cathy Connolly[3], Victoria Kasprowicz[2], Allimudin Zumla[4], Thumbi Ndungu[2], Keertan Dheda[4,5,6]*





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Comparison of a Clinical Prediction Rule and a LAM Antigen-Detection Assay for the Rapid Diagnosis of TBM in a High HIV Prevalence Setting

Vinod B. Patel[1], Ravesh Singh[2], Cathy Connolly[3], Victoria Kasprowicz[2], Allimudin Zumla[4], Thumbi Ndungu[2], Keertan Dheda[4,5,6]*

- N= 150; 84 HIV+ve (median CD4= 132); n=39 culture +ve
- LAM ELISA CSF sensitivity= 31%; specificity= 94%
- CD4< 100; sensitivity 50%; specificity 95%

- However, clinical prediction rule (glucose; lymphocytes> 200, CD4< 200; CLAT-ve) = sensitivity 47%; specificity 98%

LAM in TBM: other studies

- Autopsy study in 91 HIV-infected adults

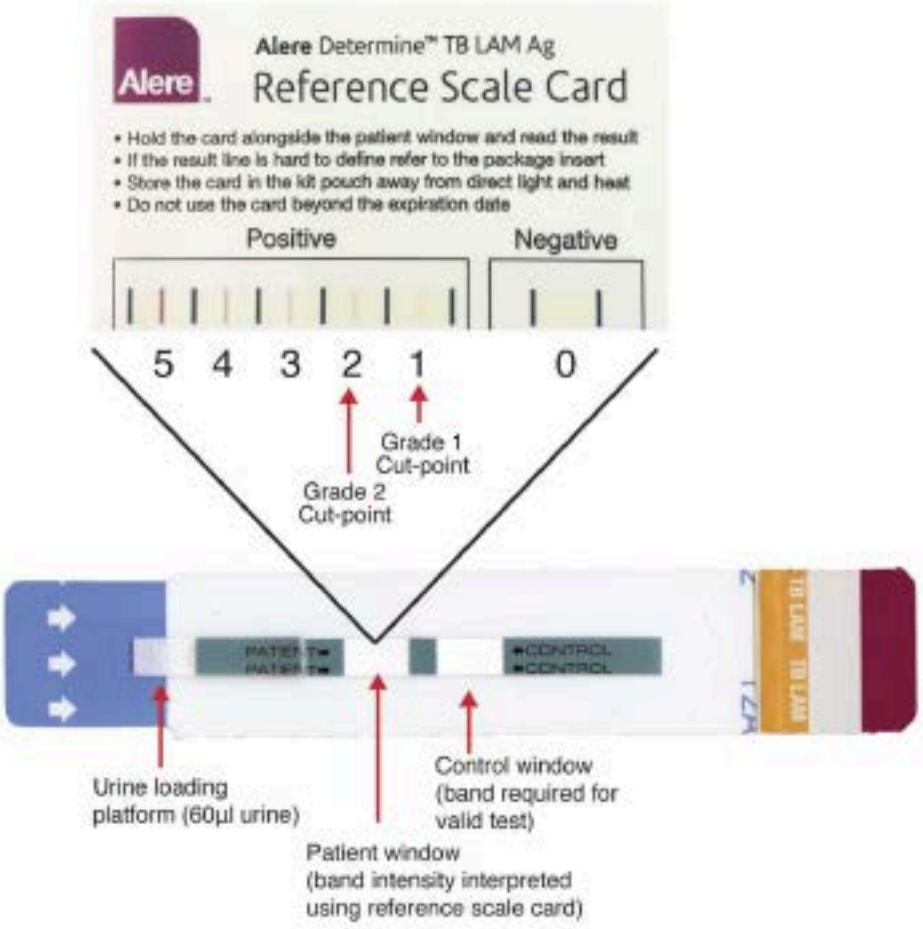
  LAM LFA sensitivity= 75% in definite TB (Xpert= 100%)

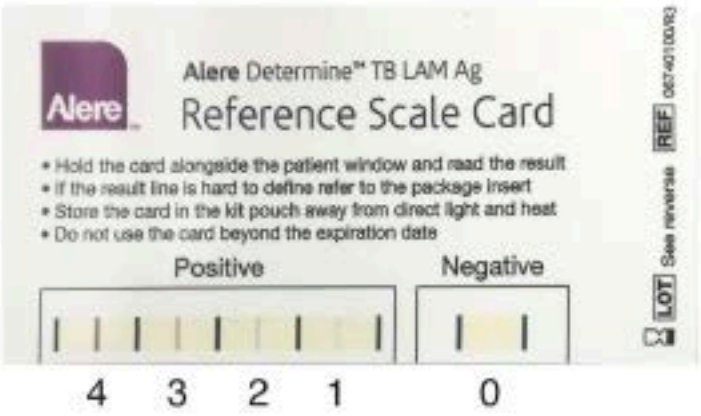
  sensitivity = 50% in definite and probable TB

  22% to 78% where not on anti-TB treatment

Cox JA, JCM, 2015

Post Jan 2014: grade 2 became grade 1





LAM utility in the CSF

Dr Omar Siddiqi

- 418 patients recruited (n= 93 culture +ve); 225 males (53.6%)
- Median age - 35 years
- HIV prevalence - 80%
- Median CD4 count - 101 cells/µl

Preliminary Results

Definite TBM		
	Sensitivity	Specificity
GeneXpert	48%	98%
CSF LAM	24%	95%
Urine LAM	29%	72%

- CSF LAM performed best in CD4 < 100
  - Sensitivity 35%
  - Specificity 91%

- CSF LAM picked up 4 cases missed by Xpert (3 of these died).
- There were 9 cases of TBM that were detected by urine LAM and missed by both Xpert and CSF LAM (CD4= 62).

RESEARCH ARTICLE Open Access

Early morning urine collection to improve urinary lateral flow LAM assay sensitivity in hospitalised patients with HIV-TB co-infection

Phindile Gina[1], Philippa J. Randall[1], Tapuwa E. Muchinga[1], Anil Pooran[1], Richard Meldau[1], Jonny G. Peter[1†] and Keertan Dheda[1,2*†]

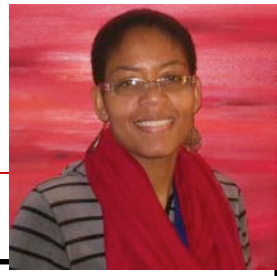
BMC Infectious Diseases

Concentration of LAM in the urine (n= 70 HIV+ve)

- ✓ Incremental significant increase in sensitivity (Figure 1)
- ✓ Sensitivity increase dependent on CD4 count (Figure 2)
- ✓ No effect on specificity (n = 31 non-TB)

Other TB-specific biomarkers in the CSF

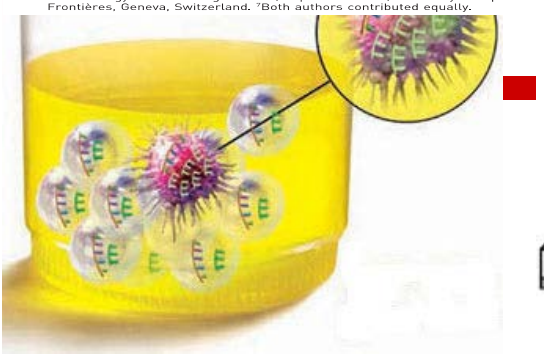
UCT patent: panel of novel urine TB-specific biomarkers

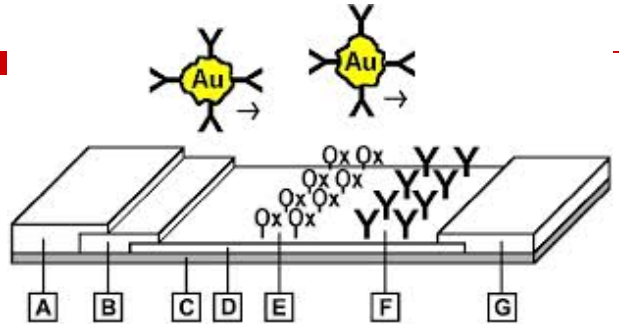


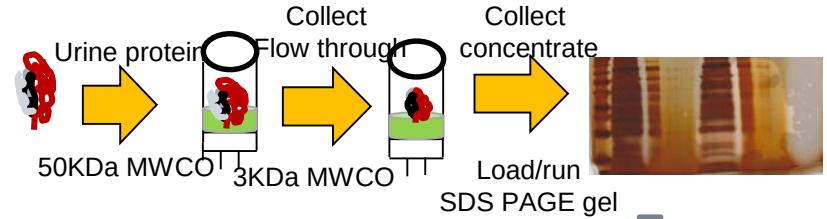
The identification of tuberculosis biomarkers in human urine samples

Brandy L. Young[1], Zandile Mlamla[2], Putuma P. Gqamana[3], Salome Smit[4], Teri Roberts[5], Jonathan Peter[6], Grant Theron[6], Ureshnie Govender[6], Keertan Dheda[1,6,7] and Jonathan Blackburn[1,2,7]

Affiliations: [1]Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, [2]Division of Medical Biochemistry, Dept of Clinical Laboratory Sciences, University of Cape Town, Cape Town, [3]University of the Western Cape, Cape Town, [4]Proteomics Laboratory, Central Analytical Facility, University of Stellenbosh, Cape Town, and [5]Lung Infection and Immunity Unit, Division of Pulmonology and UCT Lung Institute, Dept of Medicine, University of Cape Town, South Africa. [6]Médecins Sans Frontières, Geneva, Switzerland. [7]Both authors contributed equally.



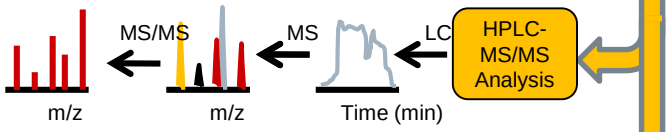




SDS PAGE Gel Digestion

1st Excise protein bands

2nd Digest protein



HPLC-MS/MS Analysis

Database Searching ‡

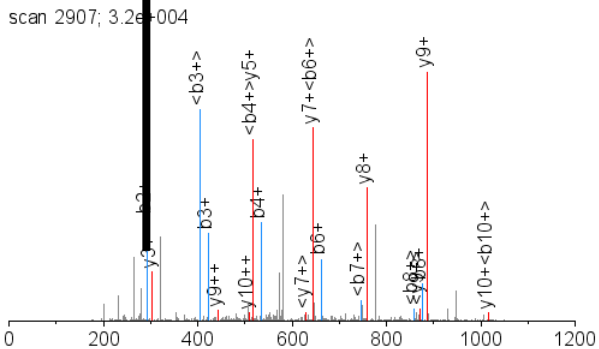
PSM

Protein Identification

Previous	R			
First	85			
Last	93			
Next	A			
Precursor charge	2			
Precursor m/z	549.2873			
Precursor m+h	1097.5673			
b+	**# AA**	**# y+**		
187.09	1	W		
301.13	2	N	8	901.49
372.17	3	A	7	797.45
443.20	4	A	6	726.41
556.29	5	I	5	655.37
687.33	6	M	4	542.29
786.40	7	V	3	411.25
923.46	8	H	2	312.18
		R	1	175.12

Pyruvate dehydrogenase E1 component OS=Mycobacterium tuberculosis

MASYLPDIDPEETSEWLESFDTLLQRCGPSRARYLML
RLLERAGEQRVAIPALTSTDYVNTIPTELEPWFPGDED
VERRYRAWIR**WNAAIMVHR**AQRPGVGVGGHISTYAS
SAALYEVGFNHFFRGK**SHPGGGDQVFIQGHASPGIYA**
RAFLEGR**LTAEQLDGFRQEHSHVGGGLPSYPHPRLM**
PDFWEFPTVSMGLGPLNAIYQARFNHYLHDRGIK**DTS**
DQHVWCFLGDGEMDEPESRGLAHVGALEGLDNLTF
VINCNLQRLDGPVRGNGK**IIQELESFFRGAGWNVIK**VV
WGR**EWDALLHADRDGALVNLMNTTPDGDYQTYKAN**
DGGYVRDHFFGRDPRTK**ALVENMSDQDIWNLK**RGG
HDYRKVYAAYRAAVDHK**GQPTVILAK**TIKGYALGKHFE
GRNATHQMKKLTLEDLKEFRDTQRIPVSDAQLEENPY
LPPYYHPGLNAPERYMLDRRR**ALGGFVPER**RTKSKA
LTLPGRDIYAPLKK**GSGHQEVATTMATVR**TFKEVLRD
IKQIGPR**IVPIIPDEARTFGMDSWFPSLK**IYNR**NGQLYT**
AVDADLMLAYKESEVGQILHEGINEAGSVGSFIAAGTS
YATHNEPMIPIYIFYSMFGFQR**TGDSFWAAADQMARG**
IFVLGATAGRTTLTGEGLQHADGHSLLLAATNPAVVAY
DPAFA
QPPEF
VAMPA
GVAIE
WMRA
YFNTD
DVAAA



‡A combined human, Mtb H37Rv and decoy database was used in X!Tandem and Omssa

Mycobacterial-specific molecules detected by LC-MS

Biomarkers for Tuberculosis Based on Secreted, Species-Specific, Bacterial Small Molecules

Shih-Jung Pan,[1,2] Asa Tapley,[1,3,4] John Adamson,[1] Tessa Little,[5]
Michael Urbanowski,[2] Keira Cohen,[1,6] Alexander Pym,[1] Deepak Almeida,[2]
Afton Dorasamy,[1] Emilie Layre,[7] David C. Young,[7] Ravesh Singh,[8]
Vinod B. Patel,[9] Kristina Wallengren,[10] Thumbi Ndung'u,[1,8,11,12]
Douglas Wilson,[13] D. Branch Moody,[7] and William Bishai[2,4]

- 2 mycobactin siderophores and tuberculosinyladenosine
- Detected in 71% of CSF samples (n= 14)
- Suggest that detection of TB-specific proteins coupled with appropriate detection platforms holds promise

Other TB-specific antigens

- ESAT-6

Feng GD, AJRCCM, 2014

Kashyap RS, Infection, 2009

Sing F, Ir J Med Sci, 2014

- Ag 85A and Ag85B

Kashyap RS, Clin Diagn Lab Immunol. 2005

- 65kd HSP

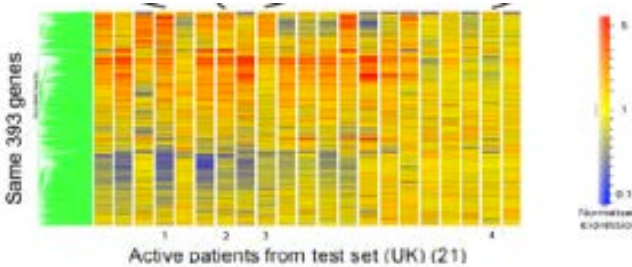
Mudaliar AV, BMCNeurol, 2006

- Tuberculostearic acid

French GL, Lancet, 1987

Host (non-specific) biomarker readouts for TB (OMICS)

matabolomics, proteomics, transcriptomics



- TB presents as part of a differential diagnosis (several infectious or inflammatory diseases)
- Host or non pathogen-specific biomarker approach that can discriminate diseases seems counter intuitive
- Blood-based host transcriptional signatures can distinguish TB from other inflammatory or infectious diseases in adults and children (infection-associated sepsis vs. SIRS)

Bloom CI, PLoS One, 2013

Anderson ST, NEJM, 2014

Roe JK, PloS One, 2016

Kaforou M, PLoS Med, 2013

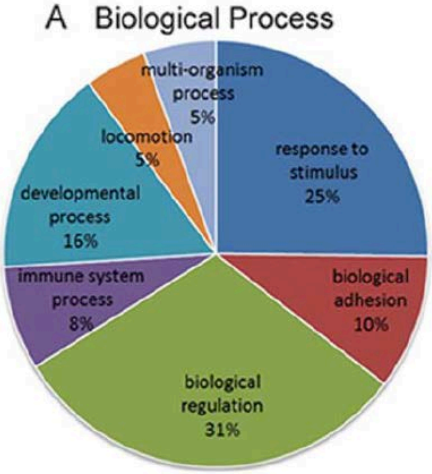
Berry MP, Nature, 2010

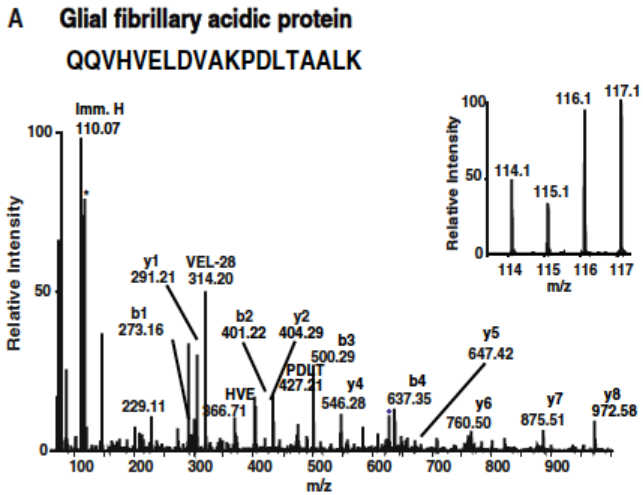
Zak DE, Lancet, 2016

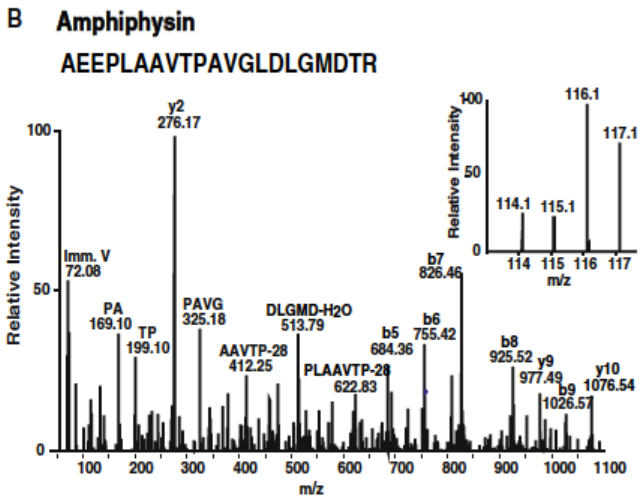
Reviews: Clif JM, Imm Rev, 2015

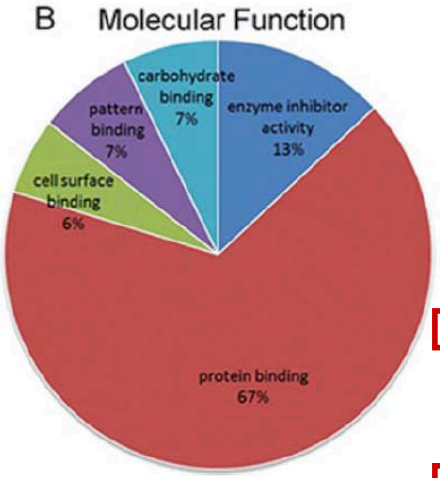
Ko ER, Expert Rev. Mol. Diag, 2015

Human proteome in the CSF (iTRAC LC-MS/MS)







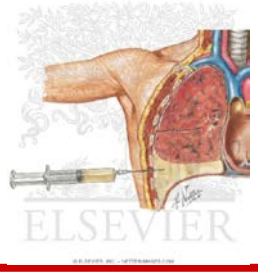


Kuma SK, Clin Proteomics, 2012
Ou Q, BioScience Trends, 2013
Yang Y, I J Mol Med, 2015
Mu J, Biochem Biophys Res Commun, 2015
Li Z, Clinica Chimica Acta, 2016 (metabolomics)

- Inappropriate controls (healthy or selected disease phenotypes)
- Small numbers
- No proven diagnostic value demonstrated

Pleural TB

IFN- gamma



Comparison of IFN-g with Xpert MTB/RIF in **pleural fluid**

- **N= 93; suspected pleural TB (20% HIV-infected)**
- **43%** (40/93) TB **culture- or biopsy-positive**

Meldau & Dheda, BMC Infect Dis, 2014

	Sensitivity using uncentrifuged pleural fluid	Sensitivity using centrifuged pleural fluid (15 ml)
Smear microscopy	0% (0/40)	-
Xpert in all patients	25% (9/40)	23% (9/40)

*

- Uniformly good specificity of 98%

	Sensitivity	Specificity
IFN-γ in all patients	93% (37/40)	96% (46/48)

Pleural TB

IFN- gamma

- 22 studies; 2101 patients
- Pooled sensitivity= 89%;
  specificity= 97% (ROC= 0.98)

Jiang J, Chest, 2007

Pericardial TB and peritoneal TB unstimulated IFN-g is the most accurate assay

- Peritoneal TB: SENS = 97% SPEC= 100% (n= 119)

Sharma SK, J Interf & Cytokine Research, 2006

- Pericardial TB: SENS= 95.7% and SPEC= 96.3% (n= 151)

Pandie & Dheda & Mayosi, BMC Med, 2014

Compartment-specific transport mechanisms and trapping of biomarkers

- Transport across serosal membranes incompletely understood
- Some biomarkers equilibrate rapidly with blood; others not
- Not size related

- Ion channels and exchanges; osmotic pressures; transport proteins, receptor mediated, transcellular lipid pathways, adsorbtive transcytosis, water soluble agents through tight junctions, active transport mechanisms, exosomes, membrane pores

- TB modulates these mechanisms ‘trapping’ IFN-g

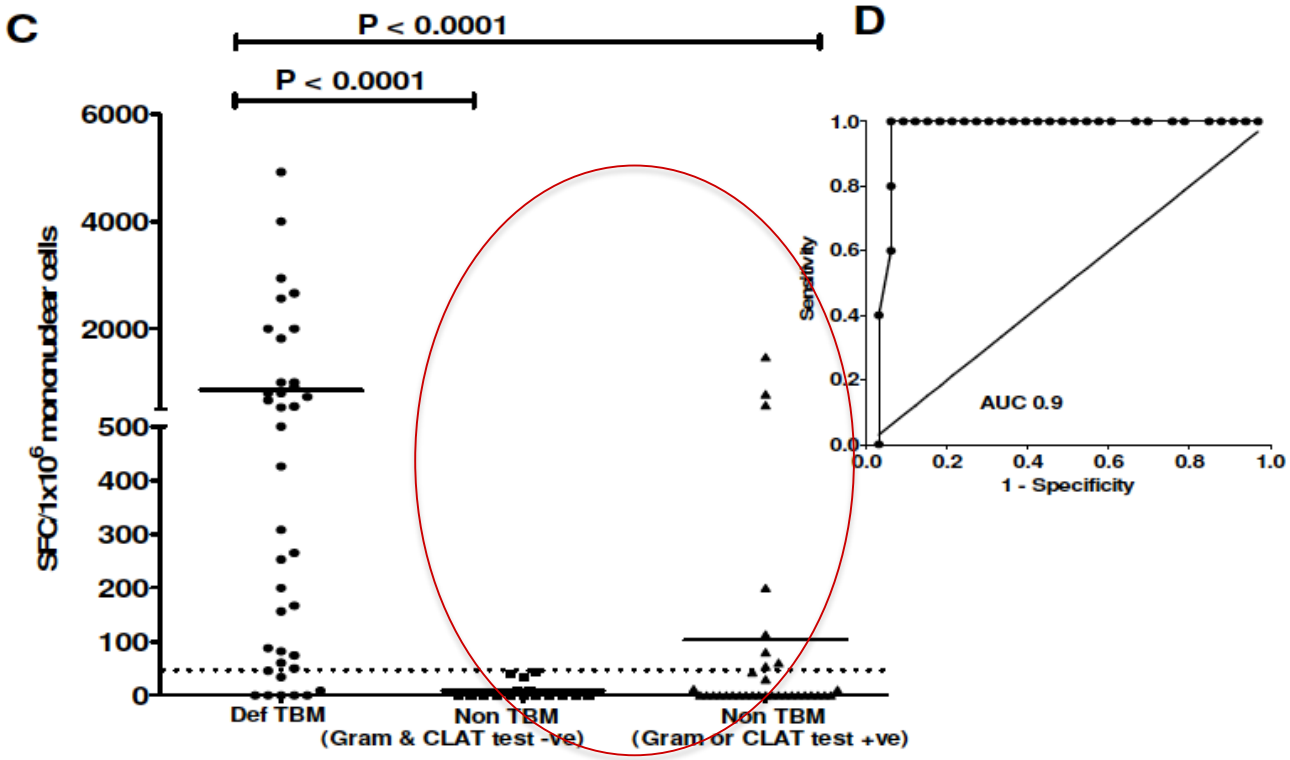
Li ZL, J Phys Condens Matter, 2016

Saunders NR, F1000Res, 2015

IGRA (T-SPOT-TB ELISPOT) in TBM (using cells from the CSF; n= 150)

- Sensitivity= 82%; specificity= 100% (n= 140)
- Works very well in TBM when used in conjunction with CLAT and Gram stain

Patel and Dheda, AJRCCM, 2010



TBM: unstimulated IFN-g when combined with low cost POC lab tests (Gram stain and CLAT)

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2011, p. 3971–3976 Vol. 49, No. 11
0095-1137/11/$12.00 doi:10.1128/JCM.01128-11
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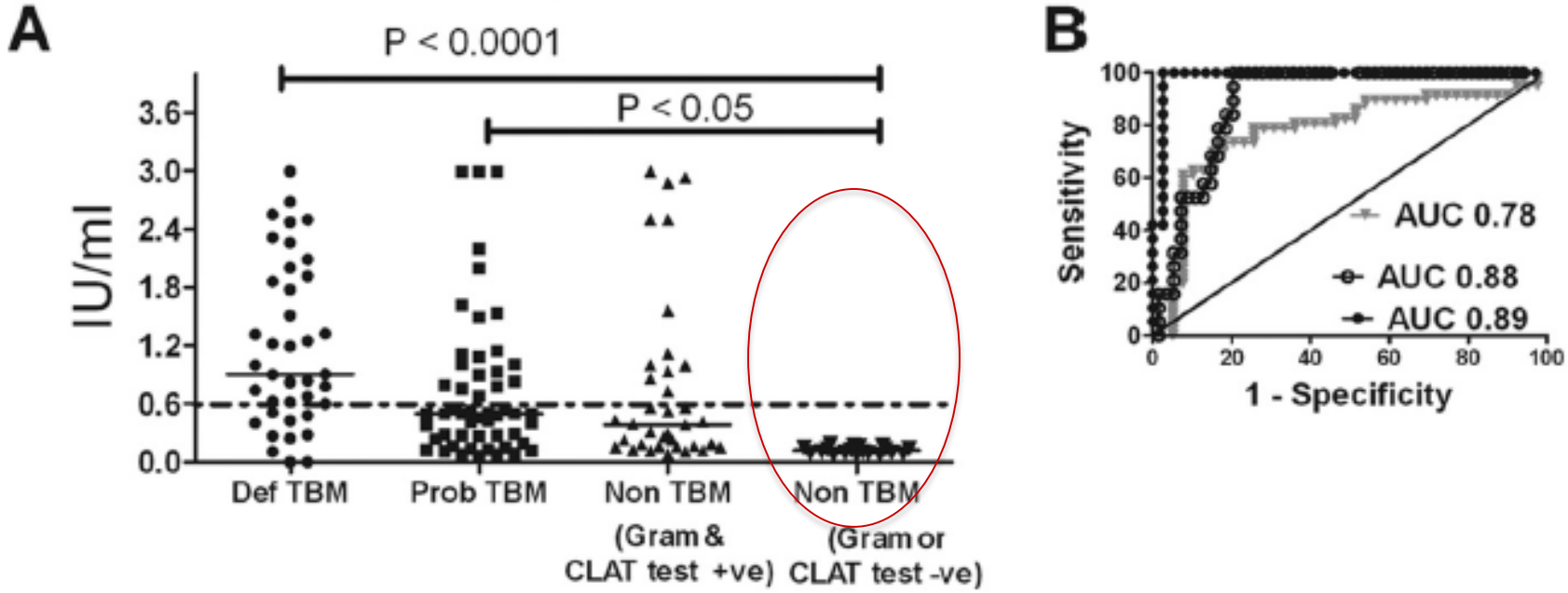
Comparative Utility of Cytokine Levels and Quantitative RD-1-Specific T Cell Responses for Rapid Immunodiagnosis of Tuberculous Meningitis[▽]

Vinod B. Patel,[1] Ravesh Singh,[2] Cathy Connolly,[3] Victoria Kasprowicz,[2] Thumbi Ndung'u,[2] and Keertan Dheda[4,5,6]*



- Unstimulated and unprocessed IFN-Y when used in conjunction with CLAT and Gram stain; (n= 140)

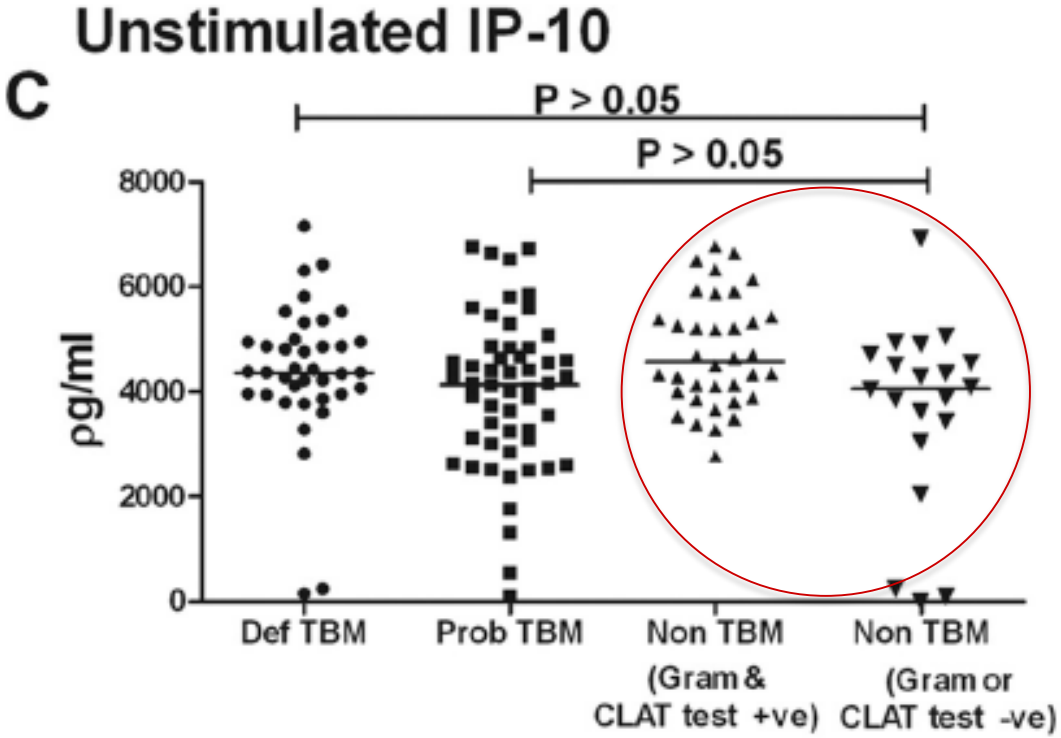
- Sensitivity= 92% (78-98); specificity= 100% (78-100); NPV= 83%

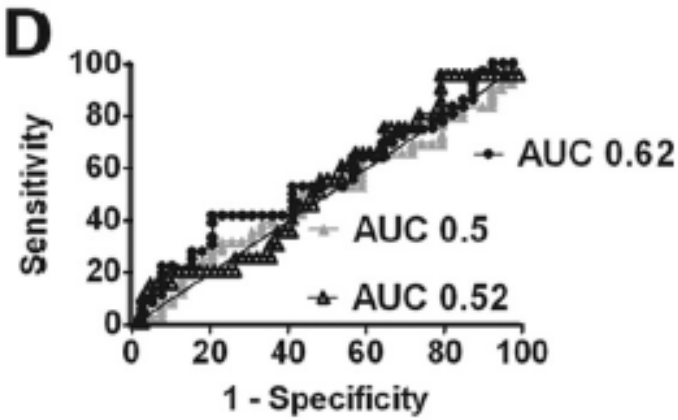
Patel and Dheda, JCM, 2011

Test			IFN-γ levels ≥0.244 iu/ml	
	Sensitivity	Specificity	Sensitivity (94%)	Specificity (89%)
Test			P value	P value
GeneXpert (All)	61%	95%	0.002	0.4

N= 184 suspected TBM; NPV= 91% in preparation

Other biomarkers





Multicentre clinical trial (IFN-g) now in progress……..

Summary

- New diagnostics must have incremental yield over clinical findings + basic CSF lab tests including Gram stain and CLAT (high NPV)
- Not a ‘one size fits all’

- Urine LAM should be performed in hospitalised advanced HIV with or without suspected TBM when sputum tests are unavailable or negative (low cost and simple test)
- CSF LAM- need more data; promising option if Xpert unavailable

- Unstimulated IFN-g in combination with simple adjunct lab tests is a promising approach that requires further evaluation
- Further work on the TB-specific proteome in combination with amplification technologies is required

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