14th National Conference on
Laboratory Aspects of Tuberculosis

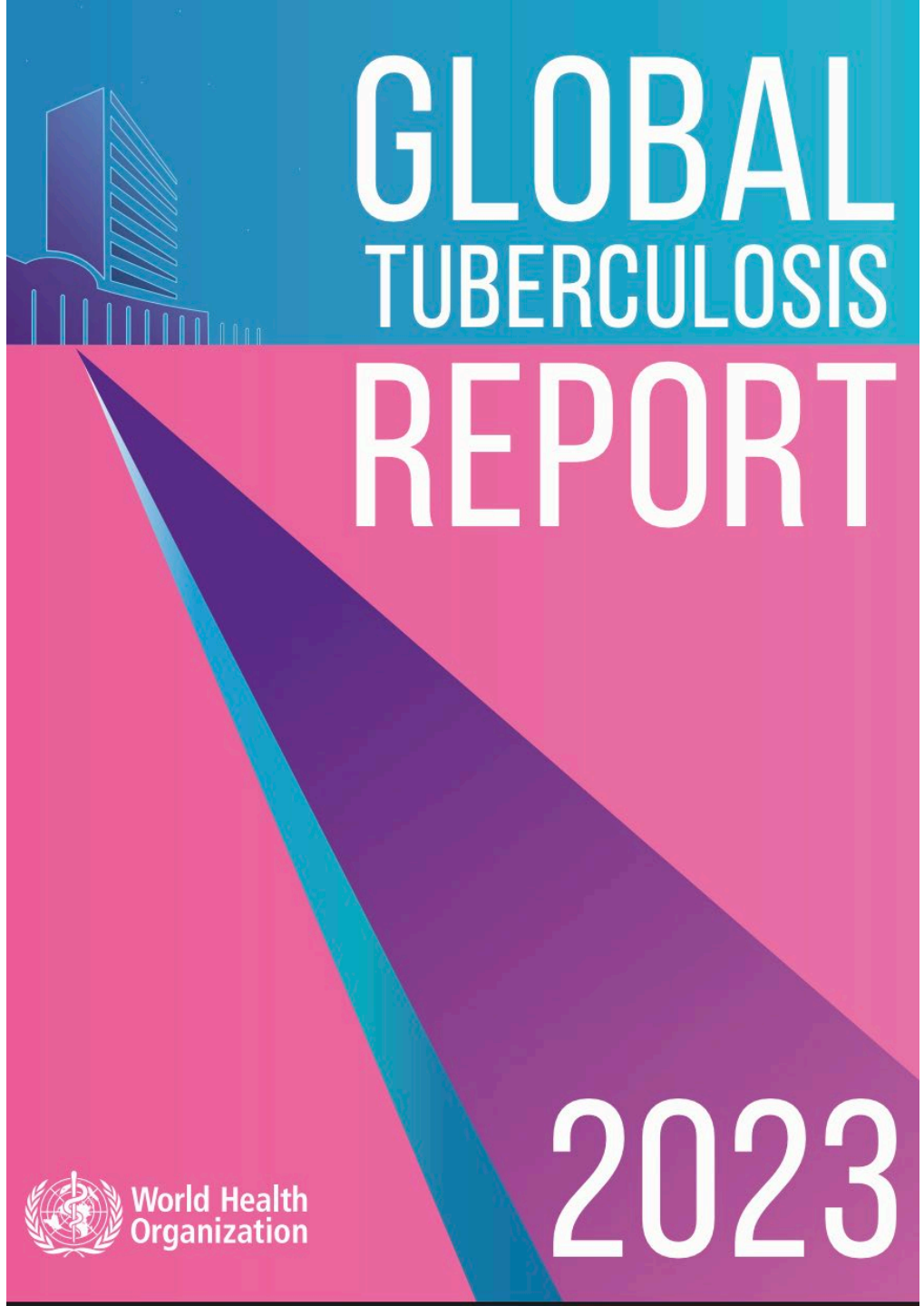
March 10–12, 2026 | Atlanta, Georgia

New Routes Ahead: Exploring the Future of TB Diagnostics

Novel Diagnostics for Closing the TB Diagnostic Gap

Niaz Banaei MD
Professor of Pathology and Medicine
Stanford University
nbanaei@stanford.edu

Stanford MEDICINE | Pathology


GLOBAL
TUBERCULOSIS
REPORT
2023
World Health
Organization

Diagnostics are Necessary but Not Sufficient: Need for a Healthcare System

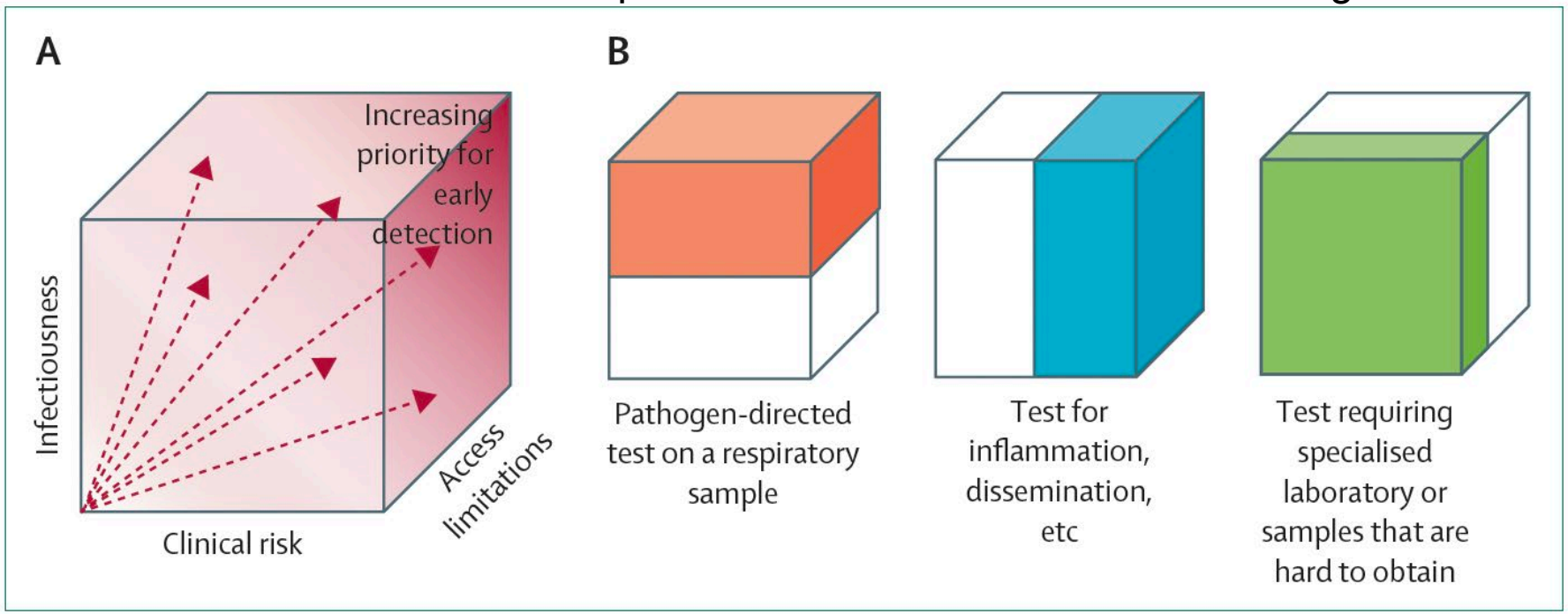
Question

What TB diagnostics do we need?

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests

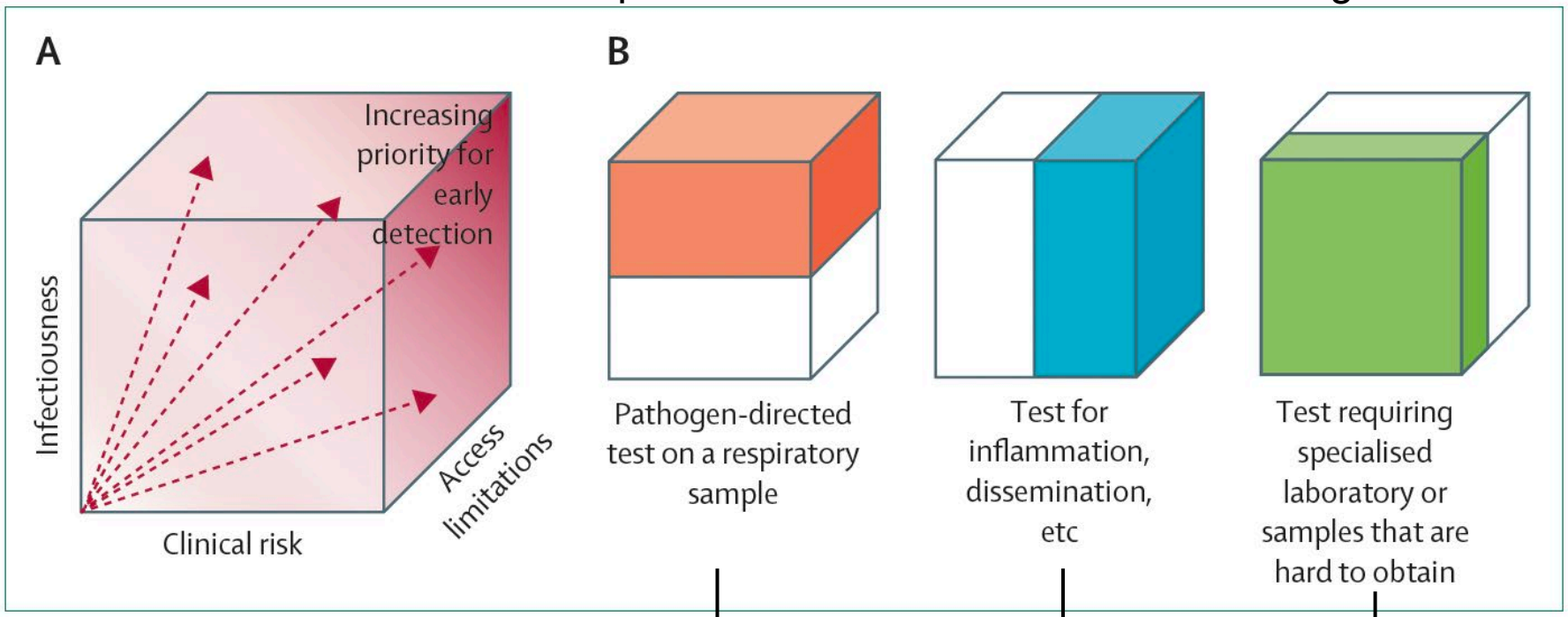


PMID: 41115442

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed</u>
Xpert MTB/RIF
Non-sputum tests
Assisted microscopy

<u>Host-directed</u>
Host response

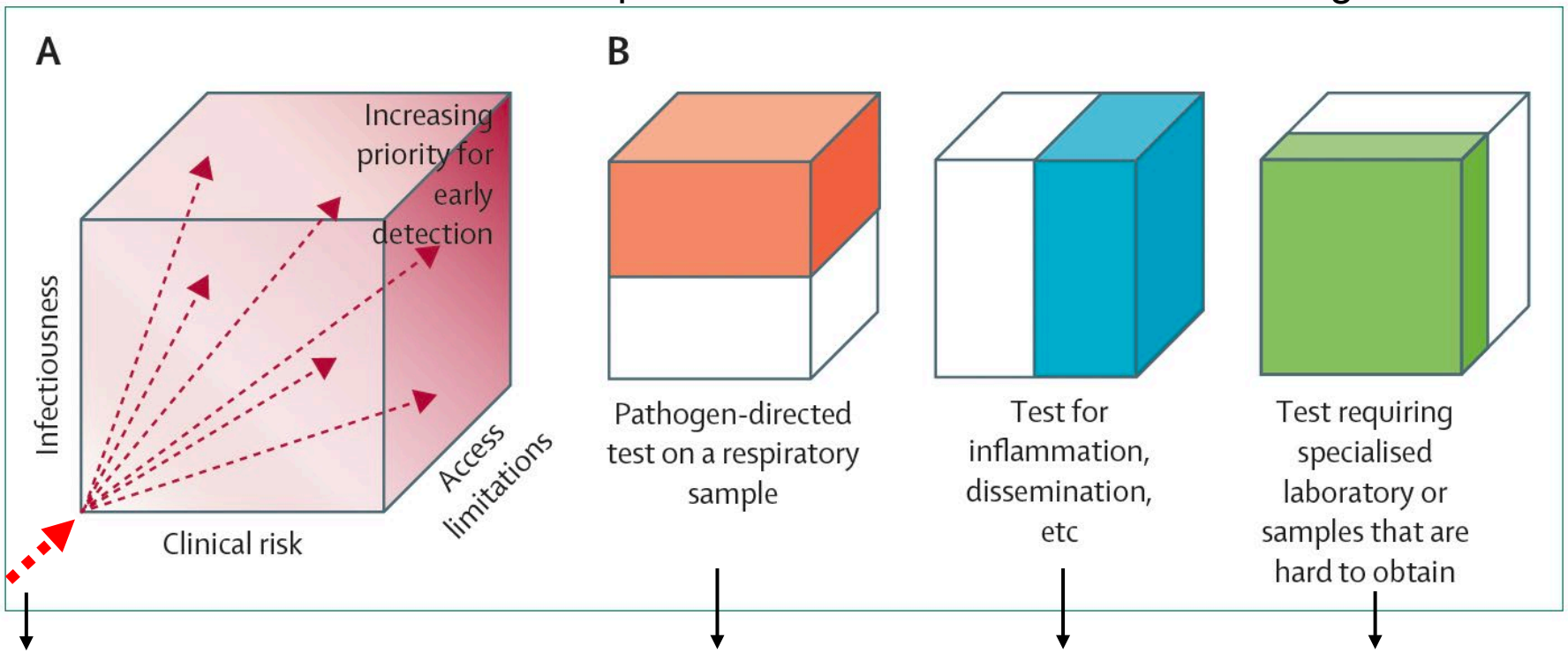
<u>Pathogen-directed</u>
LAM
cell-free DNA

PMID: 41115442

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

<u>Pathogen-directed</u>
Xpert MTB/RIF
Non-sputum tests
Assisted microscopy

<u>Host-directed</u>
Host response

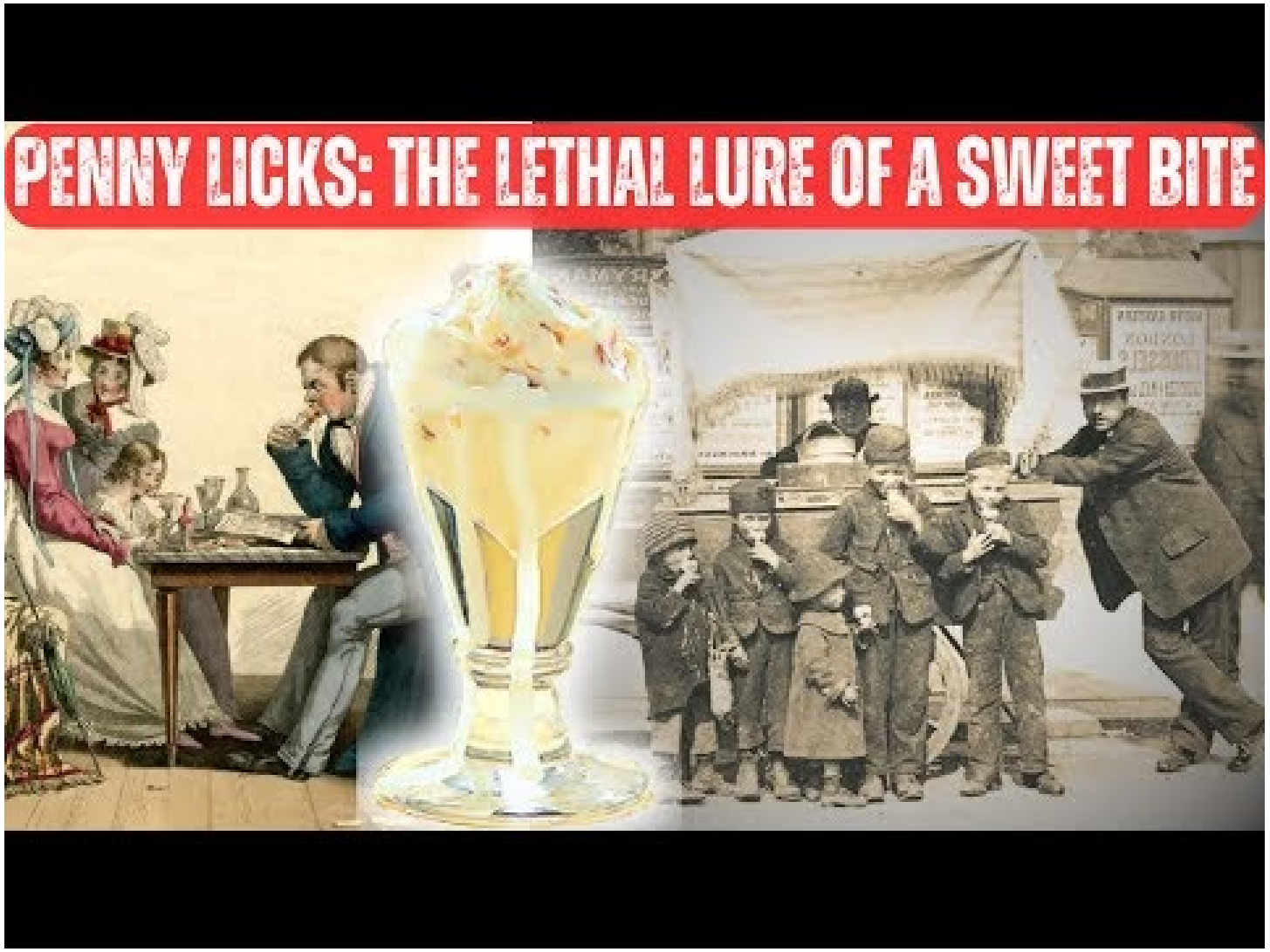
<u>Pathogen-directed</u>
LAM
cell-free DNA

PMID: 41115442

Penny Lick: Perceived link with the spread of TB

A "penny lick" was a small, reusable glass used by street vendors in the 19th century to sell a single lick of ice cream for a penny, with customers licking the glass clean before returning it to the vendor for reuse. This unsanitary practice was banned in many places, like London in 1898, due to the risk of spreading diseases like tuberculosis, leading to the invention of a hygienic alternative.

Penny Lick banned to stop the spread of TB



The Solution: Edible Vessels

Italo Marchiony (1903): An Italian immigrant in New York patented a machine to mass-produce edible pastry cone.

Ernest Hamwi (1904 World's Fair): A Syrian waffle vendor invented waffle cone.

American Journal of Primatology 74:676–686 (2012)

RESEARCH ARTICLE

From the Mouths of Monkeys: Detection of *Mycobacterium tuberculosis* Complex DNA From Buccal Swabs of Synanthropic Macaques

ALICIA K. WILBUR[1], GREGORY A. ENGEL[1,2], AIDA ROMPIS[3], I.G.A. A PUTRA[3], BENJAMIN P. Y.-H. LEE[4], NANTIYA AGGIMARANGSEE[5], MUKESH CHALISE[6], ERIC SHAW[7], GUNWHA OH[1], MICHAEL A. SCHILLACI[8], AND LISA JONES-ENGEL[1]*
[1]*National Primate Research Center, University of Washington, Seattle, Washington*
[2]*Swedish Cherry-Hill Family Medicine, Seattle, Washington*
[3]*University of Udayana, Bali, Indonesia*
[4]*Nature Parks, Parks Division, National Parks Board, Singapore, Singapore*
[5]*Chiang Mai University, Chiang Mai, Thailand*
[6]*Tribhuvan University, Kathmandu, Nepal*
[7]*Gibraltar Ornithological and Natural History Society, Gibraltar, Gibraltar*
[8]*University of Toronto Scarborough, Toronto, Canada*

Although the *Mycobacterium tuberculosis* complex (MTBC) infects a third of all humans, little is known regarding the prevalence of mycobacterial infection in nonhuman primates (NHP). For more than a century, tuberculosis has been regarded as a serious infectious threat to NHP species. Advances in the detection of MTBC open new possibilities for investigating the effects of this poorly understood pathogen in diverse populations of NHP. Here, we report results of a cross-sectional study using well-described molecular methods to detect a nucleic acid sequence (IS*6110*) unique to the MTBC. Sample collection was focused on the oral cavity, the presumed route of transmission of MTBC. Buccal swabs were collected from 263 macaques representing 11 species in four Asian countries and Gibraltar. Contexts of contact with humans included free ranging, pets, performing monkeys, zoos, and monkey temples. Following DNA isolation from buccal swabs, the polymerase chain reaction (PCR) amplified IS*6110* from 84 (31.9%) of the macaques. In general, prevalence of MTBC DNA was higher among NHP in countries where the World Health Organization reports higher prevalence of humans infected with MTBC. This is the first demonstration of MTBC DNA in the mouths of macaques. Further research is needed to establish the significance of this finding at both the individual and population levels. PCR of buccal samples holds promise as a method to elucidate the mycobacterial landscape among NHP, particularly macaques that thrive in areas of high human MTBC prevalence. Am. J. Primatol. 74:676–686, 2012.

"we hypothesized that DNA from MTBC would be present in the oral cavities of macaques and could be detected"

PMID: 22644580

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
INFECTIOUS-DISEASE DIAGNOSTICS
TUBERCULOSIS
DIAGNOSTIC MARKERS

Received
15 October 2014

Accepted
29 January 2015

Detection of *Mycobacterium tuberculosis* DNA on the oral mucosa of tuberculosis patients

Rachel C. Wood[1]*, Angelique K. Luabeya[2]*, Kris M. Weigel[1], Alicia K. Wilbur[3], Lisa Jones-Engel[3], Mark Hatherill[2]* & Gerard A. Cangelosi[1]*

[1]Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA, USA, [2]South African Tuberculosis Vaccine Initiative (SATVI), School of Child & Adolescent Health, University of Cape Town, Cape Town, South Africa, [3]Evolutionary Emergence of Infectious Diseases Laboratory, National Primate Research Center, University of Washington, Seattle, WA, USA.

"we hypothesized that some bacilli that pass through the mouths of TB patients might accumulate on the oral epithelium and be detectable by analysis of oral swab samples."

"The swabs(OmniSwab, Whatman) were brushed along the inside of the subject's cheek for about 10 seconds (7–8 times) to collect cells and saliva."

PMID: 25727773

38 publications since 2014

PubMed®

mycobacterium tuberculosis oral swab

Search

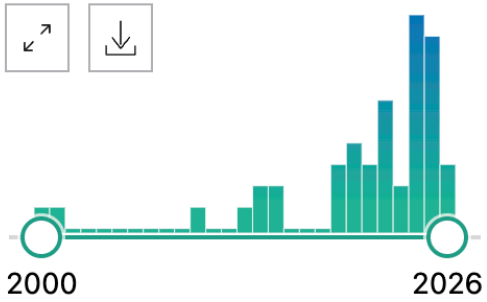
Advanced Create alert Create RSS User Guide

Save Email Send to Sort by: Most recent Display options

MY CUSTOM FILTERS

42 results Page 1 of 5

RESULTS BY YEAR



1 Cite

Improving **tuberculosis** diagnosis: evaluation of tongue swabbing as a complementary method to sputum analysis in Guinea.

Hassane-Harouna S, Condé M, Bah K, Braet SM, Van Dyck-Lippens M, Ruhwald M, Gaichiri M, De Vos M, De Jong BC, Rigouts L.

Ann Clin Microbiol Antimicrob. 2025 Nov 18;24(1):65. doi: 10.1186/s12941-025-00835-2.

PMID: 41254781 **Free PMC article.**

Minimally invasive **oral** sampling methods, such as tongue swabs (TS), have been proposed as alternatives. ...RESULTS: In the prospective study, among 99 smear-positive patients, Ultra detected

Sample Sources and Types Tested with PCR

Buccal Swab

Tongue Swab

Oral Wash

Sample Sources and Types Tested with PCR

Buccal Swab

Tongue Swab

Oral Wash

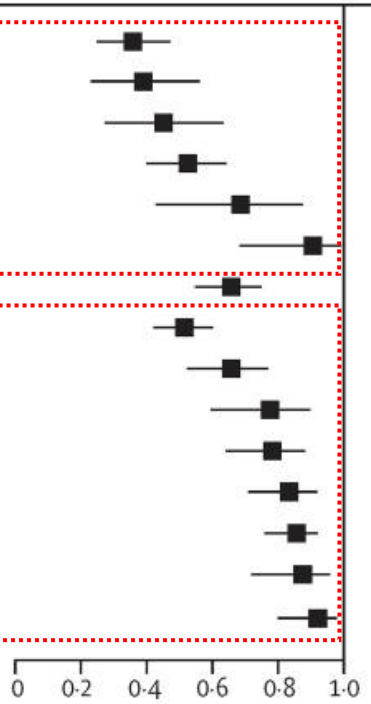
Performance of PCR on Oral Samples

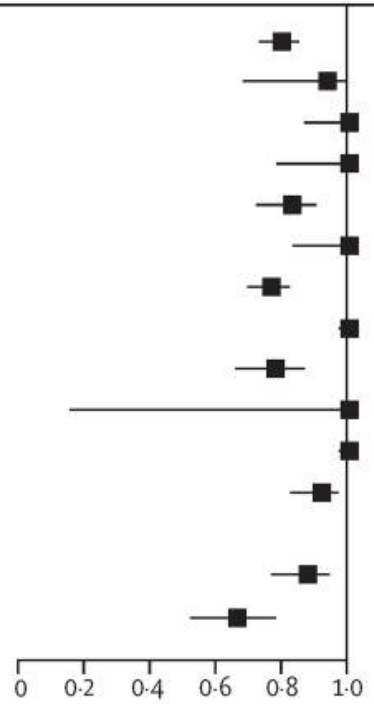
Oral Swab (PMID: 38097297)
Sensitivity:
- In adults: 36% to 91% in adults
- In children: 5% to 42% in children

Specificity:
- 66% to 100%
- Most studies reporting >90%

Performance of PCR on Oral Samples

	True positive	False positive	False negative	True negative	Location swabbed	Sensitivity (95% CI)	Specificity (95% CI)
Molina-Moya et al, 2020[31]	29	38	51	148	Buccal mucosa	0·36 (0·26–0·48)	0·80 (0·73–0·85)
Mesman et al, 2020a[17]	15	1	23	14	Buccal mucosa	0·39 (0·24–0·57)	0·93 (0·68–1·00)
Mesman et al, 2019[35]	15	0	18	26	Buccal mucosa	0·45 (0·28–0·64)	1·00 (0·87–1·00)
Mesman et al, 2020b[17]	38	0	34	15	Buccal mucosa	0·53 (0·41–0·65)	1·00 (0·78–1·00)
LaCourse et al, 2022[23]	13	13	6	62	Buccal mucosa	0·68 (0·43–0·87)	0·83 (0·72–0·90)
Wood et al, 2015[16]	18	0	2	20	Buccal mucosa	0·90 (0·68–0·99)	1·00 (0·83–1·00)
Kang et al, 2022[20]	61	42	32	136	Gingiva	0·66 (0·55–0·75)	0·76 (0·69–0·82)
Lima et al, 2020[14]	66	0	62	128	Tongue	0·52 (0·43–0·60)	1·00 (0·97–1·00)
Shapiro et al, 2022[7]	42	15	22	52	Tongue	0·66 (0·53–0·77)	0·78 (0·66–0·87)
Andama et al, 2022b[22]	27	0	8	2	Tongue	0·77 (0·60–0·90)	1·00 (0·16–1·00)
Andama et al, 2022a[22]	42	0	12	129	Tongue	0·78 (0·64–0·88)	1·00 (0·97–1·00)
Luabeya et al, 2019b[19]	49	6	10	65	Tongue	0·83 (0·71–0·92)	0·92 (0·83–0·97)
Luabeya et al, 2019a[19]	74	0	13	0	Tongue	0·85 (0·76–0·92)	Not estimable
Song et al, 2021[32]	33	8	5	55	Tongue	0·87 (0·72–0·96)	0·87 (0·77–0·94)
Wood et al, 2021[18]	43	19	4	37	Tongue	0·91 (0·80–0·98)	0·66 (0·52–0·78)





(PMID: 38097297)

Performance of PCR on Oral Samples

Oral Wash vs. Tongue Swab (PMID: 40686067)

Sensitivity:

Oral Wash: 80% (95% CI, 56%–94%)

Tongue swab: 65% (95% CI, 58%–72%)

Tongue Foam Swab vs. ESwab (PMID: 40686067)

Sensitivity:

Foam swab: 65% (95% CI, 58%–72%)

Flocked ESwab: 59% (95% CI, 53%–65%)

Other Performance Factors

Bacterial Load: Sensitivity higher in smear-positive or high-Xpert-result patients

Diagnostic accuracy of swab-based molecular tests for tuberculosis using near-point-of-care platforms: a multi-country evaluation

Amy Steadman,[a,o] Kingsley Manoj Kumar,[b,o] Lucy Asege,[c] Midori Kato-Maeda,[d,e] Job Mukwatamundu,[c] Kinari Shah,[d,e] Trinh Trang,[f,g] Alexey Ball,[a] Khushboo Khimani,[a] Dao Thi Kim Dung,[h] Joy Sarojini Michael,[i] Devasahayam J. Christopher,[j] Ha Phan,[f,g] Seda Yerlikaya,[k,l] Payam Nahid,[d] Claudia M. Denkinger,[k,l] Adithya Cattamanchi,[d,m,*,p] and Alfred Andama[c,n,p]

MTB Ultima (Molbio Diagnostics, India)

MiniDock MTB (Pluslife Biotech, China)

Diagnostic accuracy of swab-based molecular tests for tuberculosis using near-point-of-care platforms: a multi-country evaluation

Amy Steadman,[a,o] Kingsley Manoj Kumar,[b,o] Lucy Asege,[c] Midori Kato-Maeda,[d,e] Job Mukwatamundu,[c] Kinari Shah,[d,e] Trinh Trang,[f,g] Alexey Ball,[a] Khushboo Khimani,[a] Dao Thi Kim Dung,[h] Joy Sarojini Michael,[i] Devasahayam J. Christopher,[j] Ha Phan,[f,g] Seda Yerlikaya,[k,l] Payam Nahid,[d] Claudia M. Denkinger,[k,l] Adithya Cattamanchi,[d,m,,p] and Alfred Andama[c,n,p]*

Truenat MTB Ultima (Molbio Diagnostics, India)

	Sensitivity n/N, % (95% CI)	Specificity n/N (95% CI)
Tongue	116/149, **77.9%** (70.3-84.2)	805/820, **98.2%** (97.0-99.0)
Sputum	44/48, **91.7%** (80.0, 97.7)	129/132, **97.7%** (93.5, 99.5)

MiniDock MTB (Pluslife Biotech, China)

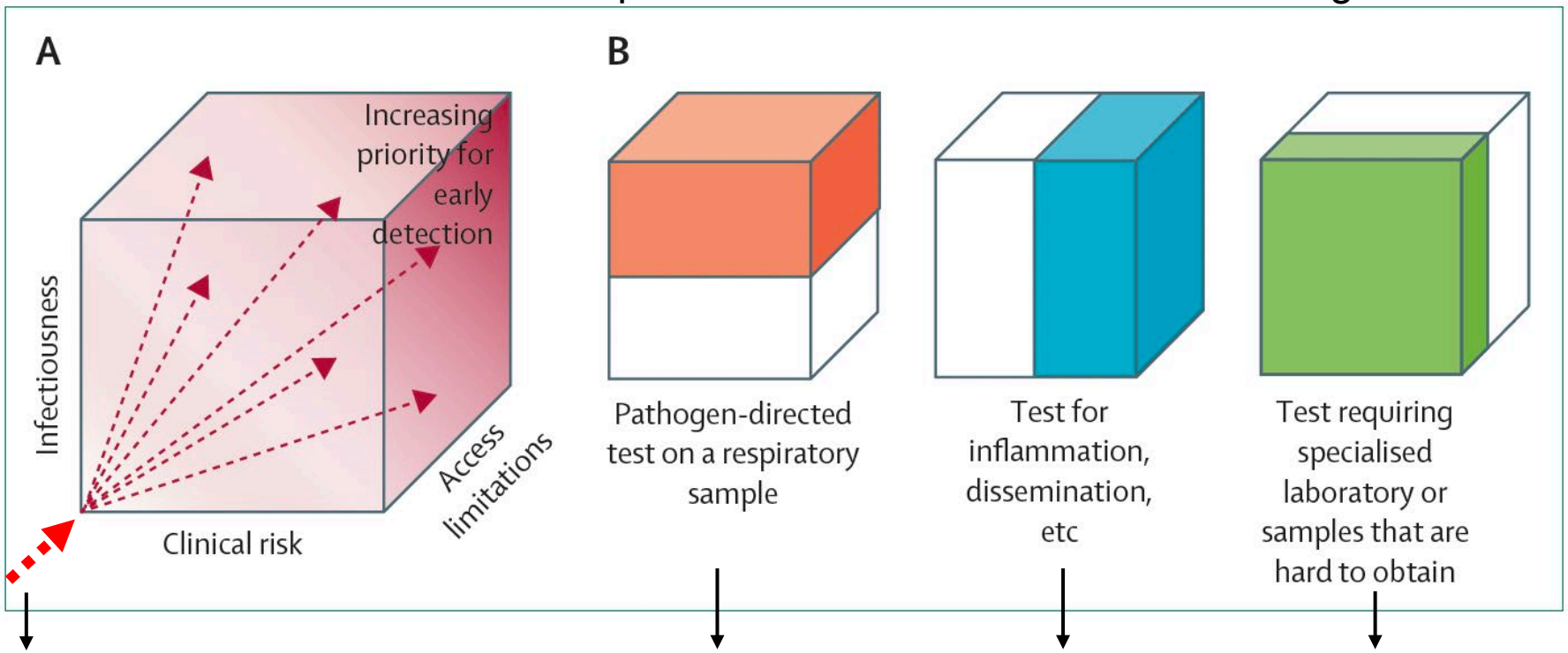
	Sensitivity n/N, % (95% CI)	Specificity n/N (95% CI)
Tongue	60/70, **85.7%** (75.3-92.9)	226/226, **100%** (98.4-100.0)
Sputum	62/69, **89.9%** (80.2, 95.8)	219/223, **98.2%** (95.5, 99.5)

PMID: 41175672

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

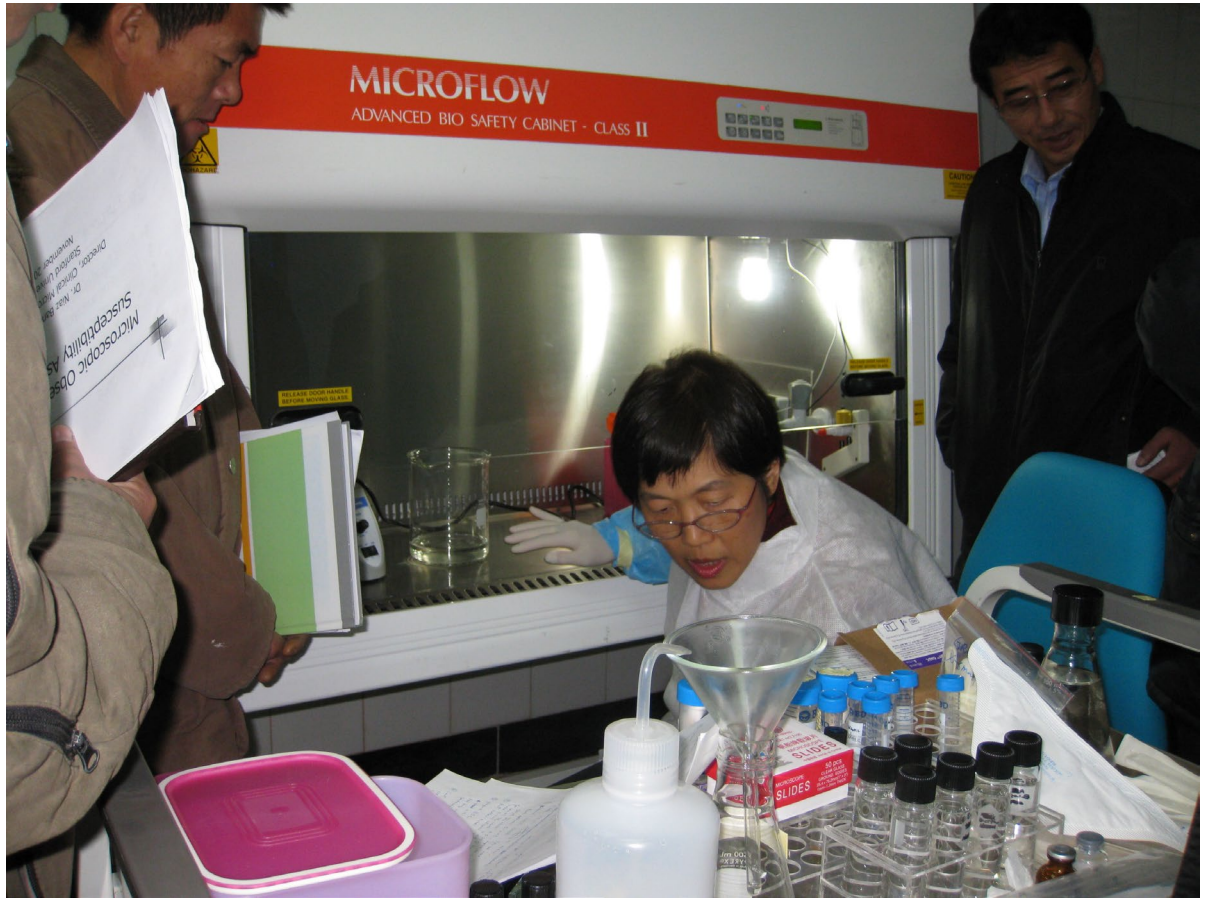
<u>Pathogen-directed</u>
Non-sputum tests
Assisted microscopy

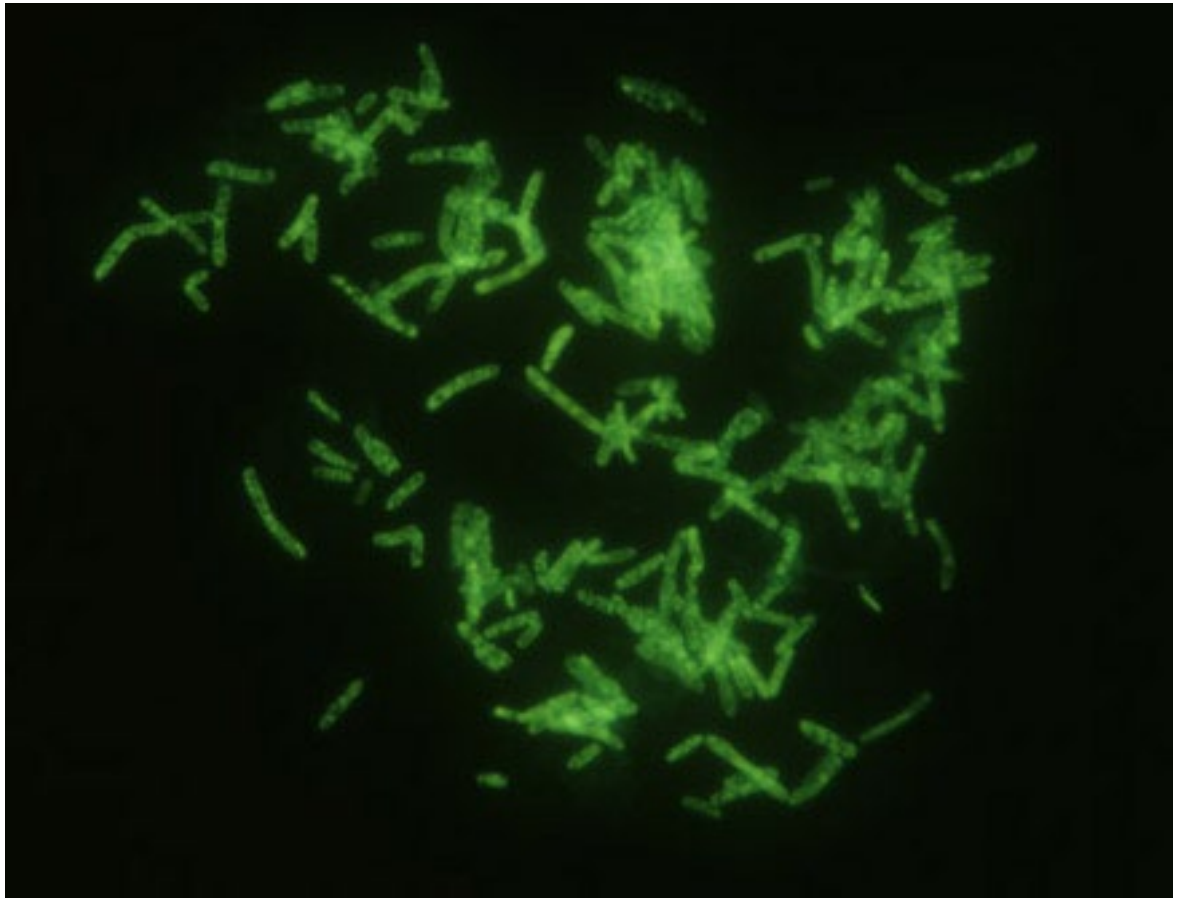
<u>Host-directed</u>
Host response

<u>Pathogen-directed</u>
LAM
cell-free DNA

PMID: 41115442

Assisted AFB Microscopy: Are We There Yet?





PMID: 36121216

Assisted AFB Microscopy: Are We There Yet?

Evaluation of MetaSystems Automated Fluorescent Microscopy System for the Machine-Assisted Detection of Acid-Fast Bacilli in Clinical Samples

Gianna Tomasello,[a] Farnaz Foroughi,[a] Danielle Padron,[a] Angel Moreno,[b] Niaz Banaei[a,b,c]

TABLE 1 Performance of MetaSystems automated AFB microscopy

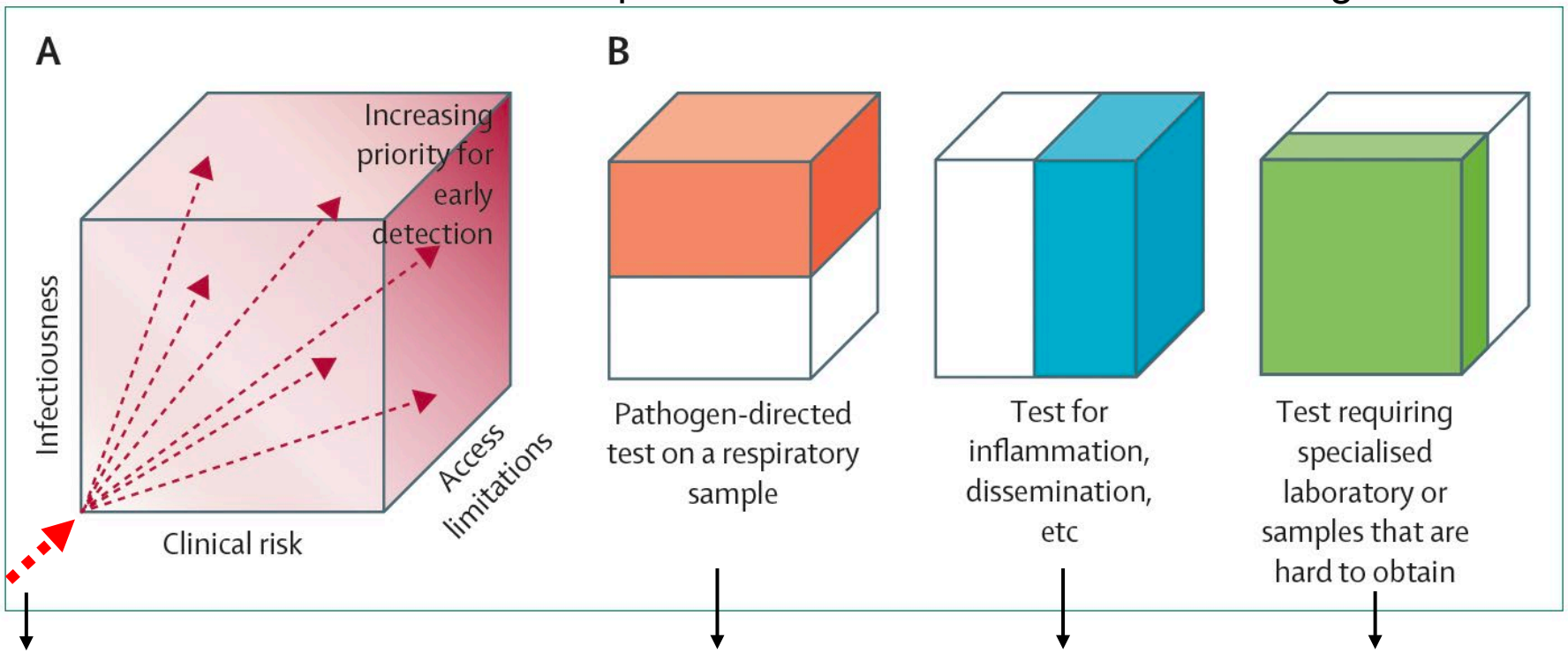
Microscopy method evaluated	Reference standard			
	Culture		Manual microscopy	
	Sensitivity (n[a]/N[b]; 95% CI)	Specificity (n/N; 95% CI)	Sensitivity (n/N; 95% CI)	Specificity (n/N; 95% CI)
Manual	79.7% (106/133; 71.9–86.2)	98.6% (358/363; 96.8–99.6)	NA[c]	NA
MetaSystems automated	97.0% (129/133; 92.5–99.2)	12.7% (46/363; 9.4–16.5)	97.3% (108/111; 92.3–99.4)	12.0% (46/385; 8.9–15.6)
MetaSystems automated assisting CLS	70.7% (94/133; 62.2–78.3)	89.0% (323/363; 85.3–92.0)	85.6% (95/111; 77.7–91.5	75.3% (290/385; 70.7–79.6)

PMID: 36121216

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

<u>Pathogen-directed</u>
Non-sputum tests
Assisted microscopy

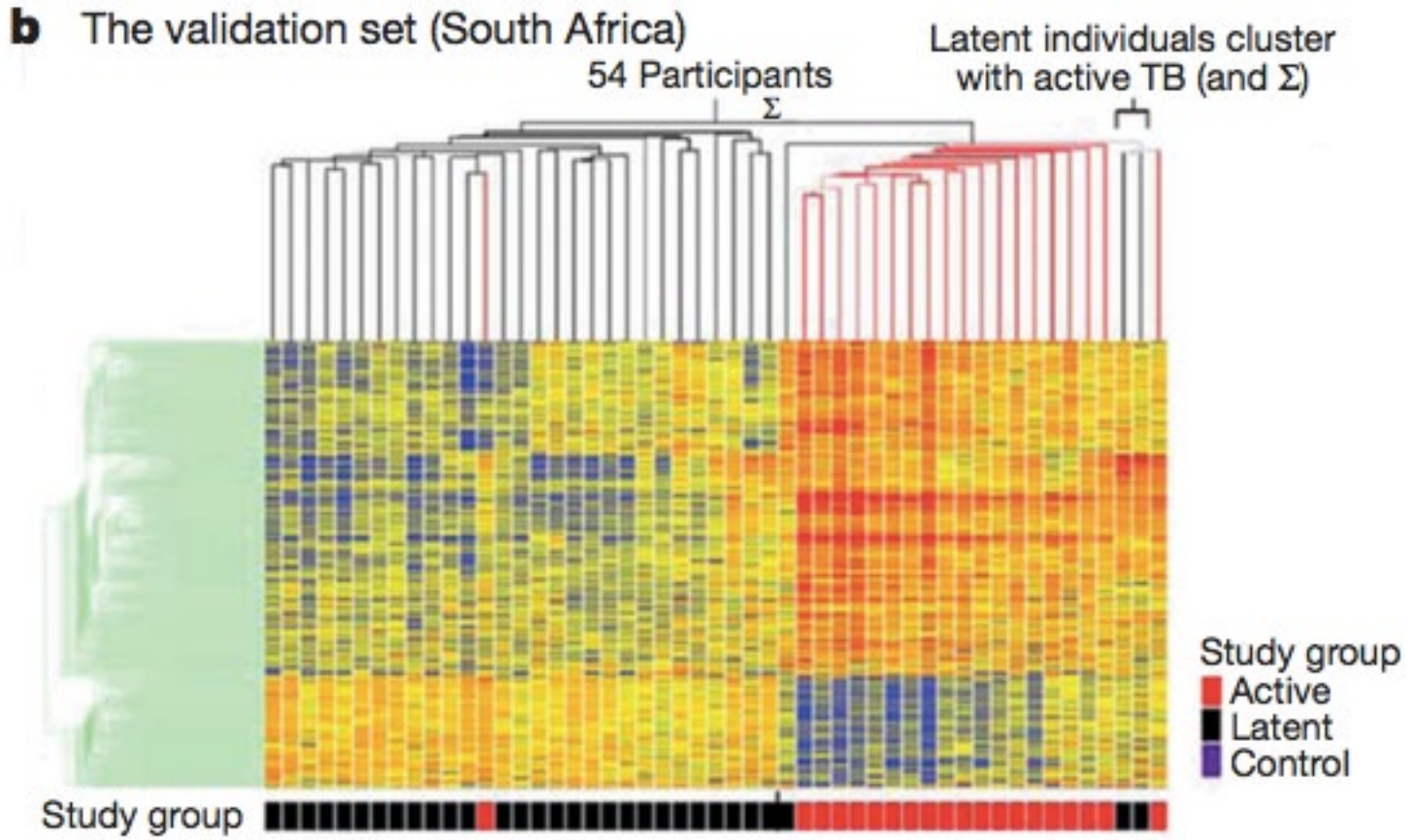
<u>Host-directed</u>
Host response

<u>Pathogen-directed</u>
LAM
cell-free DNA

PMID: 41115442

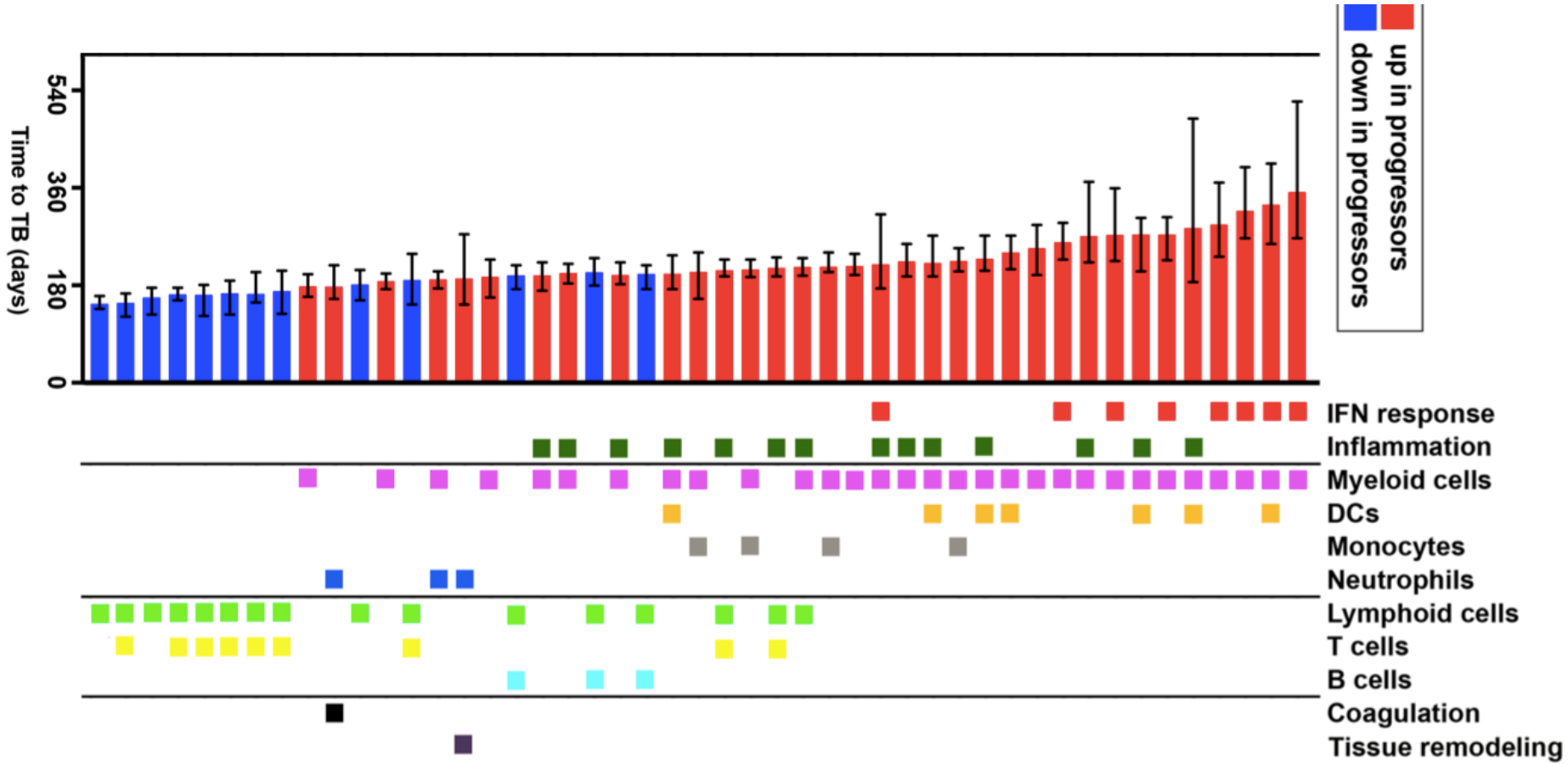
An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis

Matthew P. R. Berry[1], Christine M. Graham[1]*, Finlay W. McNab[1]*, Zhaohui Xu[6], Susannah A. A. Bloch[3], Tolu Oni[4,5], Katalin A. Wilkinson[2,4], Romain Banchereau[9], Jason Skinner[6], Robert J. Wilkinson[2,4,5], Charles Quinn[6], Derek Blankenship[7], Ranju Dhawan[8], John J. Cush[6], Asuncion Mejias[10], Octavio Ramilo[10], Onn M. Kon[3], Virginia Pascual[6], Jacques Banchereau[6], Damien Chaussabel[6] & Anne O'Garra[1]



PMID: 20725040

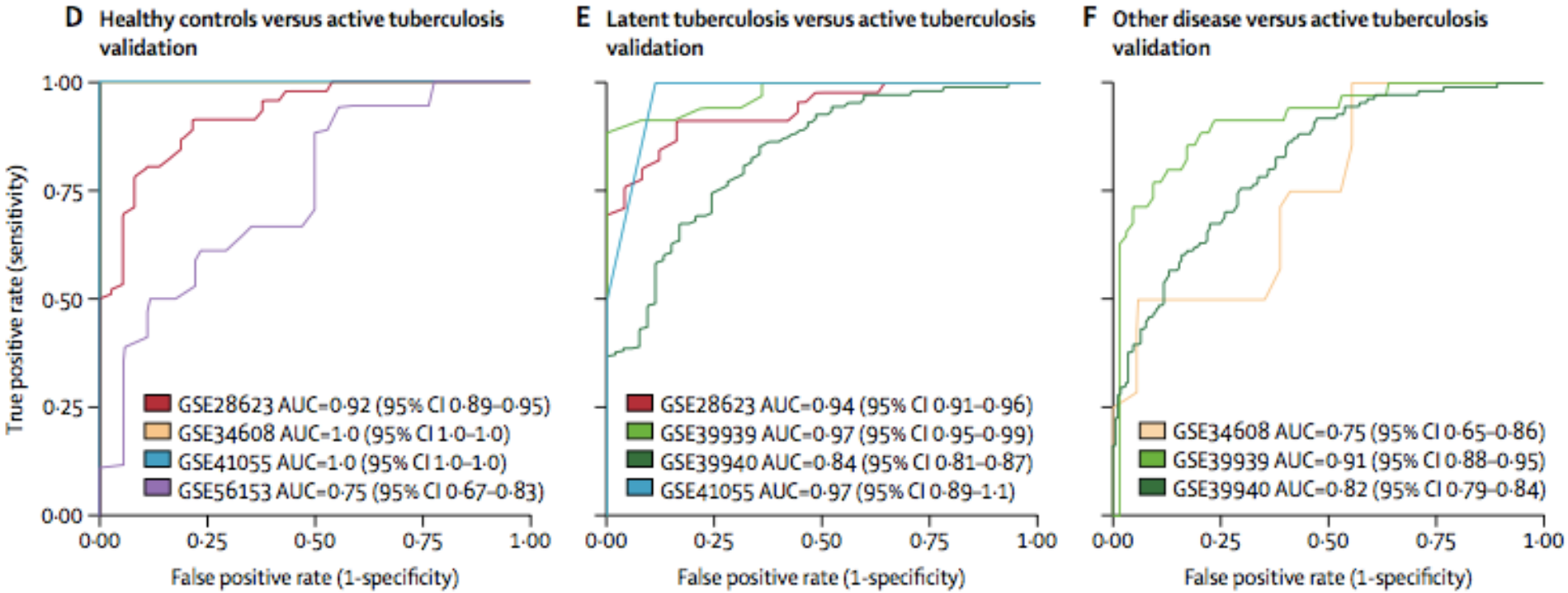
IFN I/II signalling transcripts up 18 months before TB diagnosis, while changes in myeloid , lymphoid, monocyte and neutrophil responses occurred more proximally



PMID: 29145483

Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis

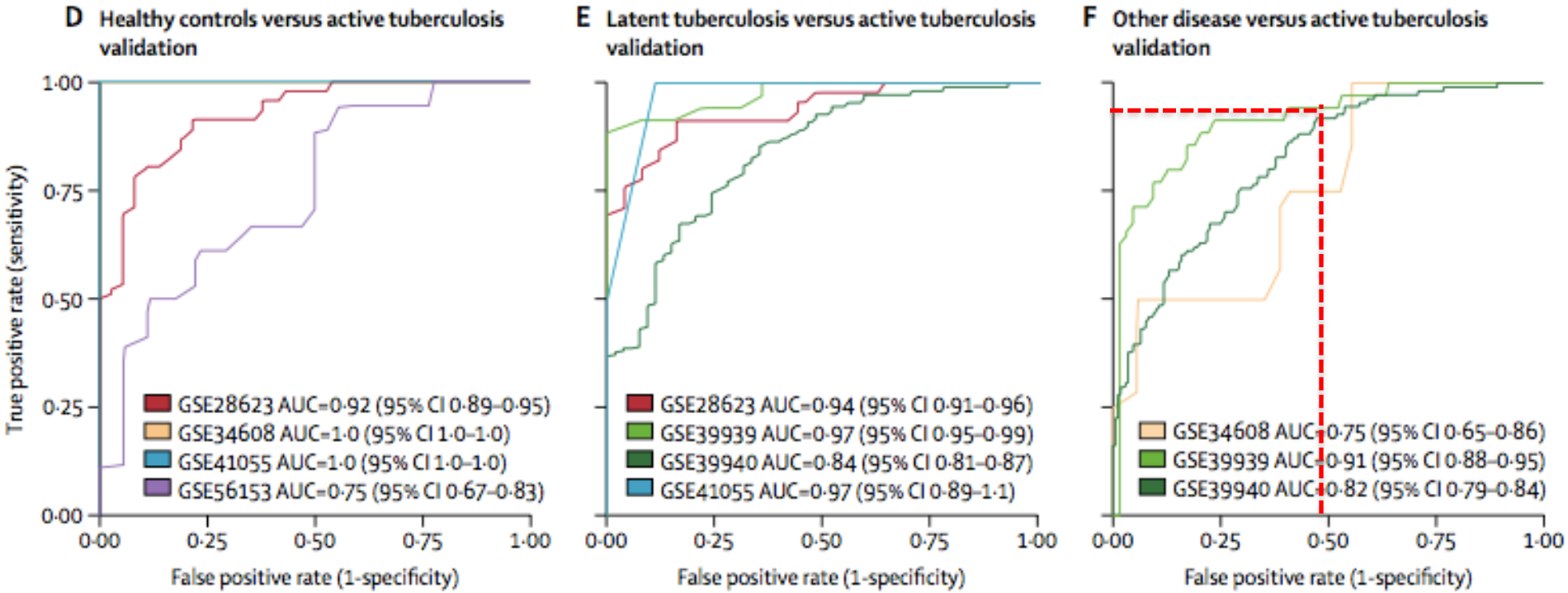
Timothy E Sweeney, Lindsay Braviak, Cristina M Tato, Purvesh Khatri



PMID: 26907218

Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis

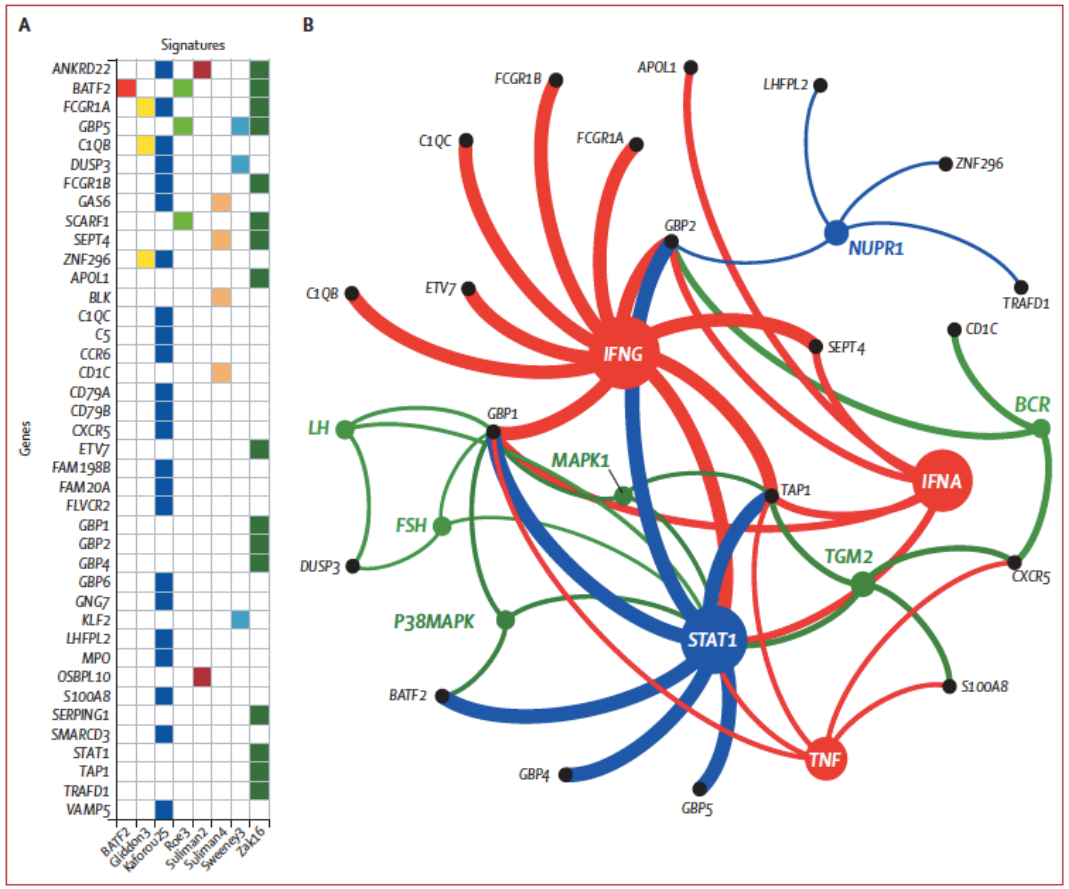
Timothy E Sweeney, Lindsay Braviak, Cristina M Tato, Purvesh Khatri



PMID: 26907218

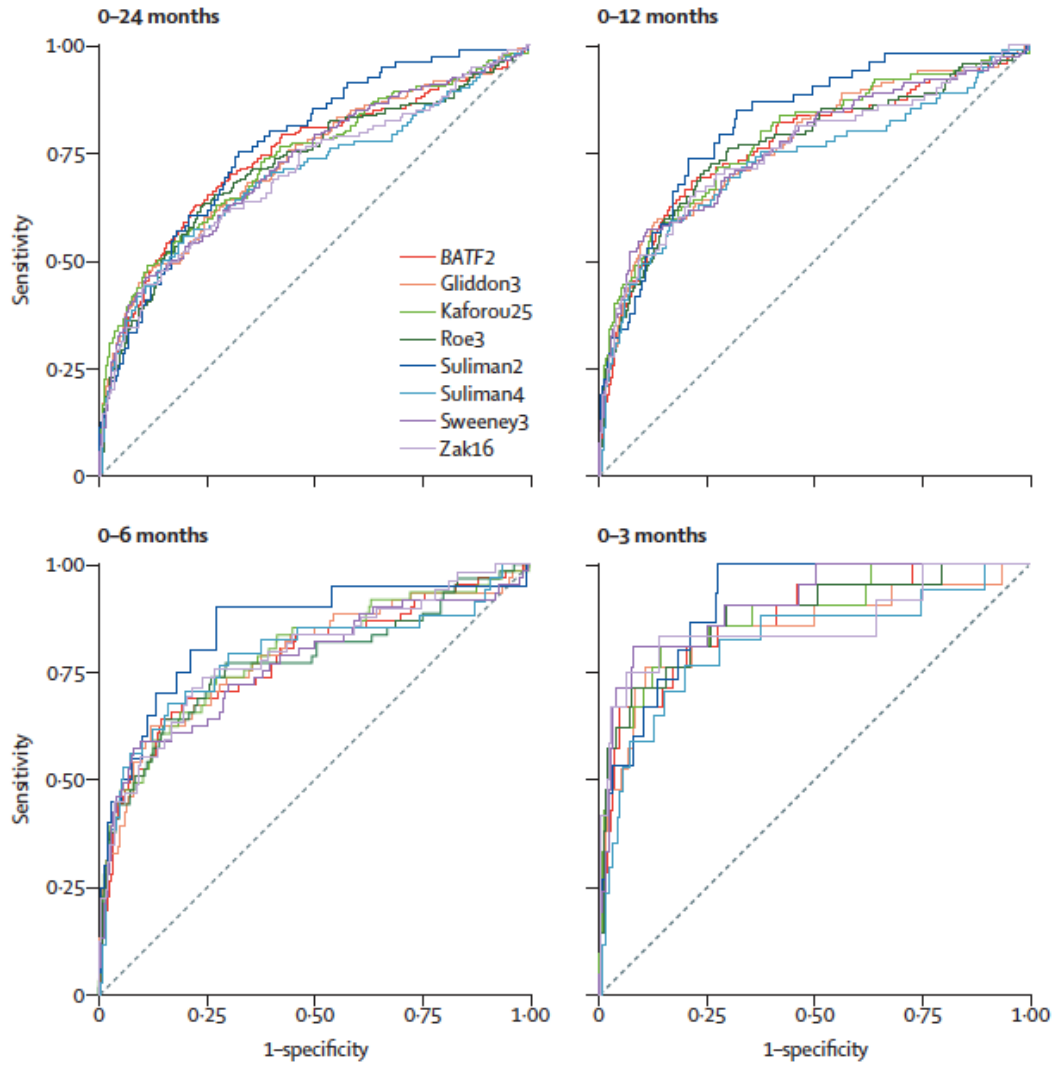
Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis

Rishi K Gupta, Carolin T Turner, Cristina Venturini, Hanif Esmail, Molebogeng X Rangaka, Andrew Copas, Marc Lipman, Ibrahim Abubakar, Mahdad Noursadeghi**



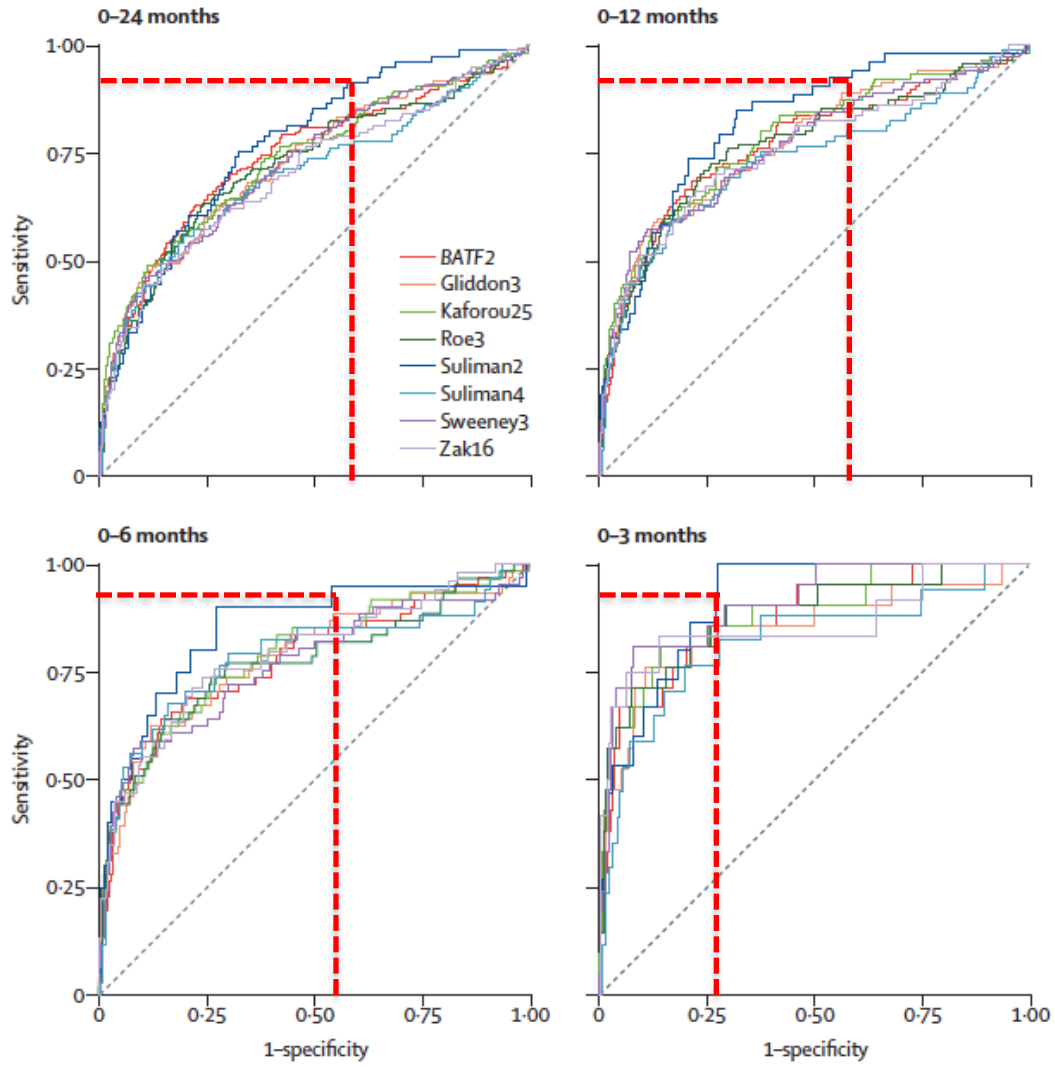
PMID: 31958400

Diagnostic Accuracy of the 8 Best Signatures for Incipient TB



PMID: 31958400

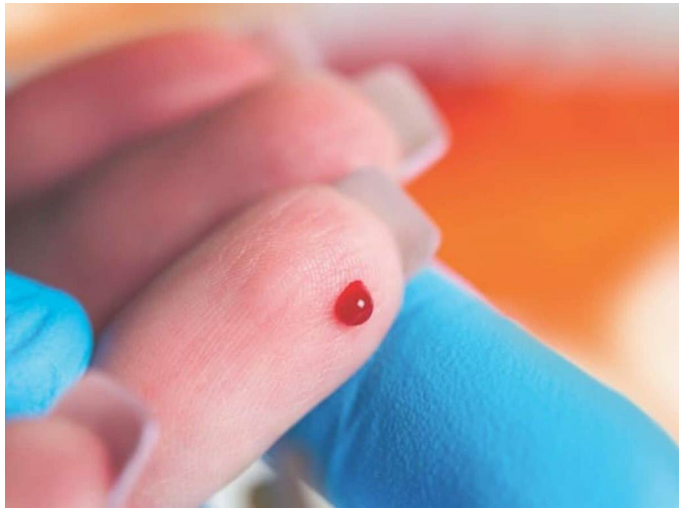
Diagnostic Accuracy of the 8 Best Signatures for Incipient TB



PMID: 31958400

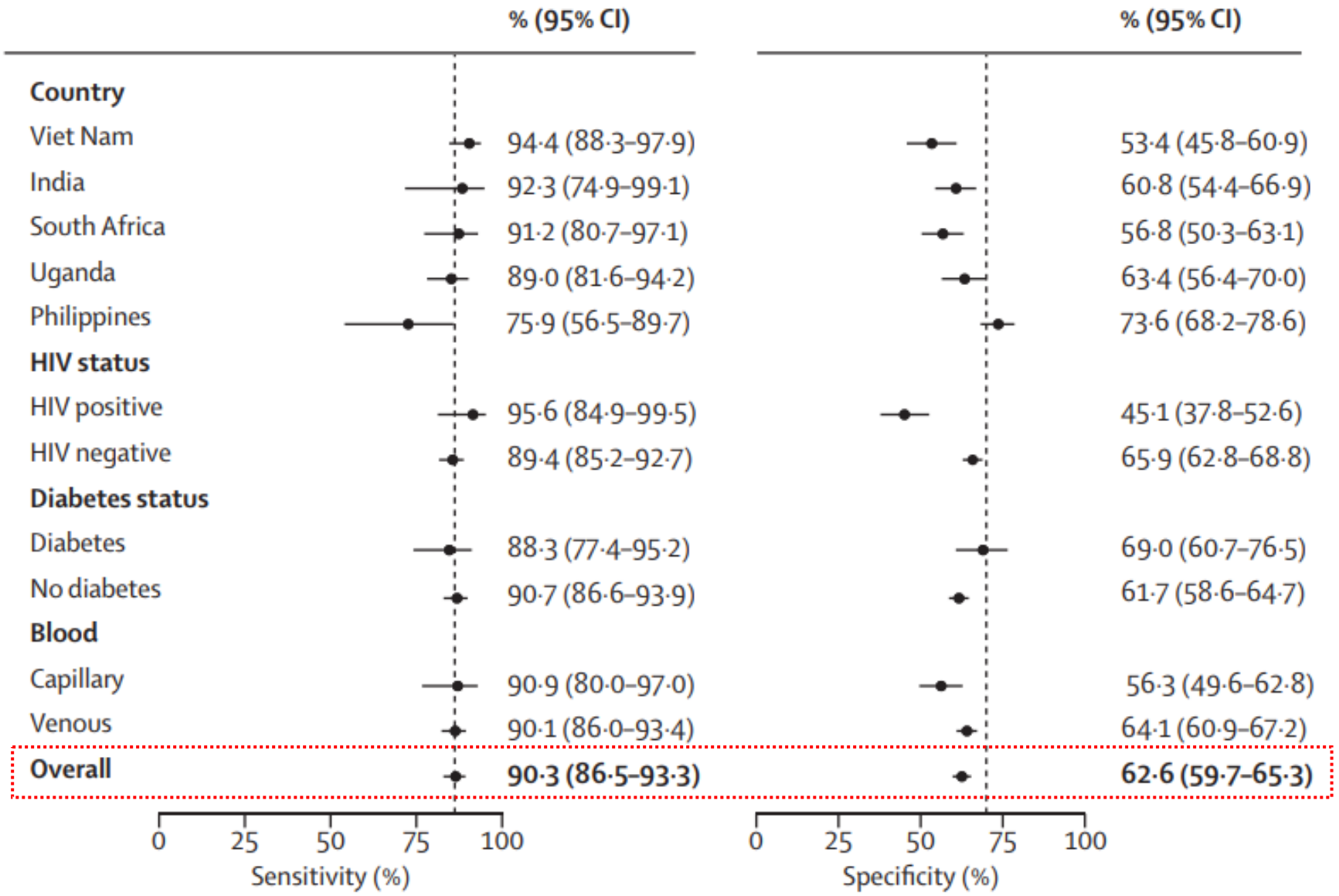
Evaluation of the Xpert MTB Host Response assay for the triage of patients with presumed pulmonary tuberculosis: a prospective diagnostic accuracy study in Viet Nam, India, the Philippines, Uganda, and South Africa

Ankur Gupta-Wright, Huy Ha*, Shima Abdulgadar, Rebecca Crowder, Jerusha Emmanuel, Job Mukwatamundu, Danaida Marcelo, Patrick P J Phillips, Devasahayam Jesudas Christopher, Nguyen Viet Nhung, Grant Theron, Charles Yu, Payam Nahid, Adithya Cattamanchi, William Worodria*, Claudia M Denkinger*, on behalf of the R2D2 TB Network†*



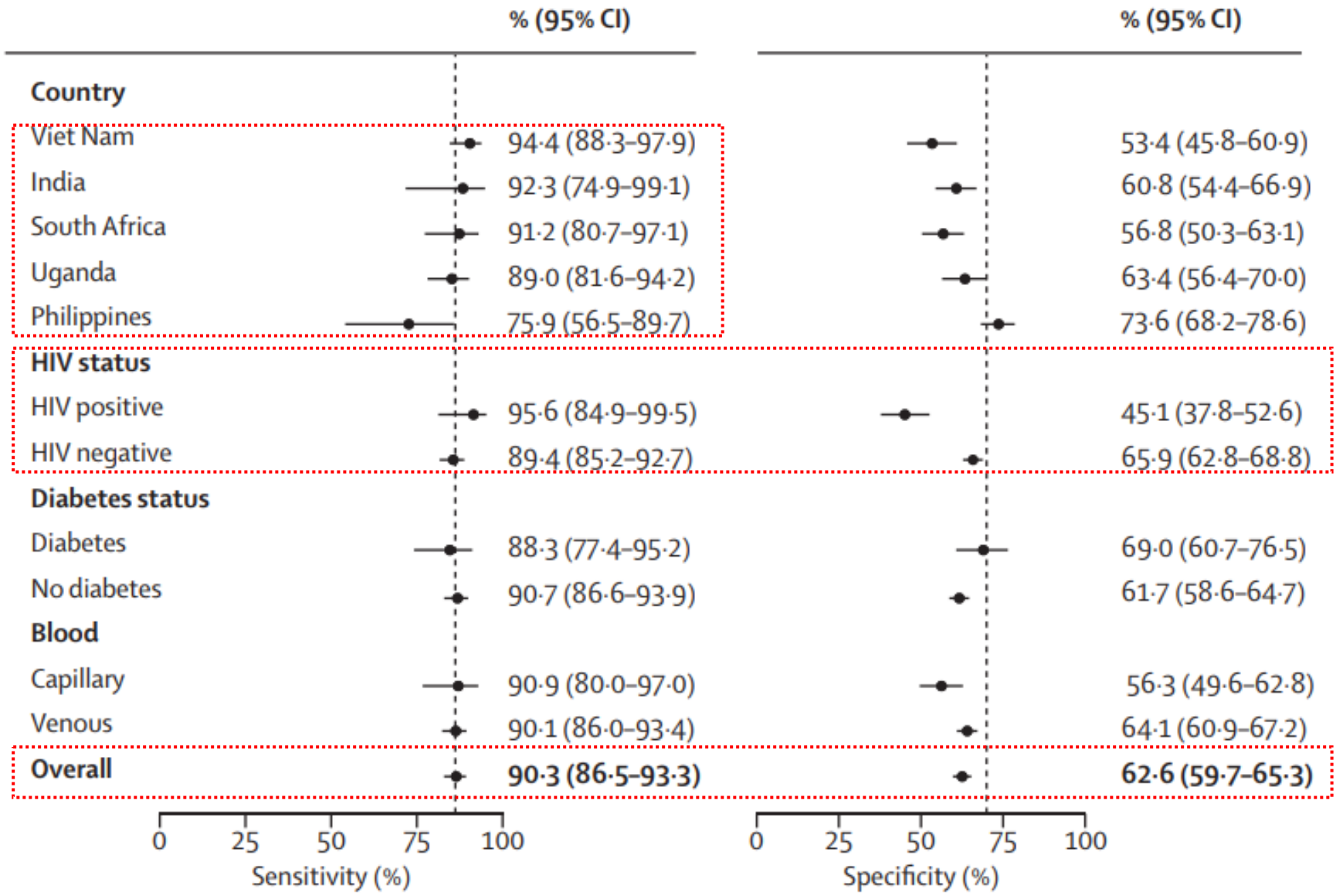
PMID: 38245113

Accuracy of the Xpert MTB Host Response



PMID: 38245113

Accuracy of the Xpert MTB Host Response for PTB



PMID: 38245113

Evaluation of the Xpert MTB Host Response assay for the triage of patients with presumed pulmonary tuberculosis: a prospective diagnostic accuracy study in Viet Nam, India, the Philippines, Uganda, and South Africa

Ankur Gupta-Wright, Huy Ha*, Shima Abdulgadar, Rebecca Crowder, Jerusha Emmanuel, Job Mukwatamundu, Danaida Marcelo, Patrick P J Phillips, Devasahayam Jesudas Christopher, Nguyen Viet Nhung, Grant Theron, Charles Yu, Payam Nahid, Adithya Cattamanchi, William Worodria*, Claudia M Denkinger*, on behalf of the R2D2 TB Network†*

Interpretation Xpert HR did not meet WHO minimum specificity targets for a non-sputum-based triage test for pulmonary tuberculosis. Despite promise as a rule-out test that could reduce confirmatory sputum testing, further cost-effectiveness modelling and data on acceptability and usability are needed to inform policy recommendations.

PMID: 38245113

Blood RNA biomarkers and C-reactive protein for triage of adult patients with tuberculosis lymphadenitis and pericarditis in South Africa: a single-centre, prospective, observational, diagnostic accuracy study

Tiffeney Mann, Stephanie Minnies*, Rishi K Gupta*, Byron W P Reeve, Georgina Nyawo, Zaida Palmer, Charissa Naidoo, Anton Doubell, Alfonso Pecoraro, Thadathilankal-Jess John, Pawel Schubert, Claire J Calderwood, Aneesh Chandran, Grant Theron†, Mahdad Noursadeghi†*

7 blood RNA signatures including Xpert MTB HR & CRP

PMID: 40675164

Accuracy of the Xpert MTB Host Response for EPTB

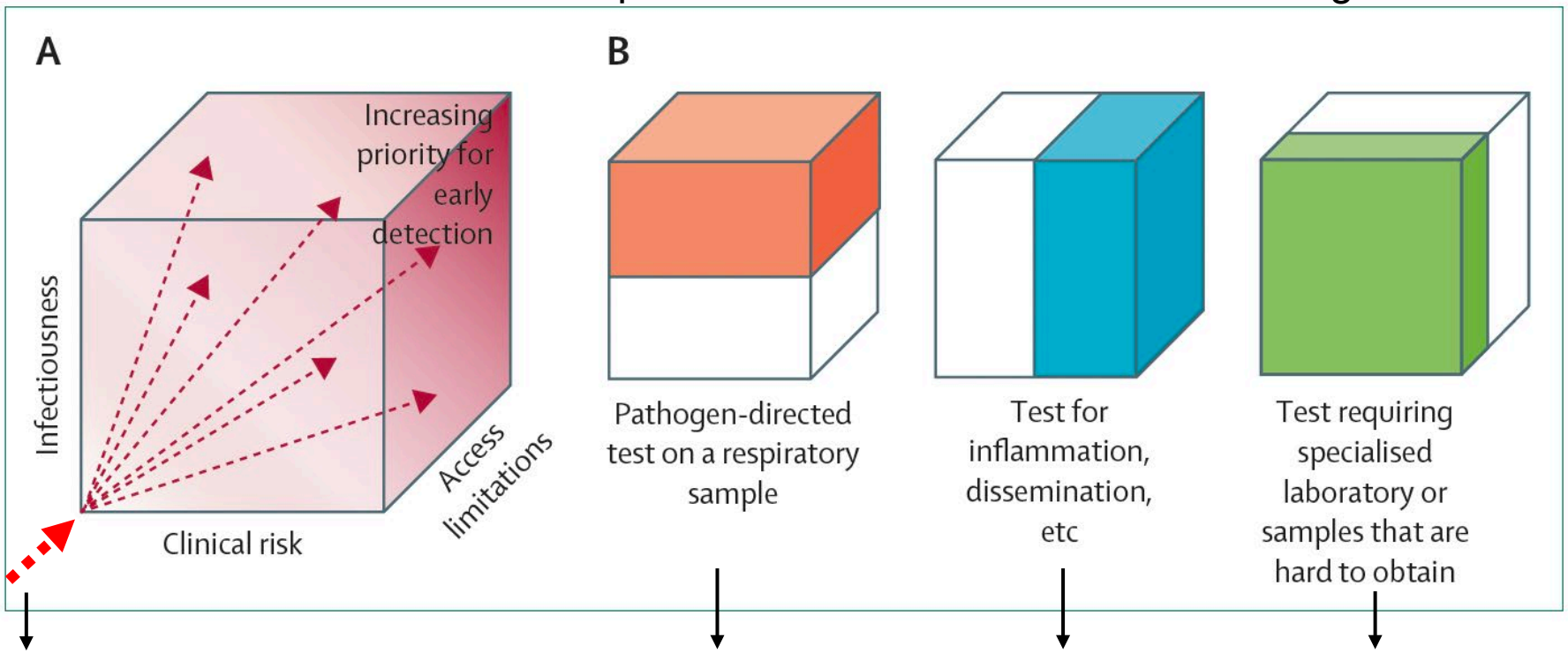
Test	Cutoff	Sensitivity	Specificity
BATF2	Sensitivity 90%	0.9 (0.85-0.93)	0.44 (0.37-0.51)
CRP	Sensitivity 90%	0.9 (0.85-0.93)	0.27 (0.21-0.34)
Gliddon3	Sensitivity 90%	0.9 (0.85-0.93)	0.39 (0.32-0.47)
Risk6	Sensitivity 90%	0.9 (0.85-0.93)	0.38 (0.31-0.45)
Roe3	Sensitivity 90%	0.9 (0.85-0.93)	0.52 (0.45-0.6)
Suliman4	Sensitivity 90%	0.9 (0.85-0.93)	0.27 (0.21-0.34)
Sweeney3	Sensitivity 90%	0.9 (0.85-0.93)	0.42 (0.35-0.5)
Darboe11	Sensitivity 90%	0.9 (0.85-0.93)	0.45 (0.37-0.52)

PMID: 40675164

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

<u>Pathogen-directed</u>
Non-sputum tests
Assisted microscopy

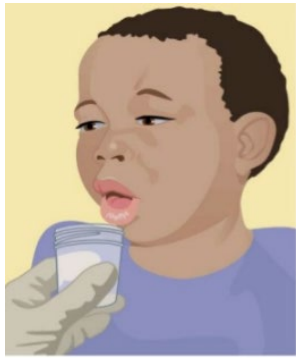
<u>Host-directed</u>
Host response

<u>Pathogen-directed</u>
LAM
cell-free DNA

PMID: 41115442

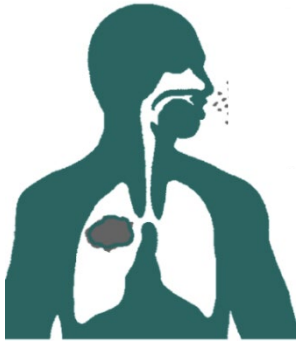
Target Population for cfDNA TB Diagnosis

Pediatric

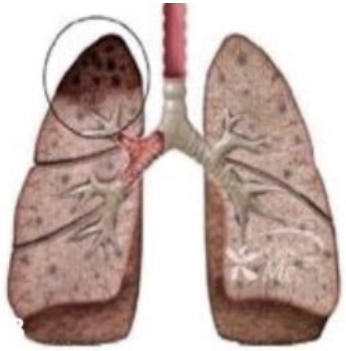


1 million

Unproductive

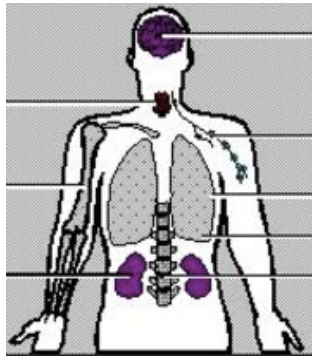


HIV/AIDS



0.9 million

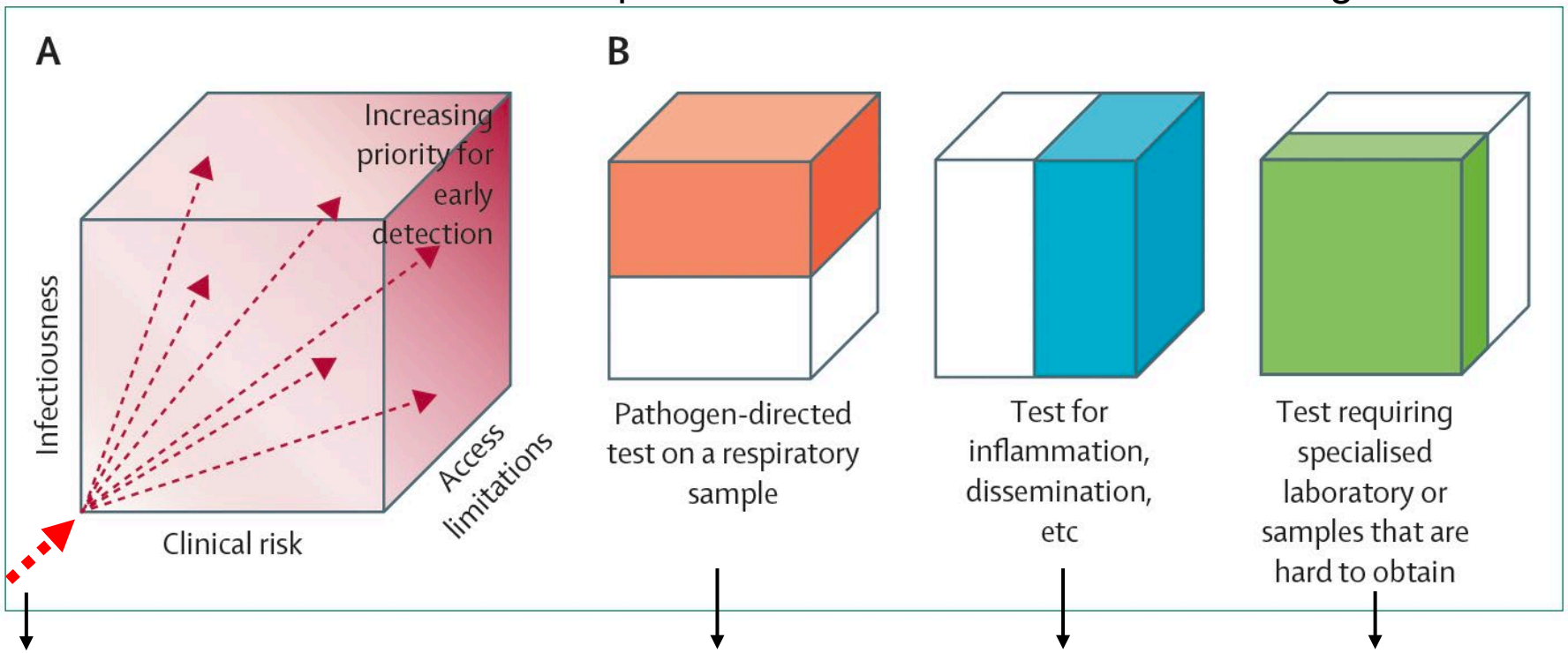
Extrapulmonary



Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

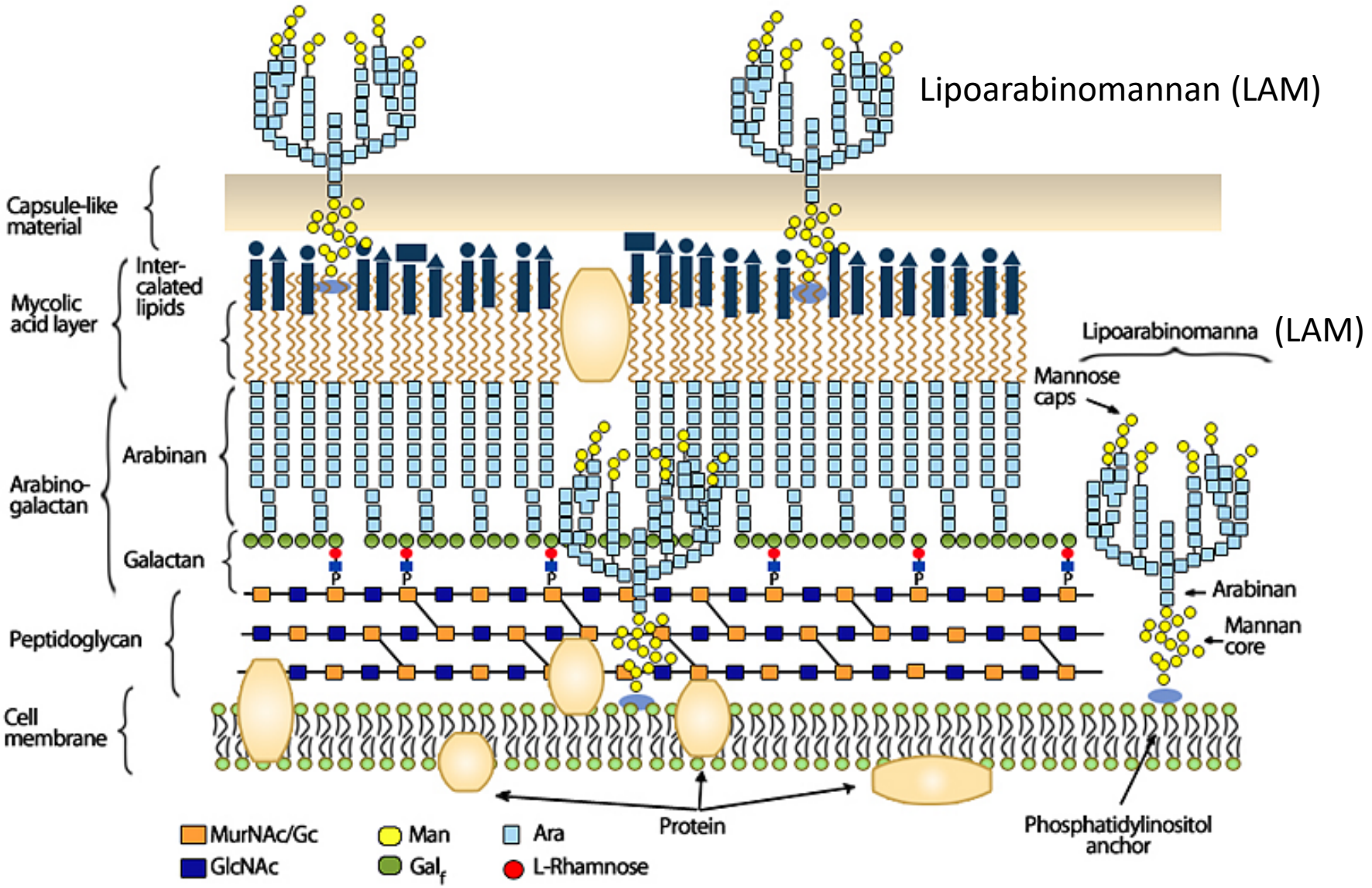
<u>Pathogen-directed</u>
Non-sputum tests
Assisted microscopy

<u>Host-directed</u>
Host response

<u>Pathogen-directed</u>
LAM
cell-free DNA

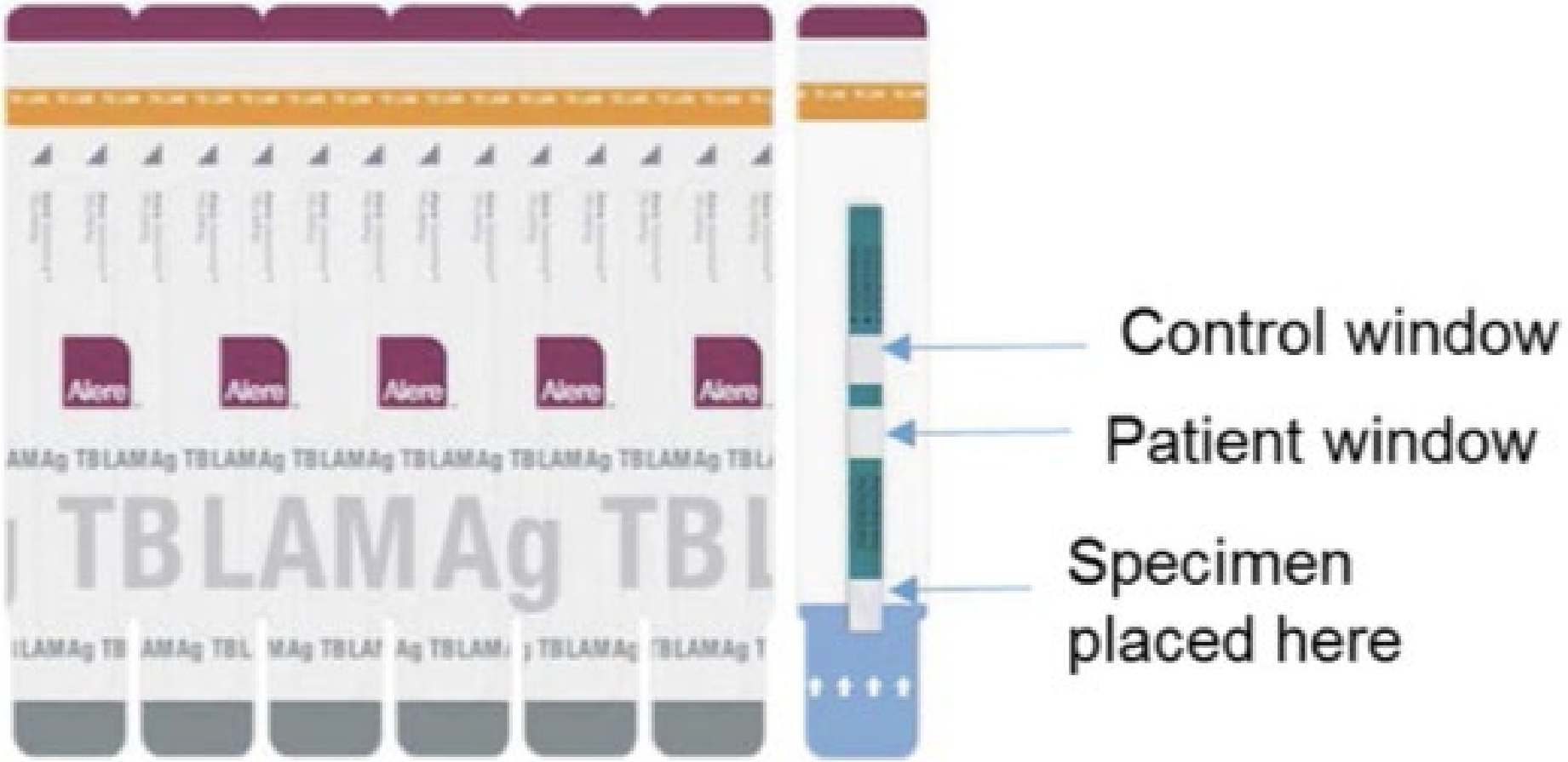
PMID: 41115442

Urinary LAM Detection with Lateral Flow



Urinary LAM Detection with Lateral Flow

Alere Determine TB LAM Ag (AlereLAM)



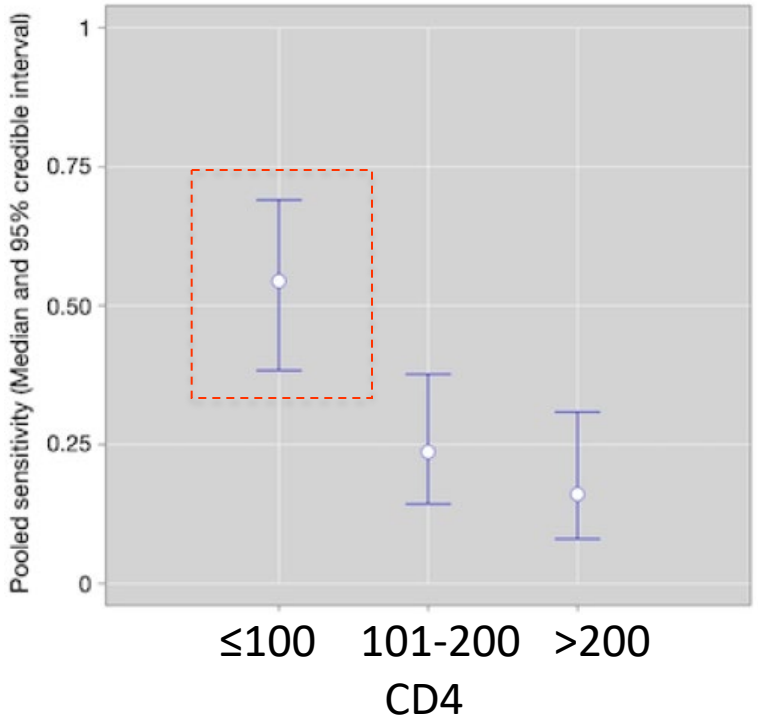
World Health Organization

TUBERCULOSIS DIAGNOSTICS

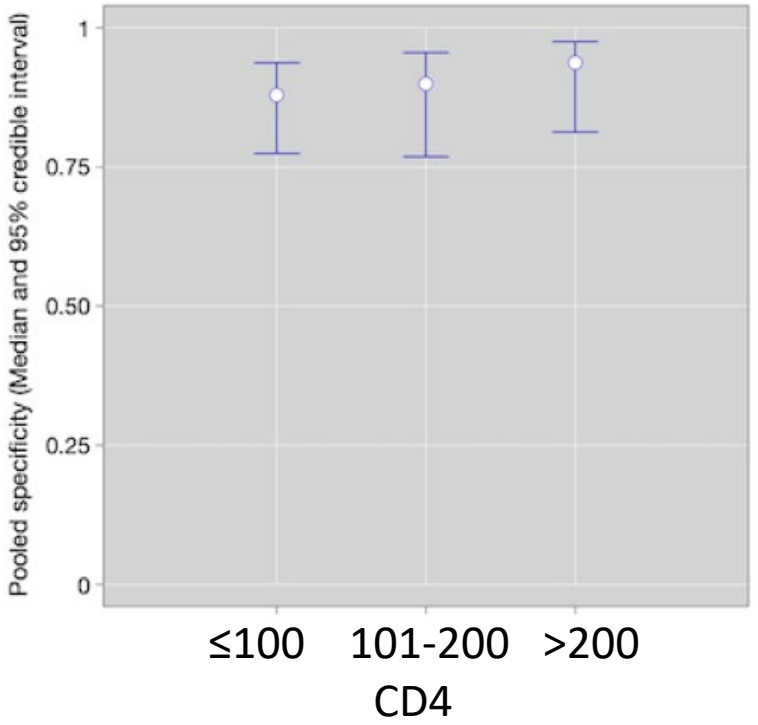
LATERAL FLOW URINE LIPOARABINOMANNAN ASSAY (LF-LAM)

FOR THE DIAGNOSIS AND SCREENING OF ACTIVE TUBERCULOSIS IN PEOPLE LIVING WITH HIV

Sensitivity

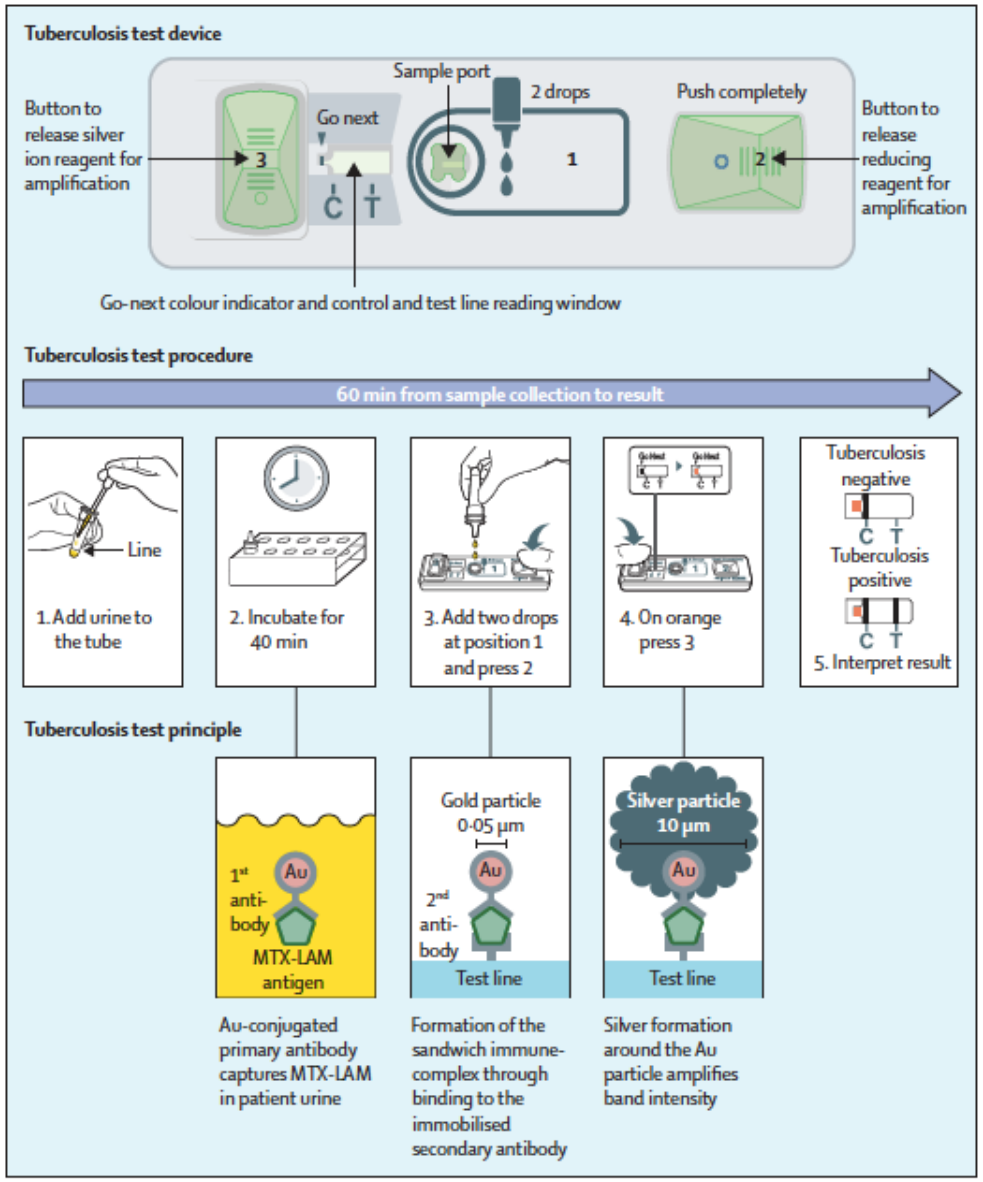


Specificity



PMID: 31633805

Novel Fujifilm SILVAMP TB LAM (FujiLAM)



Detects LAM at 30-fold lower concentrations using high affinity monoclonal antibodies and A signal amplification step

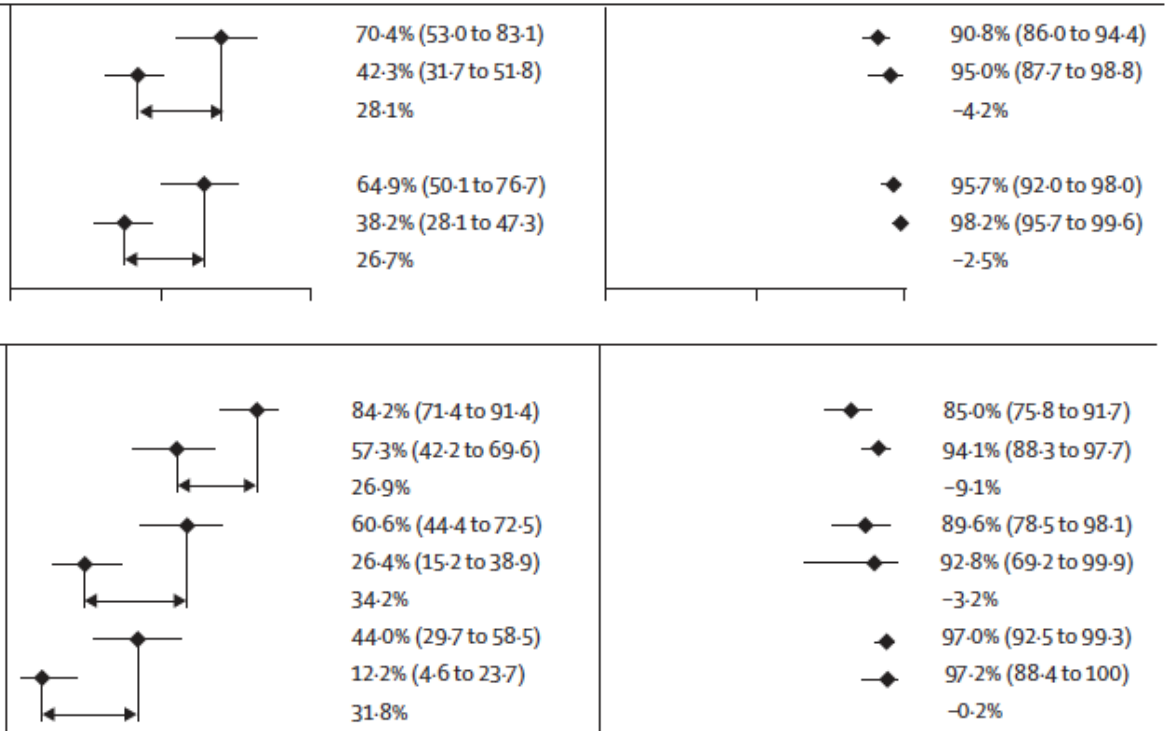
PMID: 31155318

Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study

Tobias Broger*, Bianca Sossen*, Elloise du Toit, Andrew D Kerkhoff, Charlotte Schutz, Elena Ivanova Reipold, Amy Ward, David A Barr, Aurélien Macé, Andre Trollip, Rosie Burton, Stefano Ongarello, Abraham Pinter, Todd L Lowary, Catharina Boehme, Mark P Nicol, Graeme Meintjes†, Claudia M Denkinger†

Inpatients with HIV

	Test	n	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
MRS	FujiLAM	968	455	33	145	335	70·4% (53·0 to 83·1)	90·8% (86·0 to 94·4)
	AlereLAM	968	268	18	332	350	42·3% (31·7 to 51·8)	95·0% (87·7 to 98·8)
	Difference						28·1%	−4·2%
CRS								
	FujiLAM	968	477	11	214	266	64·9% (50·1 to 76·7)	95·7% (92·0 to 98·0)
	AlereLAM	968	281	5	410	272	38·2% (28·1 to 47·3)	98·2% (95·7 to 99·6)
	Difference						26·7%	−2·5%
MRS								
0–100 cells per µL	FujiLAM	516	332	20	49	115	84·2% (71·4 to 91·4)	85·0% (75·8 to 91·7)
	AlereLAM	516	221	8	160	127	57·3% (42·2 to 69·6)	94·1% (88·3 to 97·7)
	Difference						26·9%	−9·1%
101–200 cells per µL	FujiLAM	216	83	9	49	75	60·6% (44·4 to 72·5)	89·6% (78·5 to 98·1)
	AlereLAM	216	35	7	97	77	26·4% (15·2 to 38·9)	92·8% (69·2 to 99·9)
	Difference						34·2%	−3·2%
>200 cells per µL	FujiLAM	231	37	4	46	144	44·0% (29·7 to 58·5)	97·0% (92·5 to 99·3)
	AlereLAM	231	10	3	73	145	12·2% (4·6 to 23·7)	97·2% (88·4 to 100)
	Difference						31·8%	−0·2%



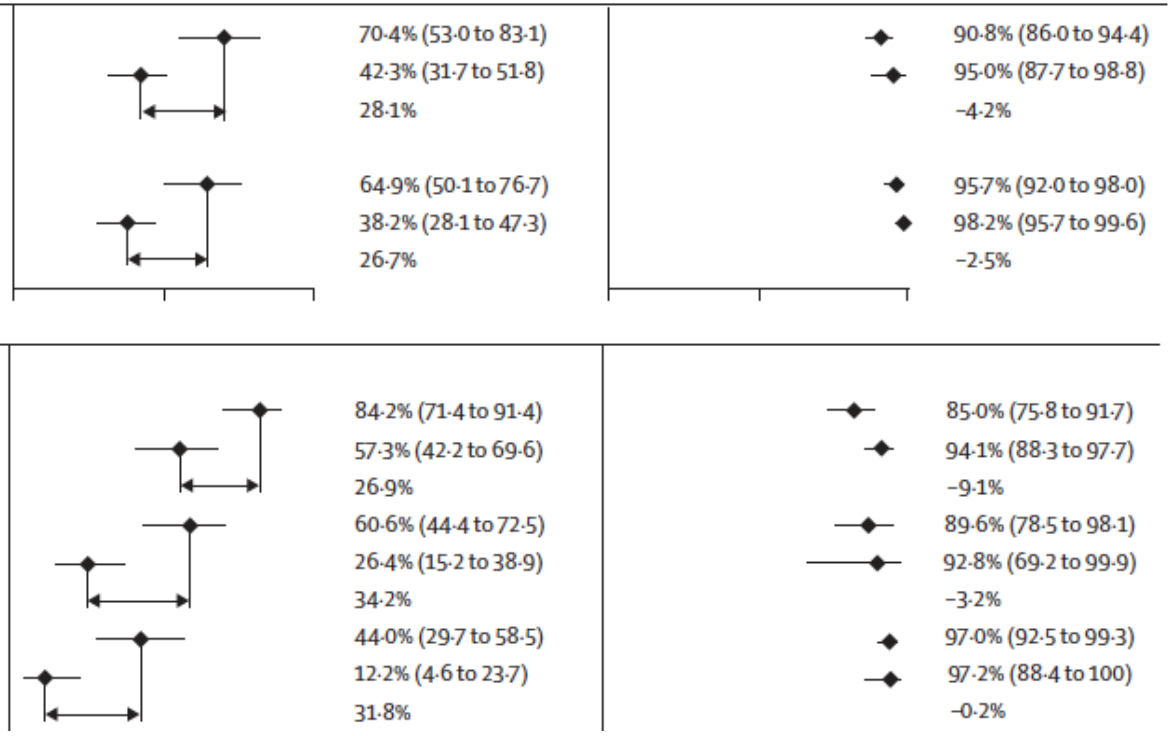
PMID: 31155318

Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study

Tobias Broger, Bianca Sossen*, Elloise du Toit, Andrew D Kerkhoff, Charlotte Schutz, Elena Ivanova Reipold, Amy Ward, David A Barr, Aurélien Macé, Andre Trollip, Rosie Burton, Stefano Ongarello, Abraham Pinter, Todd L Lowary, Catharina Boehme, Mark P Nicol, Graeme Meintjes†, Claudia M Denkinger†*

Inpatients with HIV

	Test	n	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
MRS	FujiLAM	968	455	33	145	335	70·4% (53·0 to 83·1)	90·8% (86·0 to 94·4)
	AlereLAM	968	268	18	332	350	42·3% (31·7 to 51·8)	95·0% (87·7 to 98·8)
	Difference						28·1%	−4·2%
CRS								
	FujiLAM	968	477	11	214	266	64·9% (50·1 to 76·7)	95·7% (92·0 to 98·0)
	AlereLAM	968	281	5	410	272	38·2% (28·1 to 47·3)	98·2% (95·7 to 99·6)
	Difference						26·7%	−2·5%
MRS								
0–100 cells per µL	FujiLAM	516	332	20	49	115	84·2% (71·4 to 91·4)	85·0% (75·8 to 91·7)
	AlereLAM	516	221	8	160	127	57·3% (42·2 to 69·6)	94·1% (88·3 to 97·7)
	Difference						26·9%	−9·1%
101–200 cells per µL	FujiLAM	216	83	9	49	75	60·6% (44·4 to 72·5)	89·6% (78·5 to 98·1)
	AlereLAM	216	35	7	97	77	26·4% (15·2 to 38·9)	92·8% (69·2 to 99·9)
	Difference						34·2%	−3·2%
>200 cells per µL	FujiLAM	231	37	4	46	144	44·0% (29·7 to 58·5)	97·0% (92·5 to 99·3)
	AlereLAM	231	10	3	73	145	12·2% (4·6 to 23·7)	97·2% (88·4 to 100)
	Difference						31·8%	−0·2%



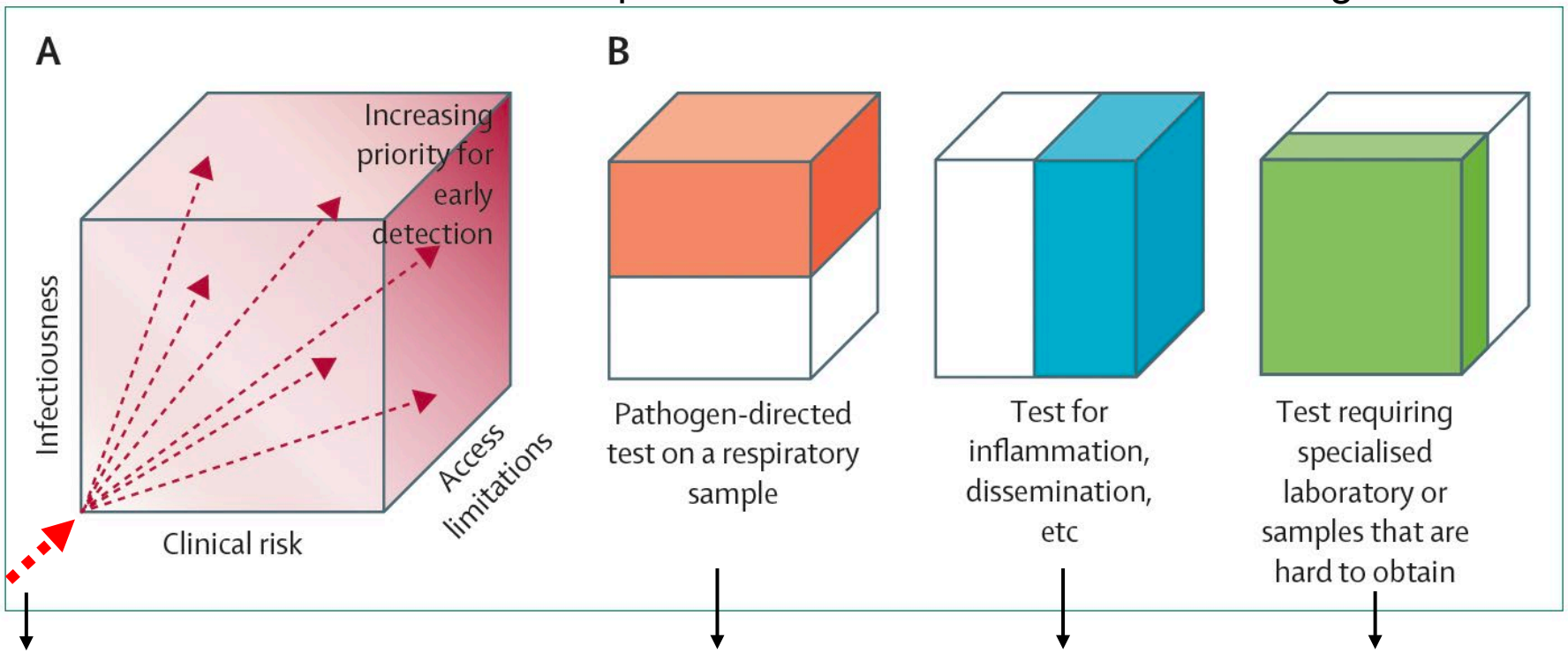
Commercialization halted due to lot to lot variability reducing performance

PMID: 31155318

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

<u>Pathogen-directed</u>
Non-sputum tests
Assisted microscopy

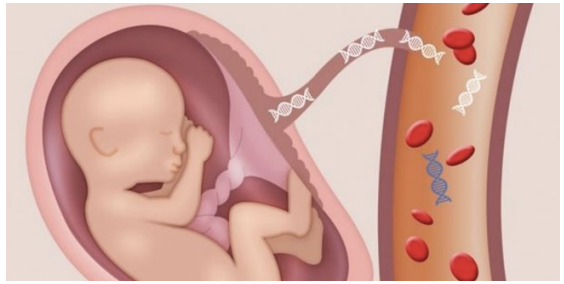
<u>Host-directed</u>
Host response

<u>Pathogen-directed</u>
LAM
cell-free DNA

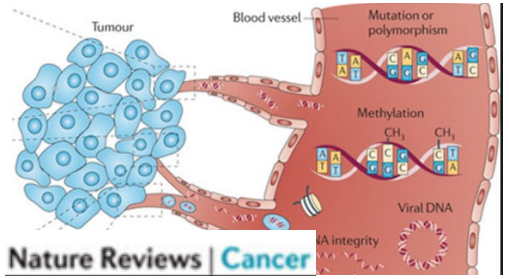
PMID: 41115442

Application of cf DNA in Diagnostics

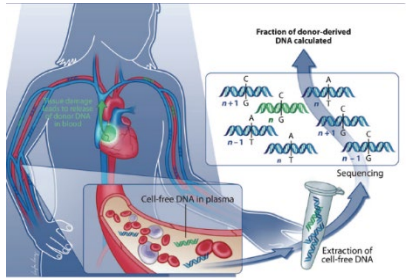
Fetal aneuploidy



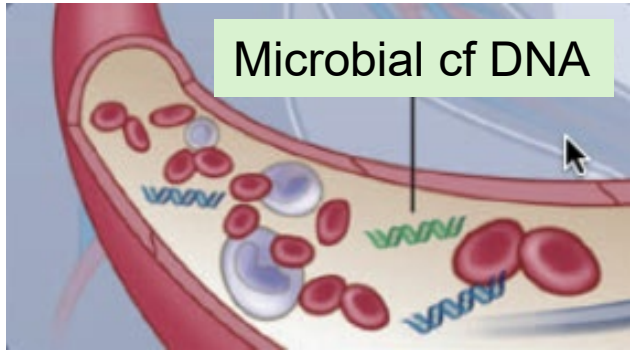
Cancer mutations



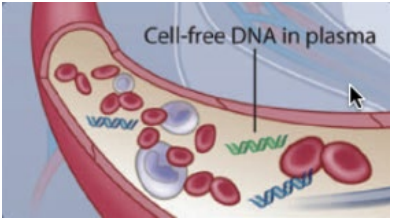
Organ rejection



Infectious diseases



- EBV cfDNA for nasopharyngeal CA
- Microbial mNGS for various infections
- Fungal cfDNA PCR for invasive fungal disease



Diagnostic Accuracy of the MTB cfDNA PCR

Publication first author	Yr	Sample type	TB presentation	HIV positive (%)	Smear positive (%)	Method of TB confirmation	% (no./total no. of samples) of indicating: Sensitivity	Specificity
Ushio	2016	Plasma	Pulmonary	0	100	Culture	65 (21/33)	93 (18/19)
							29 (10/33)	100 (19/19)
Click	2018	Plasma	Pulmonary	64	100	Culture and/or Xpert	45 (18/40)	67 (2/3)

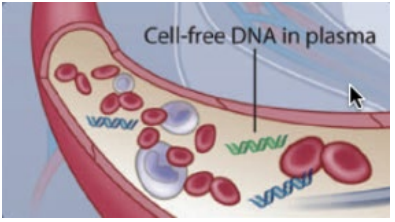
Bilateral vs Unilateral PTB

↑cfDNA

PTB+I vs. F

↑cfD

PMID: 30404942

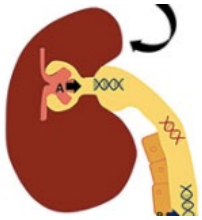


Diagnostic Accuracy of the MTB cfDNA PCR

Age	Country	TB Type	Cases	Controls	Method	Target	Sensitivity	Specificity
>18 yo	Brazil	PTB	49	69	PCR	GWiS	74% (34/49)	100% (69/69)
	USA	EPTB	8				75% (6/8)	
>18 yo	Vietenam & SA	PTB	15	15	PCR	GWiS	53% (8/15)	100% (15/15)
>18 yo	Uganda	PTB	54	40	PCR	IS6110	46% (25/54)	100% (40/40)

Day 14	Day 0+14
84%	94%

Unpublished data

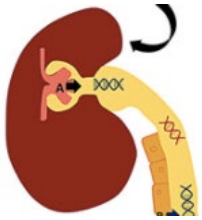


Diagnostic Accuracy of the MTB cfDNA PCR

Publication first author	Yr	Sample type	TB presentation	HIV positive (%)	Smear positive (%)	Method of TB confirmation	% (no./total no. of samples) of indicating: Sensitivity	Specificity
Cannas	2008	Urine	Pulmonary	5	95	Sputum smear or culture	79 (34/43)	100 (23/23)
Fortun	2014	Urine	Pulmonary	12	NR	Culture	18 (5/28)	NR
			Extrapulmonary	29	NR		70 (57/82)	NR
Labugger	2017	Urine	Pulmonary	0	60	Culture	64 (7/11)	100 (8/8)
Patel	2017	Urine	Pulmonary	38	33	Culture	43 (75/175)	89 (210/237)

Miliary	Multifo
90% (9/10)	67% (16/2
vs Radiology	vs Sm
↑cfDNA	No

PMID: 30404942



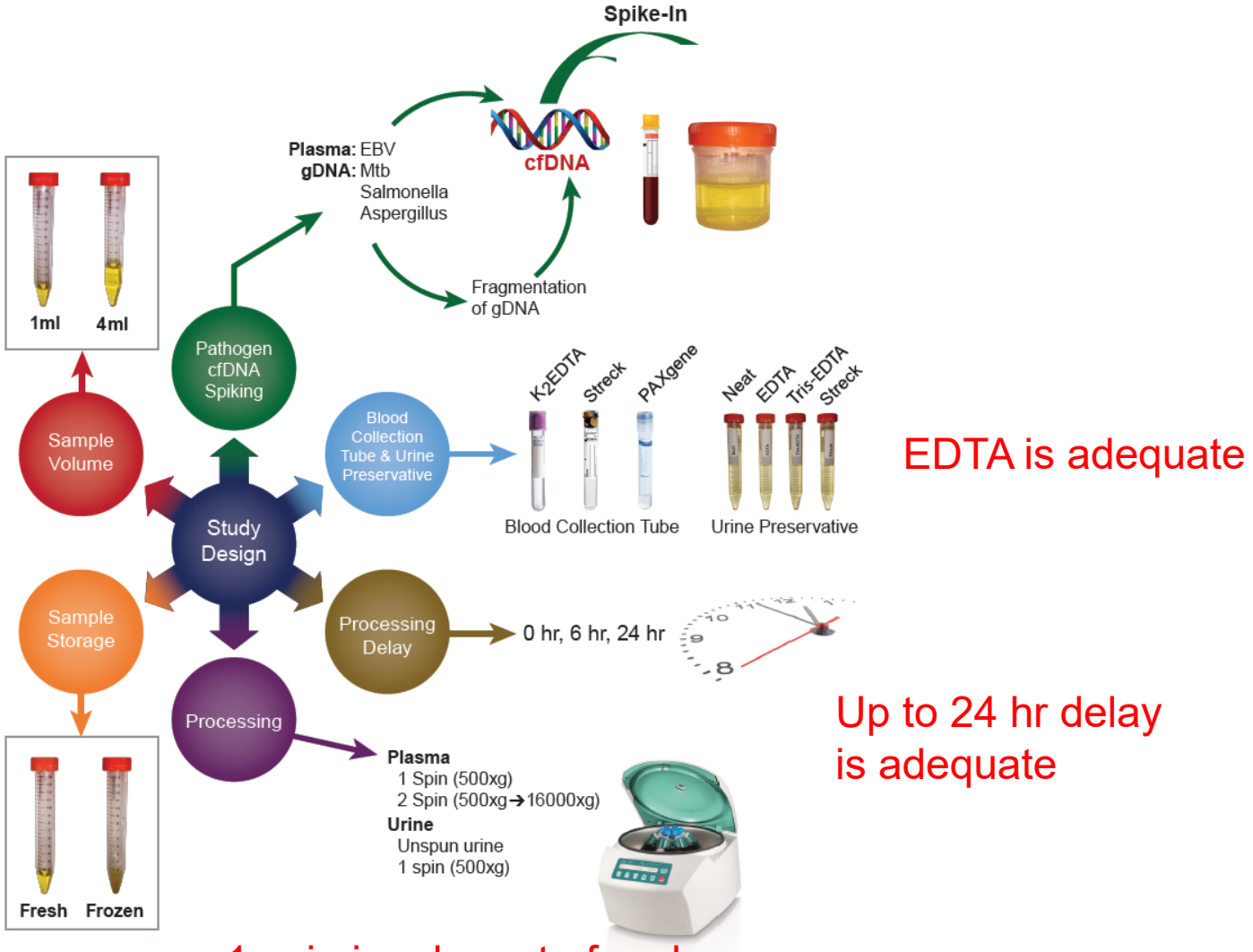
Diagnostic Accuracy of the MTB cfDNA PCR

Age	Country	TB Type	Cases	Controls	Method	Target	Sensitivity	Specificity
>18 yo	Uganda	PTB	75	59	PCR	IS6110	32% (24/75)	98% (58/59)

Unpublished data

Optimization of Preanalytical Variables Impacting Pathogen Plasma cfDNA Detection

Higher volume more sensitive



EDTA is adequate

Up to 24 hr delay is adequate

Freeze thaw has no impact

1 spin is adequate for plasma
No spin needed for urine

PMID:31511335

Bacterial and Fungal cfDNA PCR Tests Orderable

Mycobacterium tuberculosis complex PCR

Nocardia spp. PCR

Legionella spp. PCR

MOLD PCR Panel
1. *Aspergillus* species (*A. fumigatus, A. flavus, A. niger), A. terreus, A. ustus/nidulans*
2. Mucorales agents (*Rhizopus* spp., *Mucor* spp., *Rhizomucor* spp.)
3. *Fusarium* spp.
4. *Scedosporium* spp.
5. Lomentospora prolificans

Dimorphic Fungi PCR Panel
1. *Coccidioides immitis/ posadasii*
2. *Histoplasma capsulatum*
3. *Blastomyces dermatitidis*
4. *Sporothrix schenckii*

Candida PCR Panel
1. *C. albicans*
2. *C. glabrata*
3. *C. krusei*

Pneumocystis jirovecii PCR

Specimen types: Plasma, BAL/iSputum, tissue, sterile fluid

Fungal PCR Panel Workflow

Samples: Fresh EDTA plasma 4mL

Extraction: cfDNA from 4mL of plasma on Promega Maxwell® CSC Instrument
TAT: 1 ½ hours

NAAT: Multiplex PCR on Rotor-Gene
TAT: 3 hours

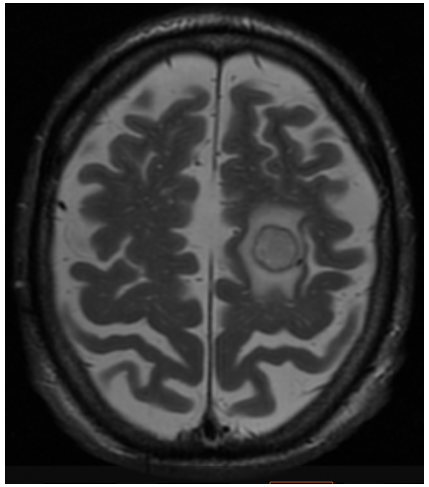
80♂ CLL with pulmonary and brain lesions

2/21 New ring-enhancing L frontal lobe brain lesions (etiology elusive despite brain bx at OSH).

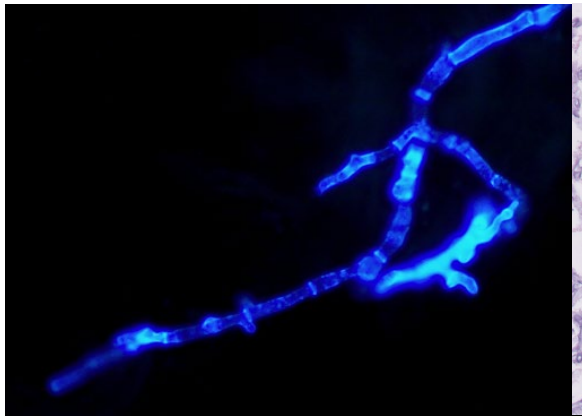
4/7 Referred to Stanford. Mold cfDNA PCR panel on plasma positive for *Aspergillus* species (*A. fumigatus, A. flavus, A. niger)*
Serum Galactomannan: negative
Serum BDG: positive 215 pg/mL

4/9 Started voriconazole

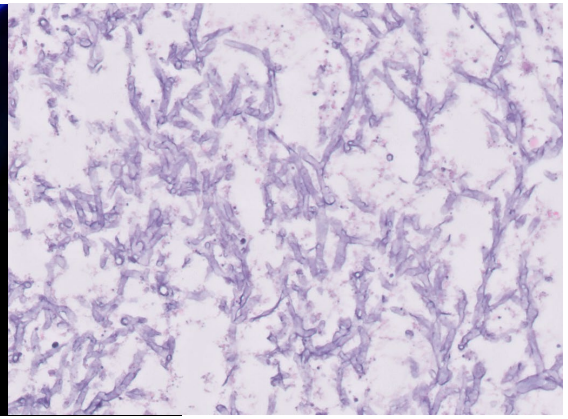
4/19 Brain biopsy performed. Fungal culture grew *Aspergillus fumigatus species complex*



Calcoflour white



H&E

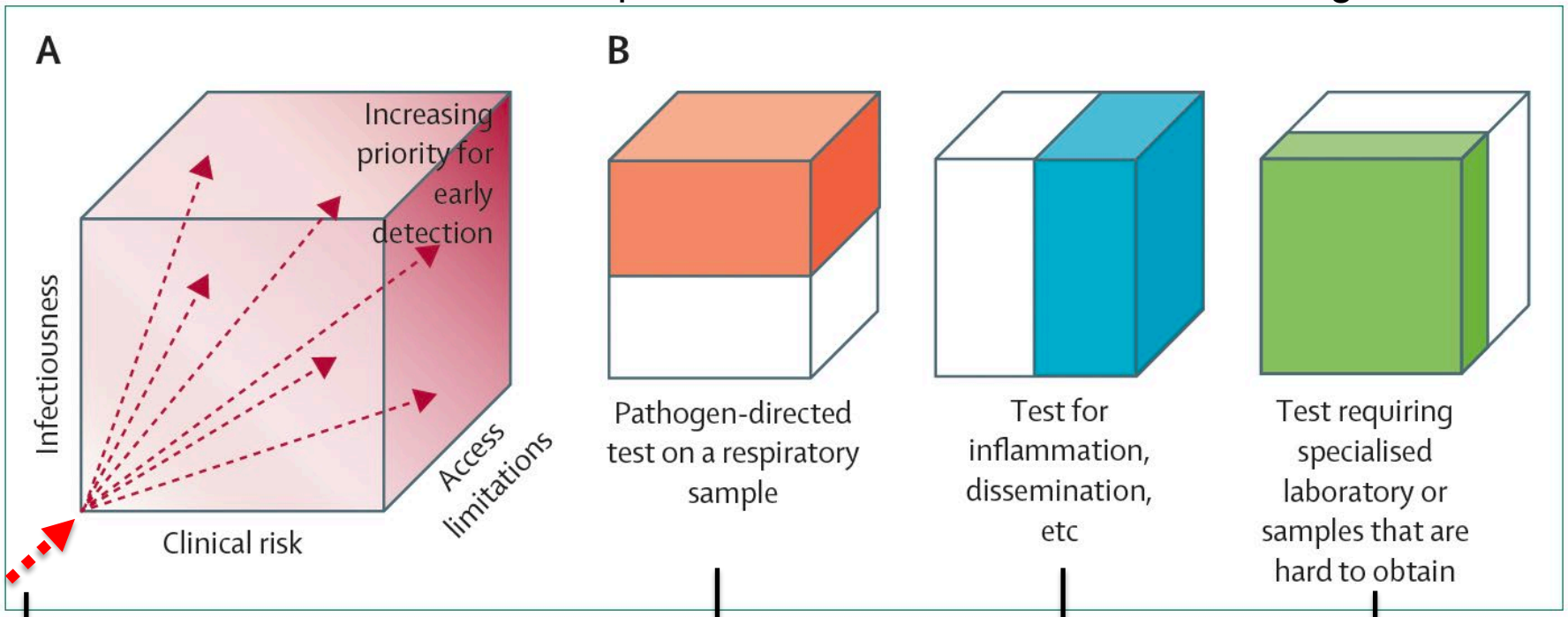


Diagnostic Accuracy of MTB Plasma cfDNA PCR

	Sensitivity % (n/N)	Specificity % (n/N)
Pulmonary overall	53.8 (28/52)	97.2 (208/214)
HIV positive (PTB)	63.6(14/22)	98.9 (88/89)
HIV negative (PTB)	46.7 (14/30)	96 (120/125)
Extrapulmonary overall	48.1 (26/54)	97.2 (208/214)
HIV positive (EPTB)	64.1 (25/39)	98.9 (88/89)
HIV negative (EPTB)	6.7 (1/15)	96 (120/125)

Unpublished data

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



Pathogen-directed or host-directed?

Pathogen-directed
Non-sputum tests
Assisted microscopy

Host-directed
Host response

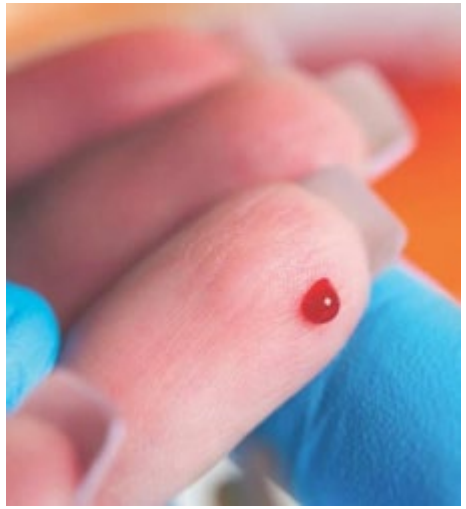
Pathogen-directed
LAM
cell-free DNA

2-Step Testing:
cfDNA First → Host Response on cfDNA-

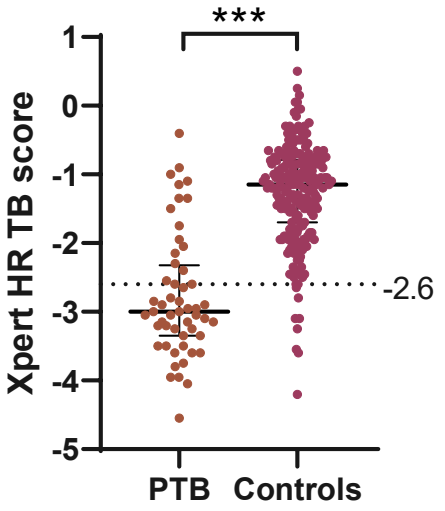
PMID: 41115442

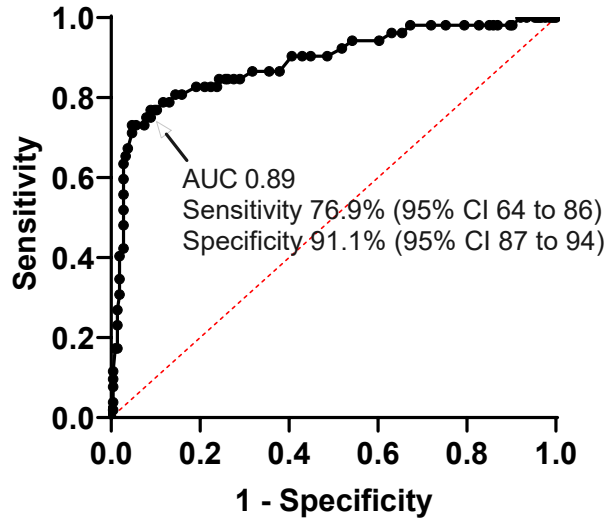
Evaluation of the Xpert MTB Host Response assay for the triage of patients with presumed pulmonary tuberculosis: a prospective diagnostic accuracy study in Viet Nam, India, the Philippines, Uganda, and South Africa

Ankur Gupta-Wright, Huy Ha*, Shima Abdulgadar, Rebecca Crowder, Jerusha Emmanuel, Job Mukwatamundu, Danaida Marcelo, Patrick P J Phillips, Devasahayam Jesudas Christopher, Nguyen Viet Nhung, Grant Theron, Charles Yu, Payam Nahid, Adithya Cattamanchi, William Worodria*, Claudia M Denkinger*, on behalf of the R2D2 TB Network†*



HR Cut-Off sets at >90% specificity





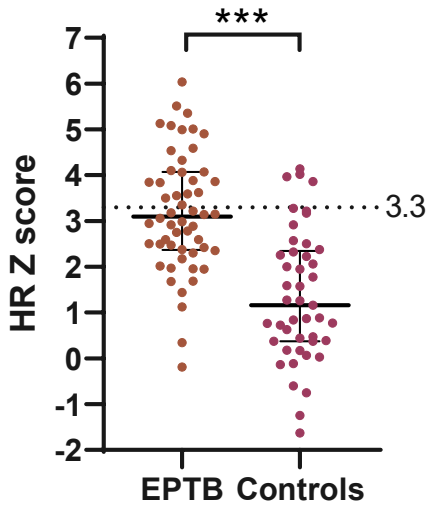
PMID: 38245113

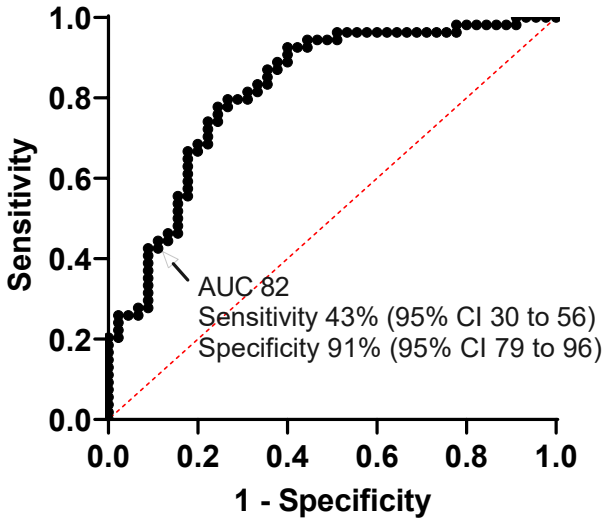
Blood RNA biomarkers and C-reactive protein for triage of adult patients with tuberculosis lymphadenitis and pericarditis in South Africa: a single-centre, prospective, observational, diagnostic accuracy study

Tiffeney Mann, Stephanie Minnies*, Rishi K Gupta*, Byron W P Reeve, Georgina Nyawo, Zaida Palmer, Charissa Naidoo, Anton Doubell, Alfonso Pecoraro, Thadathilankal-Jess John, Pawel Schubert, Claire J Calderwood, Aneesh Chandran, Grant Theron†, Mahdad Noursadeghi†*

Sweeney 3 signature

HR Cut-Off sets at >90% specificity





PMID: 40675164

Diagnostic Accuracy of 2-step Testing: MTB cfDNA → Xpert MTB Host Response

	Sensitivity % (n/N)			Specificity % (n/N)		
	Pathogen cell-free DNA (pcfDNA)	Host response (HR)	2-step test: pcfDNA→HR	Pathogen cell-free DNA (pcfDNA)	Host response (HR)	2-step test: pcfDNA→HR
Pulmonary overall	53.8 (28/52)	71.2 (37/52)	80.8 (42/52)	97.2 (208/214)	95.3 (204/214)	93.5 (200/214)
HIV positive (PTB)	63.6(14/22)	68.2 (15/22)	77.3 (17/22)	98.9 (88/89)	86.5 (77/89)	85.4 (76/89)
HIV negative (PTB)	46.7 (14/30)	73.3 (22/30)	83.3 (25/30)	96 (120/125)	94.4 (118/125)	92 (115/125)
Extrapulmonary overall	48.1 (26/54)	42.6 (23/54)	68.5 (37/54)	97.2 (208/214)	95.3 (204/214)	93.5 (200/214)
HIV positive (EPTB)	64.1 (25/39)	41 (16/39)	76.9 (30/39)	98.9 (88/89)	86.5 (77/89)	85.4 (76/89)
HIV negative (EPTB)	6.7 (1/15)	46.7 (7/15)	46.7 (7/15)	96 (120/125)	94.4 (118/125)	92 (115/125)

Unpublished data

Diagnostic Accuracy of 2-step Testing: MTB cfDNA → Xpert MTB Host Response

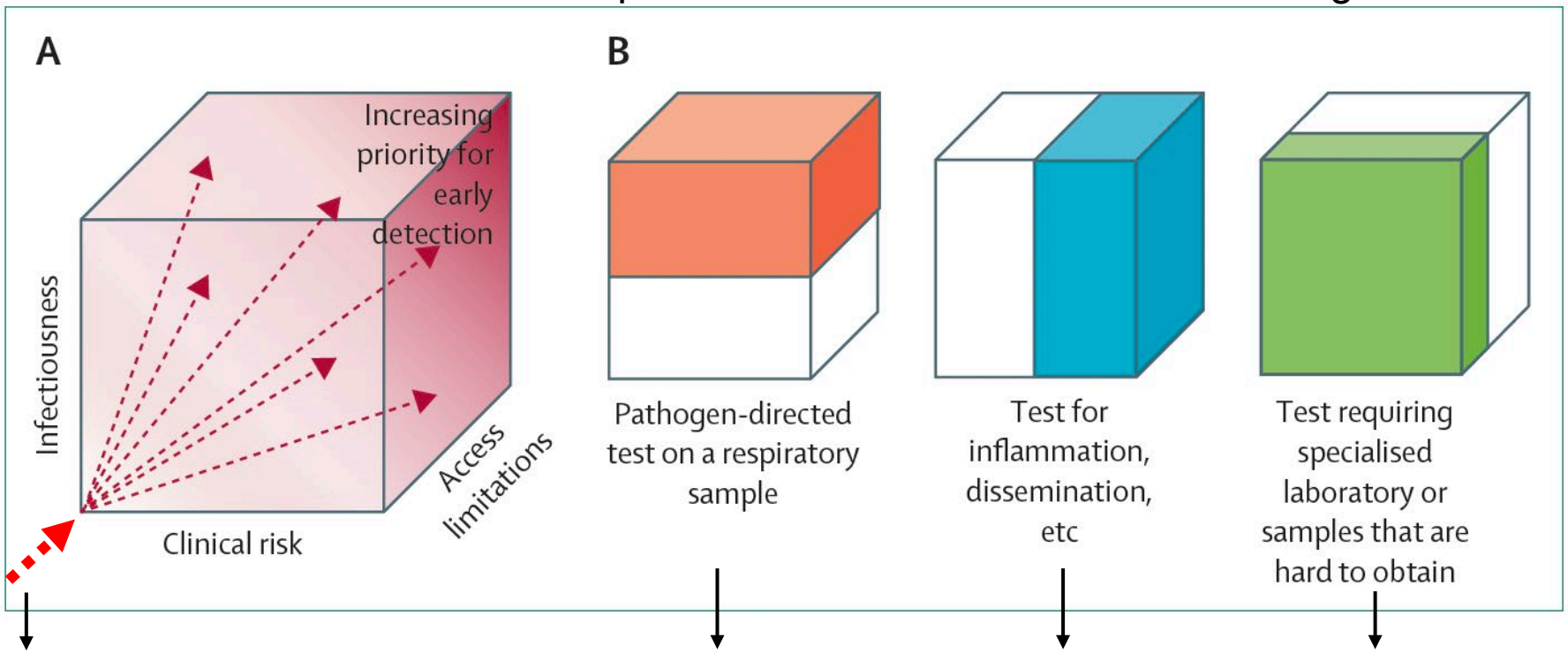
	Sensitivity % (n/N)			Specificity % (n/N)		
	Pathogen cell-free DNA (pcfDNA)	Host response (HR)	2-step test: pcfDNA → HR	Pathogen cell-free DNA (pcfDNA)	Host response (HR)	2-step test: pcfDNA → HR
Pulmonary overall	53.8 (28/52)	71.2 (37/52)	80.8 (42/52)	97.2 (208/214)	95.3 (204/214)	93.5 (200/214)
HIV positive (PTB)	63.6 (14/22)	68.2 (15/22)	77.3 (17/22)	98.9 (88/89)	86.5 (77/89)	85.4 (76/89)
HIV negative (PTB)	46.7 (14/30)	73.3 (22/30)	83.3 (25/30)	96 (120/125)	94.4 (118/125)	92 (115/125)
Extrapulmonary overall	48.1 (26/54)	42.6 (23/54)	68.5 (37/54)	97.2 (208/214)	95.3 (204/214)	93.5 (200/214)
HIV positive (EPTB)	64.1 (25/39)	41 (16/39)	76.9 (30/39)	98.9 (88/89)	86.5 (77/89)	85.4 (76/89)
HIV negative (EPTB)	6.7 (1/15)	46.7 (7/15)	46.7 (7/15)	96 (120/125)	94.4 (118/125)	92 (115/125)

Unpublished data

Whom tuberculosis tests detect and why it matters: implications for diagnostic algorithms

Emily A Kendall, Claudia M Denkinger, Adithya Cattamanchi, David W Dowdy, Jason R Andrews**

Dimensions of the tuberculosis spectrum and their interactions with diagnostic or screening tests



<u>Pathogen-directed or host-directed?</u>

<u>Pathogen-directed</u>
Non-sputum tests
Assisted microscopy

<u>Host-directed</u>
Host response

<u>Pathogen-directed</u>
LAM
cell-free DNA

PMID: 41115442

Acknowledgements

Stanford University

Kanagavel Murugesan
Karolina Bochenska
Fiona Senchyna
Catherine Hogan
Indre Budvytiene
Angel Moreno
Jordan Mah
Anthony Lieu
Tong Wang
Bosung Park

Broad Institute

Deborah Hung
Sharon Wong

UCSF/Uganda

Adithya Cattamanchi

Stellenbosch University

Grant Theron

Funding

Stanford ITI, BMGF, Stanford Global Health, ChEM-H, Global Good/IVL, NIH FEND for TB

Automated Detection of AFB in Kinyoun Smears: A Proof of Concept

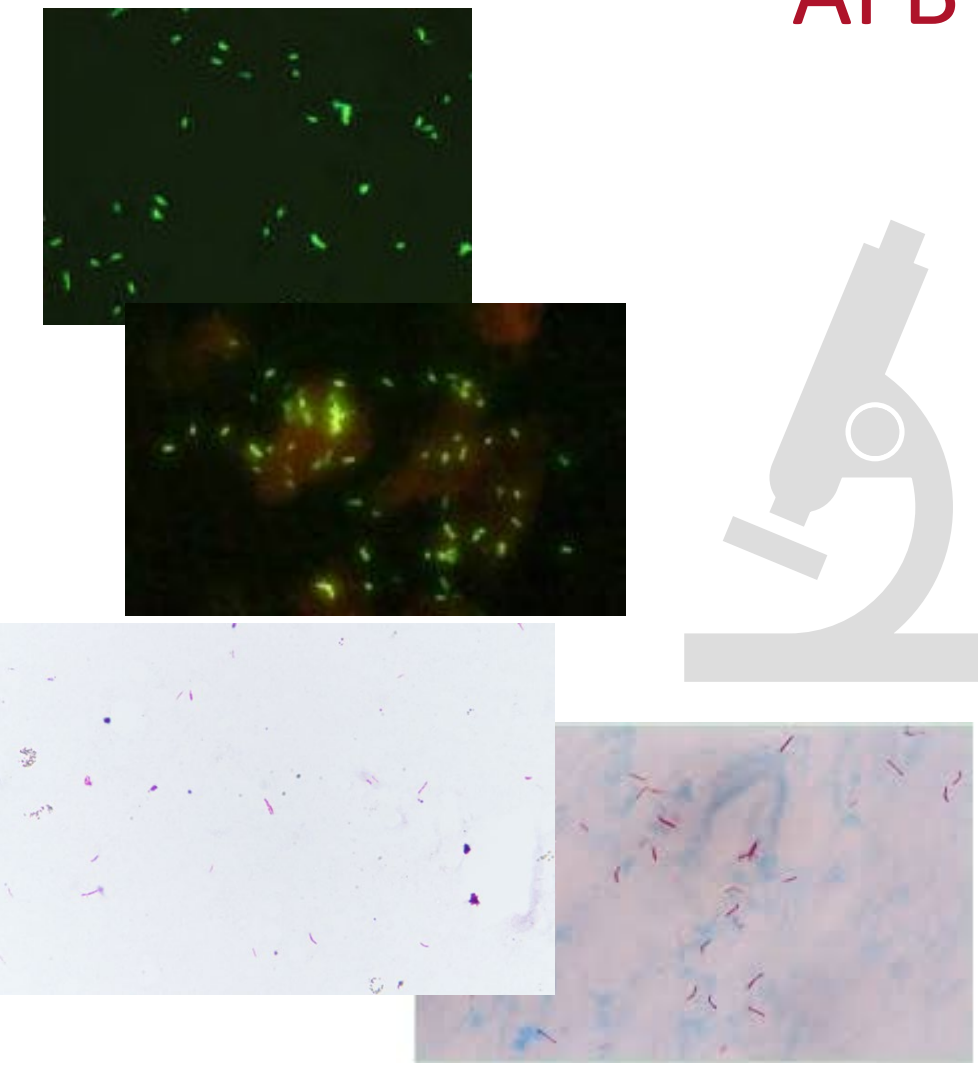
Salika Shakir PhD, D(ABMM)

University of Utah/ ARUP Laboratories

Objectives

- Review AFB workflow in a reference clinical laboratory
- Explain how artificial intelligence and machine learning are being applied to AFB testing and result interpretation
- Discuss potential benefits, limitations, and implementation considerations

AFB smear microscopy



- Cheap, easy to use
- Critical in guiding diagnosis
- Manual and subjective
- Accuracy is dependent on sample preparation
- Interpretation of results

MIC.32100 Fluorochrome Stain — Phase II

Fluorochrome staining is performed on mycobacterial smears prepared from primary respiratory specimens, either in the laboratory or by the referral laboratory.

NOTE: Such smears are easier to read than those stained with a conventional carbol-fuchsin based stain. Fluorescing organisms stand out prominently against the background of the smear, and the smears can be examined at a lower power than conventionally-stained smears, so that a larger amount of material can be examined in a given period of time. As with the interpretation of Ziehl-Neelsen- and Kinyoun-stained smears, expertise is needed for interpretation of smears stained with a fluorescent stain; not everything that fluoresces in such a stain is necessarily a mycobacterium. Particularly when only a few organism-like structures are seen, it is important to pay careful attention to their morphology before interpreting them as mycobacteria.

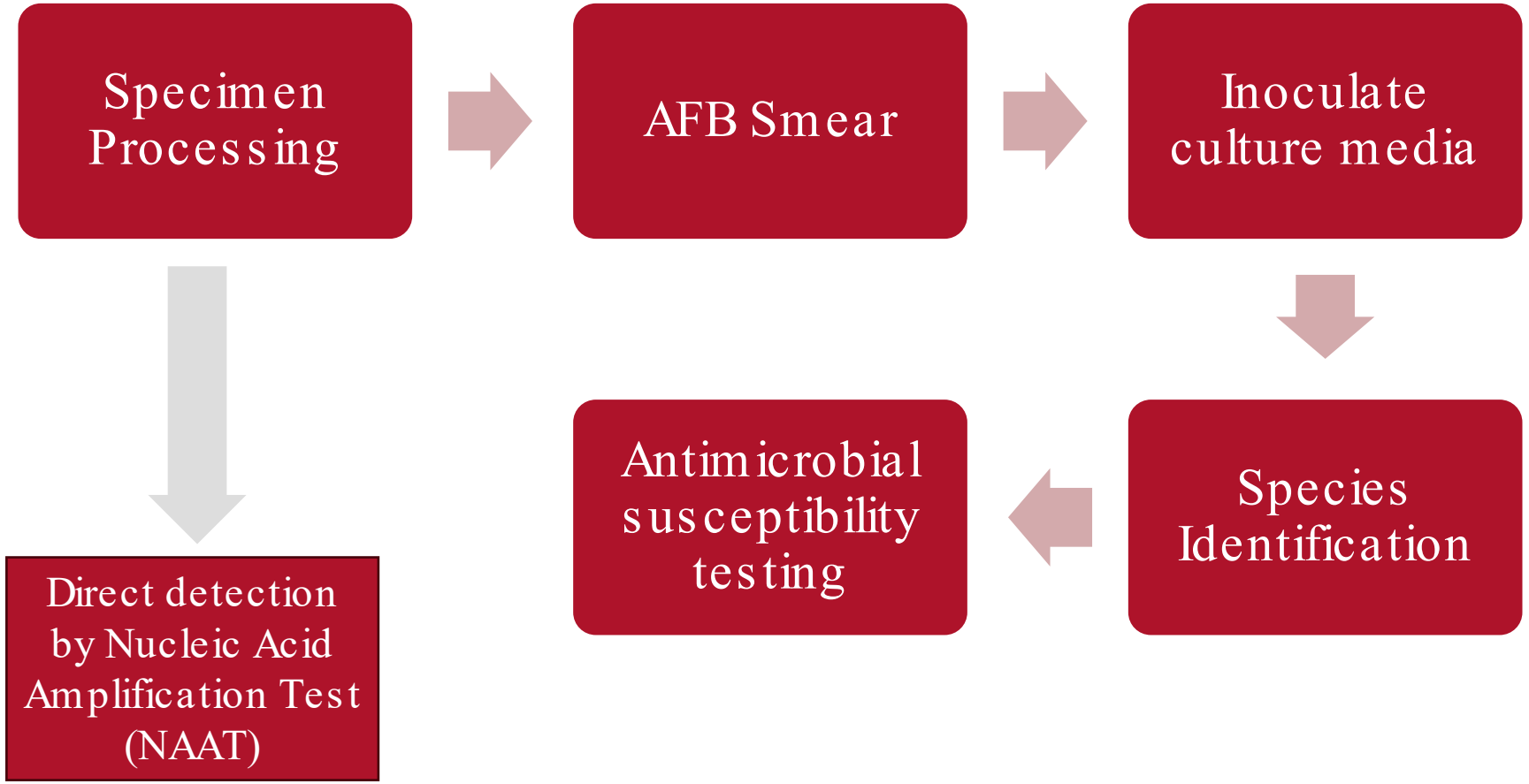
MIC.31200 Acid Fast Stain Results — Phase I

When clinically indicated, results of acid-fast stains are reported within 24 hours of specimen receipt by the testing laboratory.

Evidence of Compliance:

- ✓ Patient reports for acid-fast stain results

Mycobacterium laboratory workflow

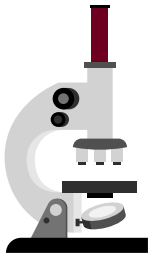


Mycobacterium Laboratory Workflow: Smear Reporting

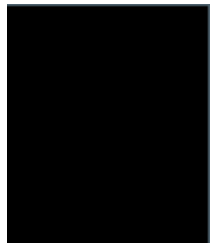
Process specimen
Decontaminate/Concentrate

AO smear preparation
(automated)

Smear manually read
(~2-4 min)



AFB Smear
Negative



Initial Smear Reported

>95% of smears are negative by AO

AFB Smear
Positive

Repeat Smear and
Report

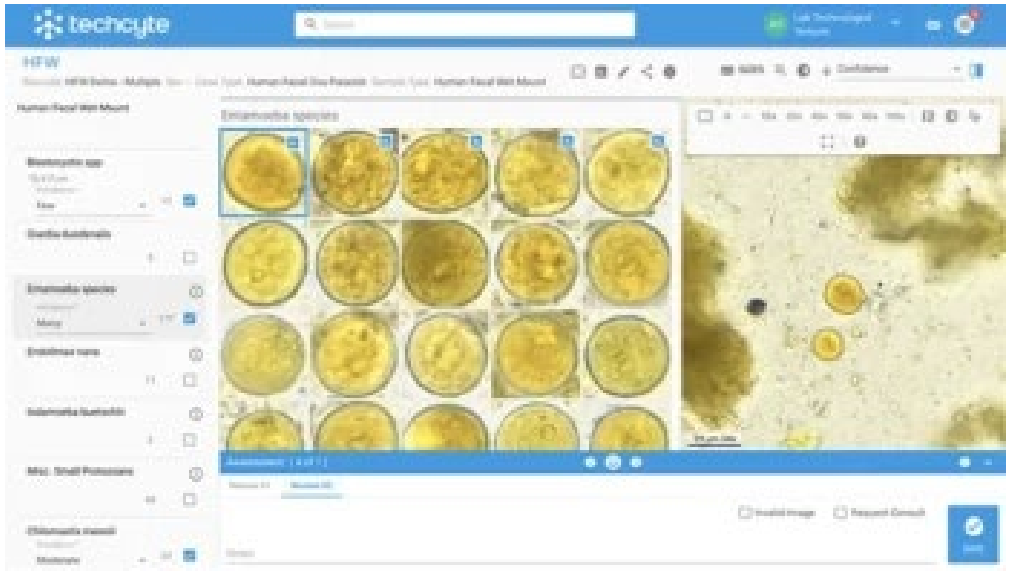
~200 samples daily
28 AO slides/batch

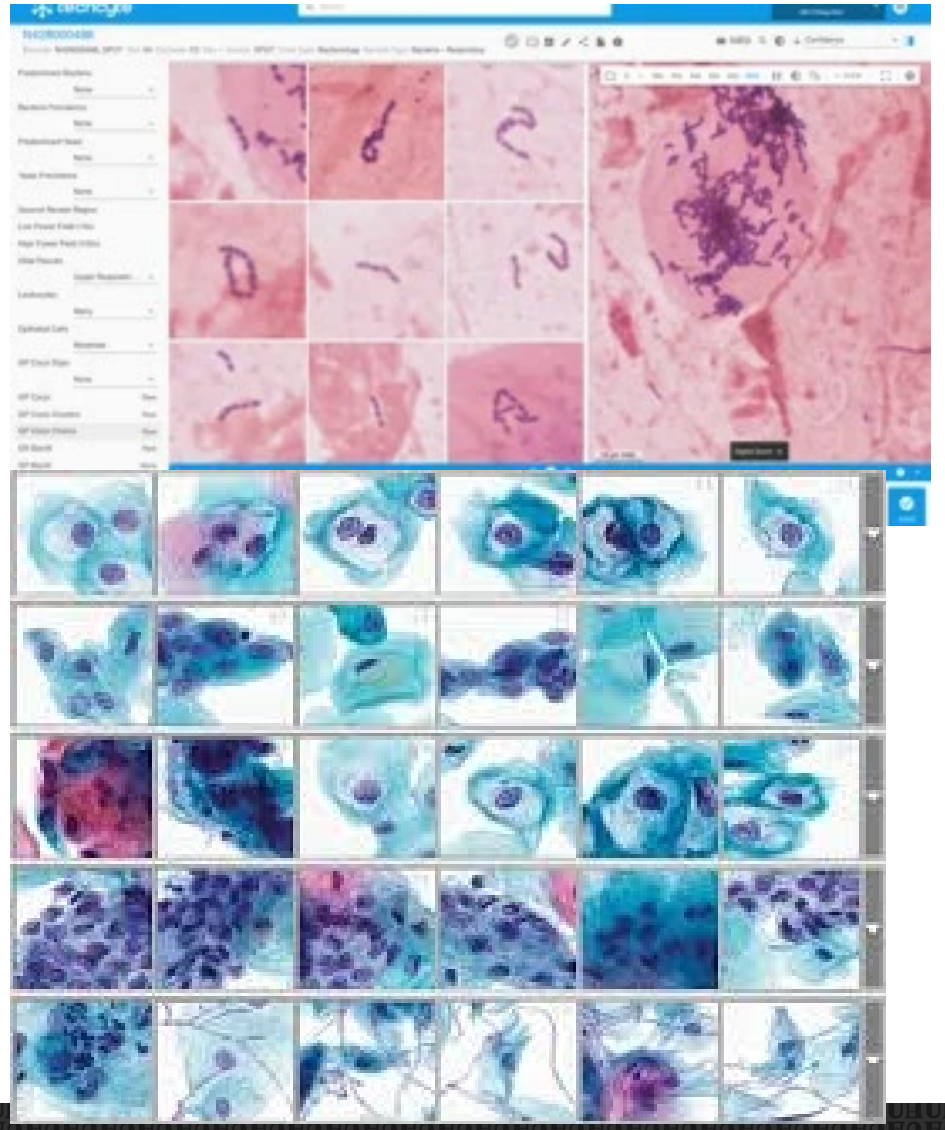
Each processor ~52 samples
60-90 minutes
6-8 runs on busy days

Could we use AI and machine learning as a screening tool?

- Detect negatives
- Improve turn-around-time
- Avoid manual review for low prevalence population
- Improve ergonomics

Use of AI in Clinical Microbiology





- Ova and Parasite Detection
- Gram stain
- Cervical PAP

https://techcyte.com/products/fusion-parasitology-suite/
https://techcyte.com/products/fusion-bacteriology-suite/
https://www.hologic.com/hologic-products/cytology/genius-cervical-ai

AI and machine learning for AFB smears

> Sci Rep. 2018 Jul 27;8(1):11308. doi: 10.1038/s41598-018-29660-8.

Automatic microscopic detection of mycobacteria in sputum: a proof-of-concept

D Zingue [1], P Weber [1], F Soltani [1], D Raoult [1], M Drancourt [2]

> Sensors (Basel). 2022 Nov 4;22(21):8497. doi: 10.3390/s22218497.

Evaluation of an AI-Based TB AFB Smear Screening System for Laboratory Diagnosis on Routine Practice

Hsiao-Ting Fu [1 2], Hui-Zin Tu [3], Herng-Sheng Lee [3], Yusen Eason Lin [4], Che-Wei Lin [2 5 6 7]

Affiliations + expand

PMID: 36366194 PMCID: PMC9657727 DOI: 10.3390/s22218497

> Diagnostics (Basel). 2022 Jun 17;12(6):1484. doi: 10.3390/diagnostics12061484.

A New Artificial Intelligence-Based Method for Identifying Mycobacterium Tuberculosis in Ziehl-Neelsen Stain on Tissue

> Microorganisms. 2024 Aug 22;12(8):1734. doi: 10.3390/microorganisms12081734.

Pulmonary Tuberculosis Diagnosis Using an Intelligent Microscopy Scanner and Image Recognition Model for Improved Acid-Fast Bacilli Detection in Smears

Wei-Chuan Chen [1 2], Chi-Chuan Chang [1 3], Yusen Eason Lin [3]

Detection of AFB in Auromine–O-stained respiratory samples

▸ J Clin Microbiol. 2024 Feb 1;62(3):e01069-23. doi: 10.1128/jcm.01069-23

Retrospective validation of MetaSystems' deep-learning-based digital microscopy platform with assistance compared to manual fluorescence microscopy for detection of mycobacteria

Claudine Desruisseaux [1,2], Conor Broderick [2], Valéry Lavergne [1,2], Kim Sy [1], Duang-Jai Garcia [1], Gaurav Barot [1], Kerstin Locher [1,2], Charlene Porter [1], Mélissa Caza [2], Marthe K Charles [1,2,✉]

- Sensitivity 90.7% vs 92% comparable to manual fluorescence microscopy
- Specificity: 91.9% vs 95.7%
  - » Statistically significant decrease in specificity (−3.8% vs MM)
- Scan failure rate (10.6%)
- AFB grading low performance
  - » Overall grading agreement: 74.8%
- Software performance varies by mycobacterial species

AI AFB smear Implementation Considerations

Assess

Identify workflows benefitting from AI

Build

Build Digital Microscopy Infrastructure

Select and Validate

Select and Validate the AI System

Ensure diagnostic performance

Integrate

Integrate AI into Routine Workflow

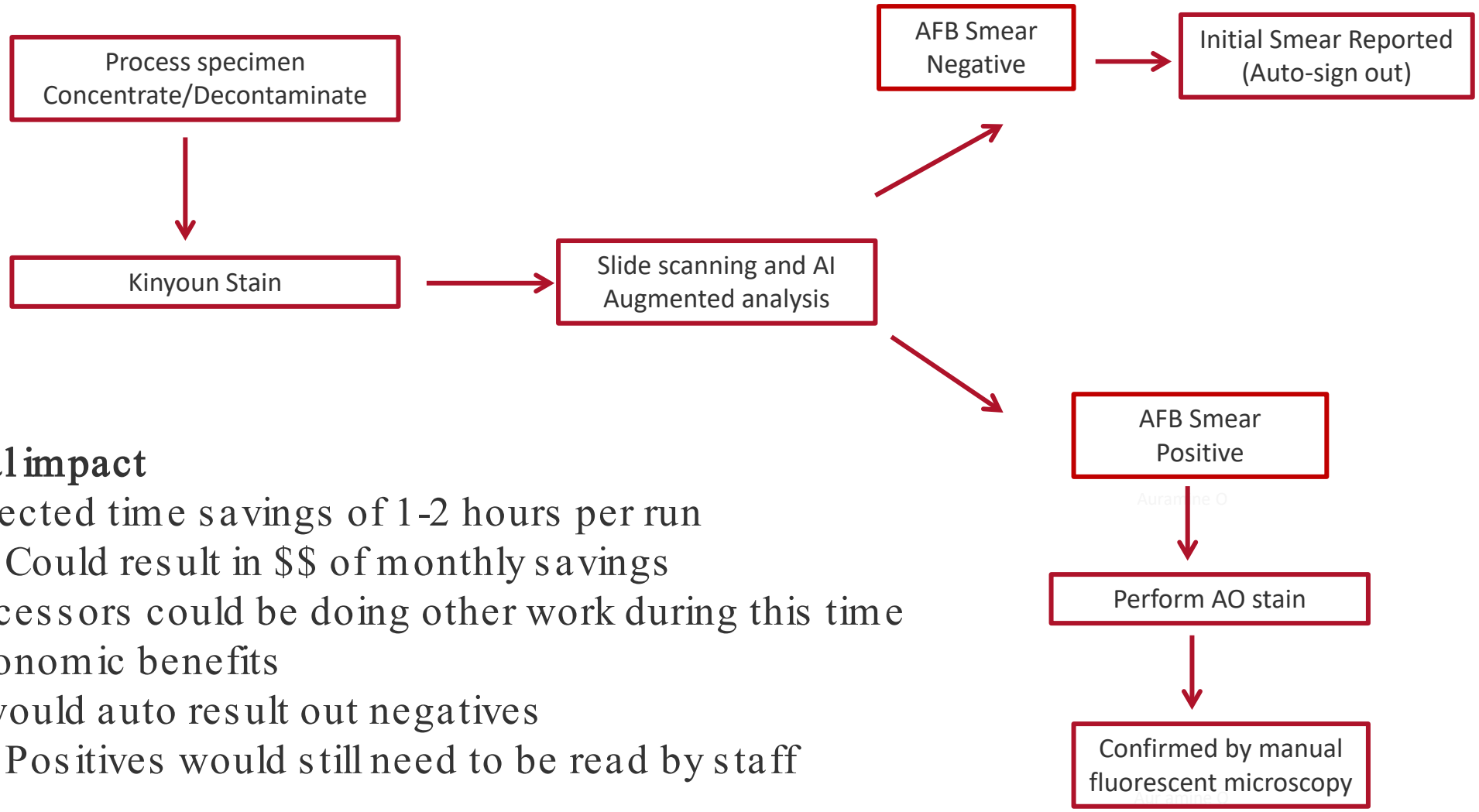
Standardize

Standardize QC, Training

Scale

Scale, Monitor Outcomes, and Expand Use Cases

AI-enabled Mycobacterium Laboratory Workflow



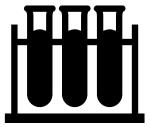
- **Potential impact**
  - Expected time savings of 1-2 hours per run
    - Could result in $$ of monthly savings
  - Processors could be doing other work during this time
  - Ergonomic benefits
  - AI would auto result out negatives
    - Positives would still need to be read by staff

Study objective

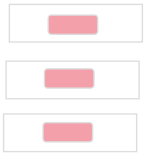
- Evaluate Kinyoun stain AI scanning with AO-stained manual smear microscopy

- Using direct clinical specimens
  - » Sputum, BAL, Tissue, body fluids, wound

Development of an AI-enabled Mycobacterium detection tool

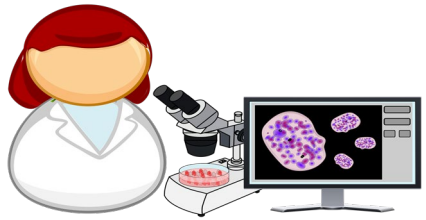


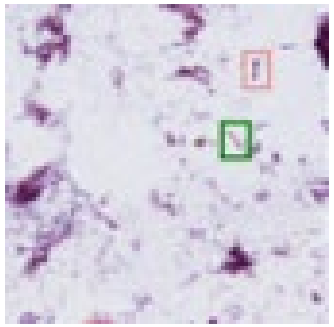
231 Samples collected and processed for routine testing



Kinyoun Smear preparation

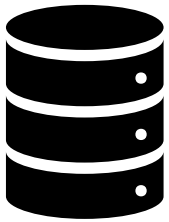
Creation of digital slides





Manually marked 11,411 AFB "answer key"

Building the AI model

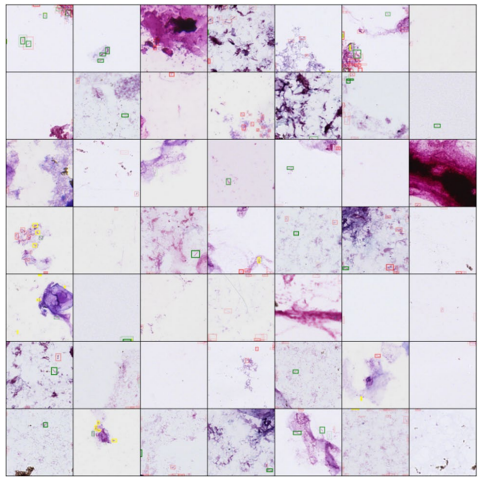


Initial positive and random negative

Second round trained using hard negatives

Convolutional Neural Network

FCOS/ConvNeXt



Whole-slide analysis
Detection on individual tiles

Detect individual AFB

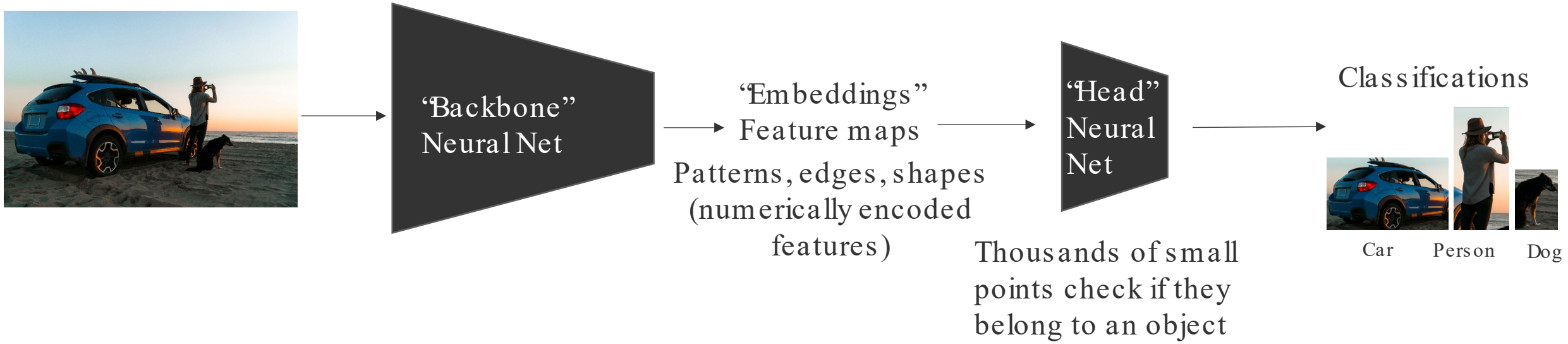
Estimate how many AFB on the whole slide

Predict whether the sample was likely positive

Machine Learning and Convolutional Neural Network: simplified

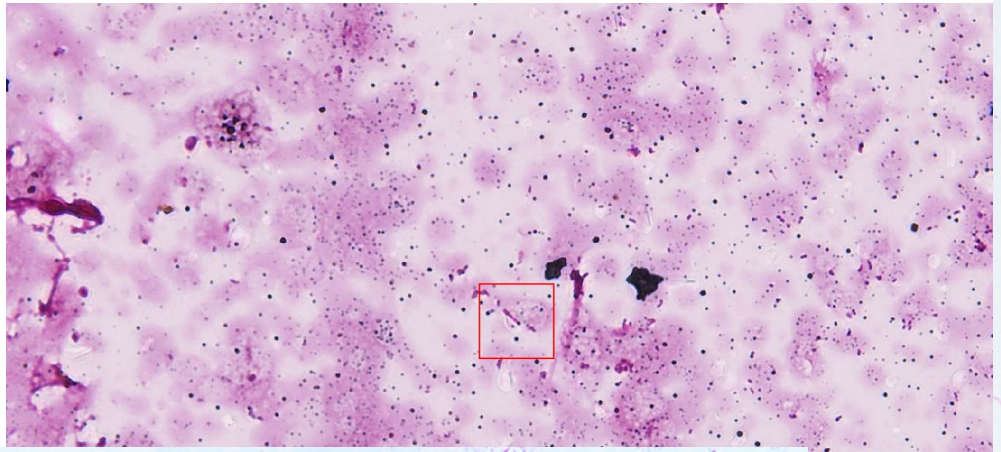
- ConvNeXt —"What is in the image?"
  - Designed to understand images by recognizing patterns such as edges, colors, shapes, and textures

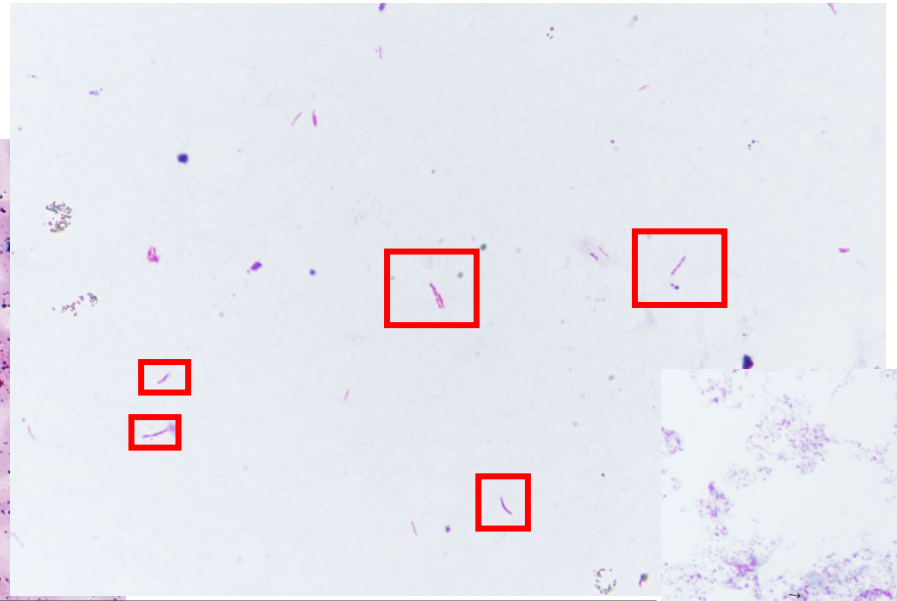
- FCOS (Fully Convolutional One-Stage Detector) —"Where are the objects?"
  - Scans an image, learns the patterns, uses patterns to classify image

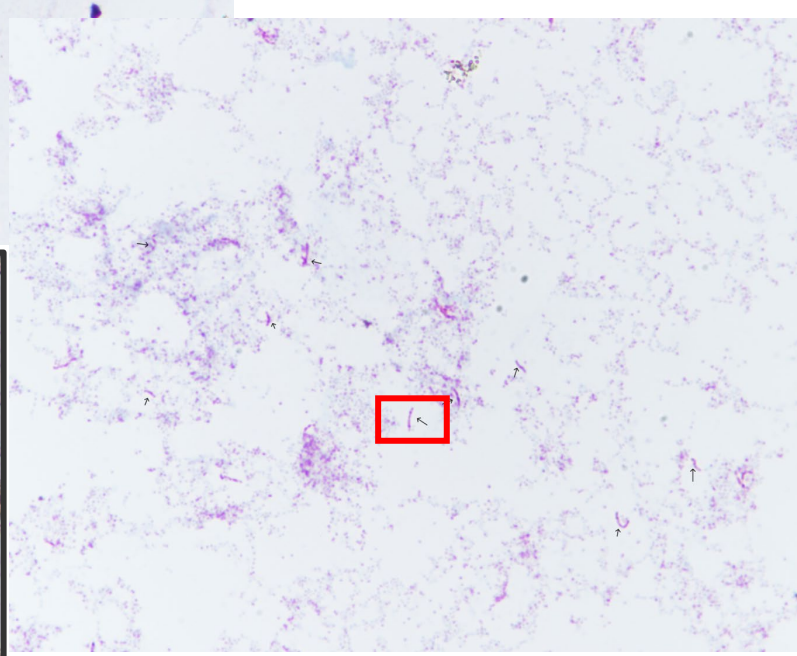


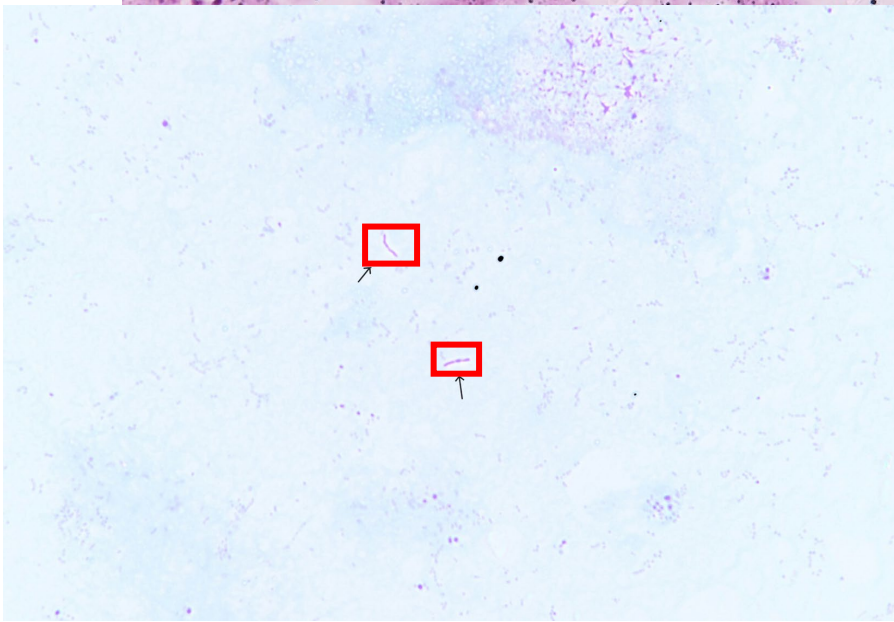
AI meets the Experts

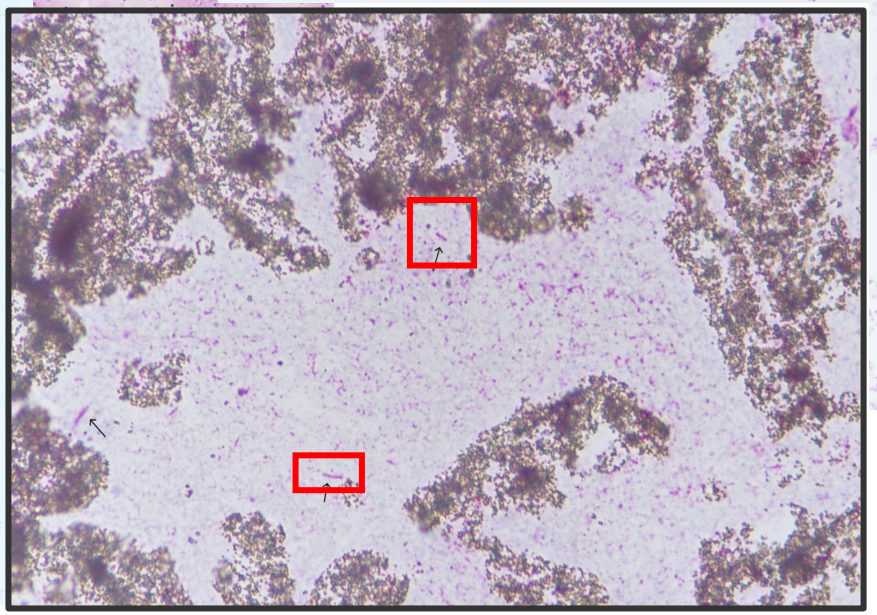
- 3 AFB laboratory trained technologists
- Objects labeled as "AFB," "mimic," "non-AFB," or "unknown"
- Almost all annotated objects were AFB
- Slides for annotation preferentially selected from AO-positive slides to enrich for objects to annotate











Distribution of sample types

Source	No. of specimens	AO positive	AO negative	MGIT culture positive	MGIT culture negative
Sputum	68	38	30	42	27
Respiratory other	68	14	54	18	50
Tissue	37	1	36	0	37
Body fluid	34	3	31	3	31
Wound	17	0	17	1	16
Not specified/other	7[a]	3	2	5	2

[a]AO not performed in two cases (direct isolate and stool).

https://doi.org/10.1128/spectrum.00602-25

Mycobacterium species in positive samples

Source	No. of positives	Organism						
		MTBC	MAC	MABS	MKC	*M. simiae*	*M. cosmeticum*	Unable to ID
Sputum	42	13	22	4	1	1	–[c]	1[b]
Respiratory other	18	1	12	3	1	–	1	–
Tissue	0	–	–	–	–	–	–	–
Body fluid	3	3	–	–	–	–	–	–
Wound	1	–	–	1	–	–	–	–
Not specified/other	4	1	3	–	–	–	–	–

[a]MTBC, *Mycobacterium tuberculosis* complex; MAC, *Mycobacterium avium* complex; MABS, *Mycobacterium abscessus*; MKC, *Mycobacterium kansasii* complex.
[b]The organism grew on culture but could not be identified by molecular techniques.
[c]–, not identified.

https://doi.org/10.1128/spectrum.00602-25

Slide Scanners

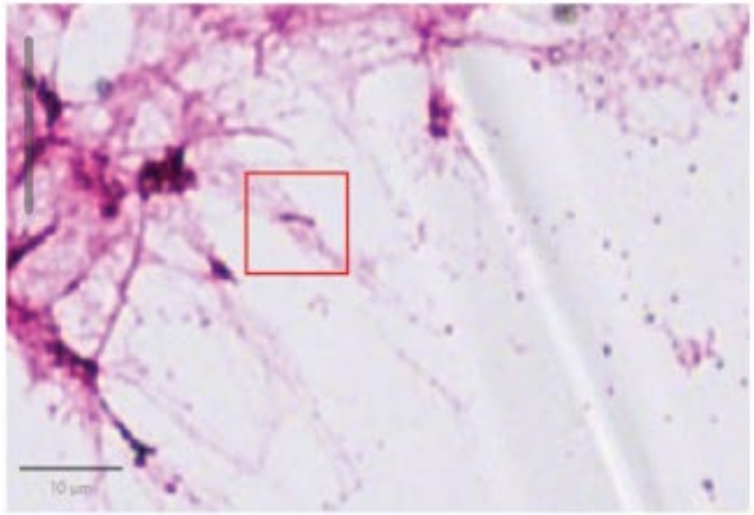
Hamamatsu NanoZoomer S360

Pramana HT2

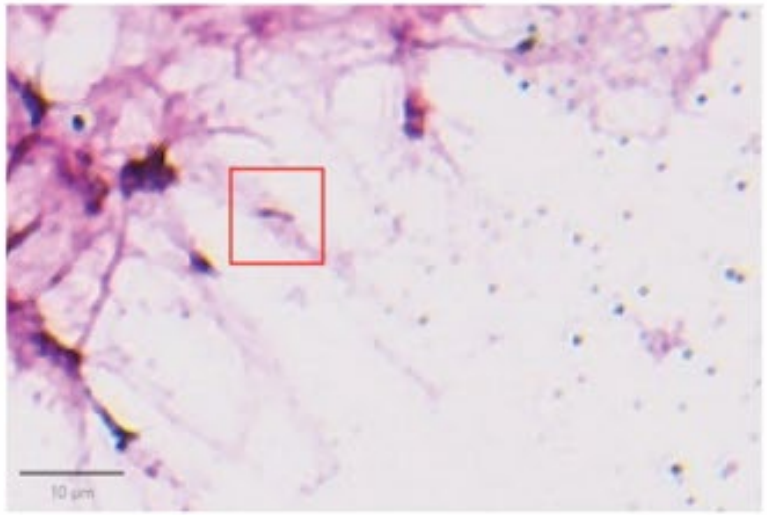
Scanner Settings and Performance Comparison

	Scanner	Pramana HT-2	Hamamatsu 360
Settings	Magnification	40x	40x
	Resolution (microns per pixel)	0.25	0.23
	Z-Layers	9	9
	Z-layer interval (um)	1.25	1.3
Performance	Average Scan Time (s)	597	330.7
	Average Scan Area (mm^2)	323.4	311.9
	Average File Size (GB)	1.6	8.3
	Scan time (s/mm^2)	1.8	1.1
	File Size (mb/mm^2)	4.9	26.5

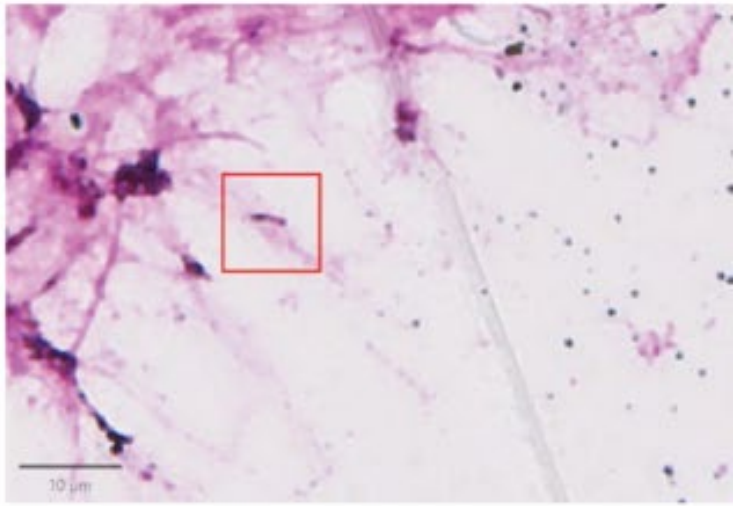
Image Quality between S360 and Pramana (40X and 80X)



Hamamatsu S360 40X

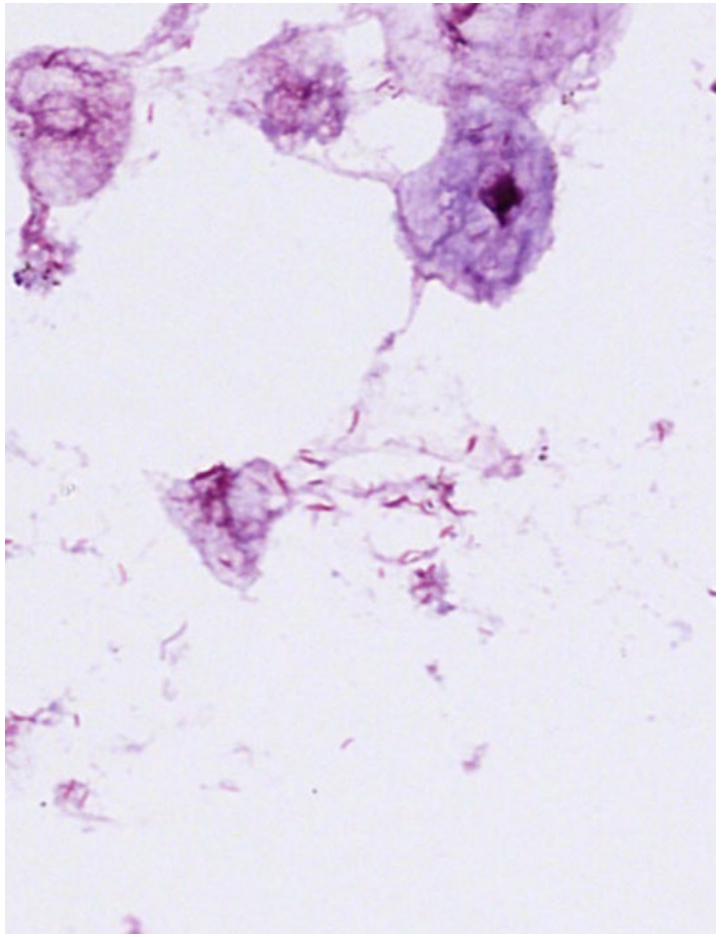


Pramana 40X

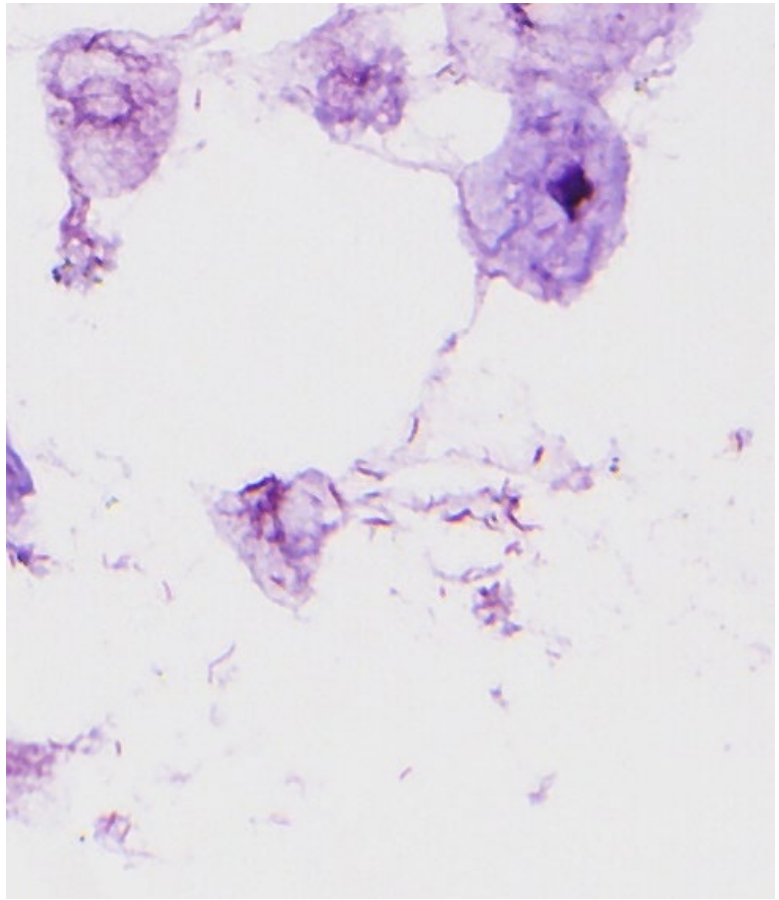


Pramana 80X

AO3+, M. tuberculosis complex

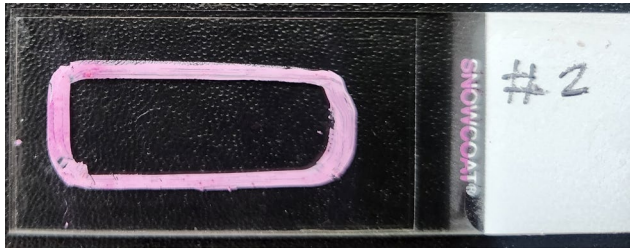


Hamamatsu S360 40X

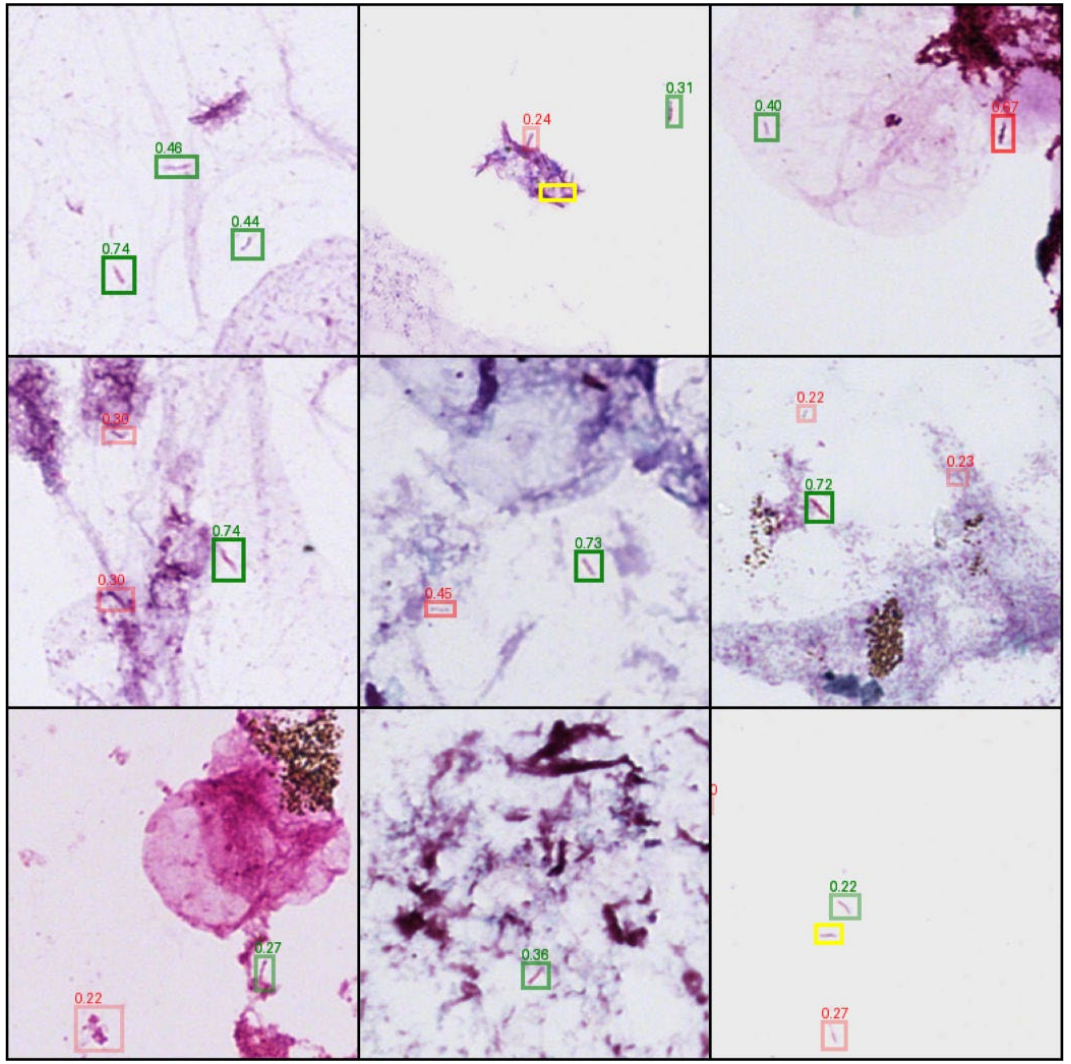


Pramana 40X

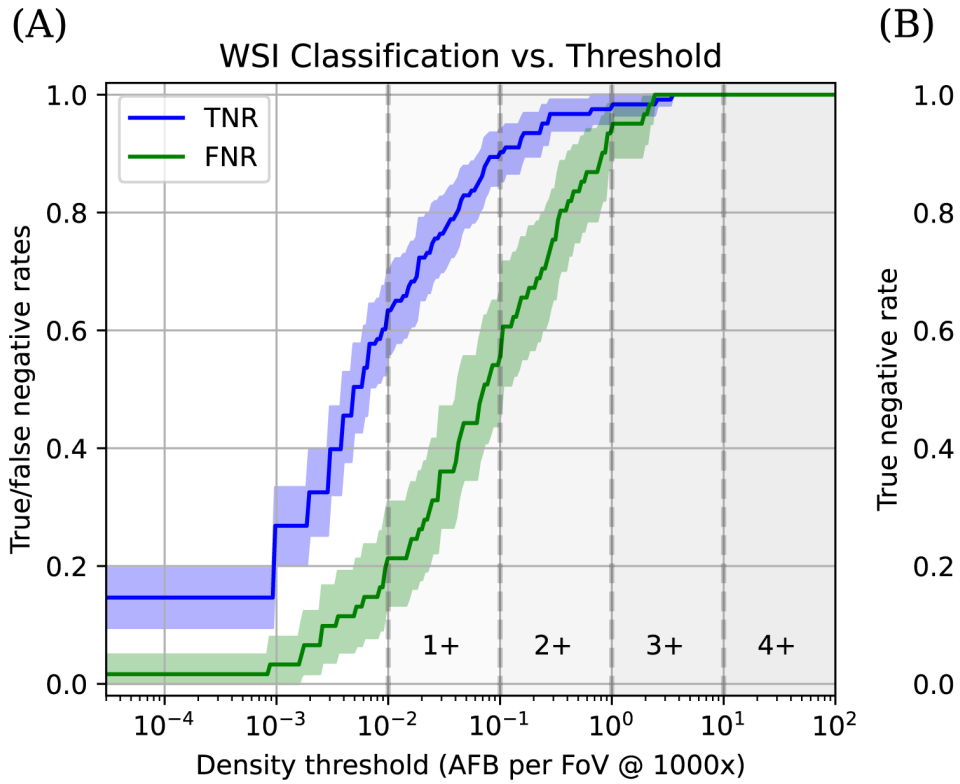
Whole slide scanning vs Tile selection



- Long scan time, >20 min
- Reduced performance
  - » Noise at edge of smear
  - » Interference from wax ring
- Randomly selected 10,000 small image tiles
  - » 50 mm^2 (minimum area of 13 mm^2)



Model-predicted slide level classification



https://doi.org/10.1128/spectrum.00602-25

Results Summary

- WSI-level performance (threshold $\geq 10^{-2}$ AFB/1000x FOV)

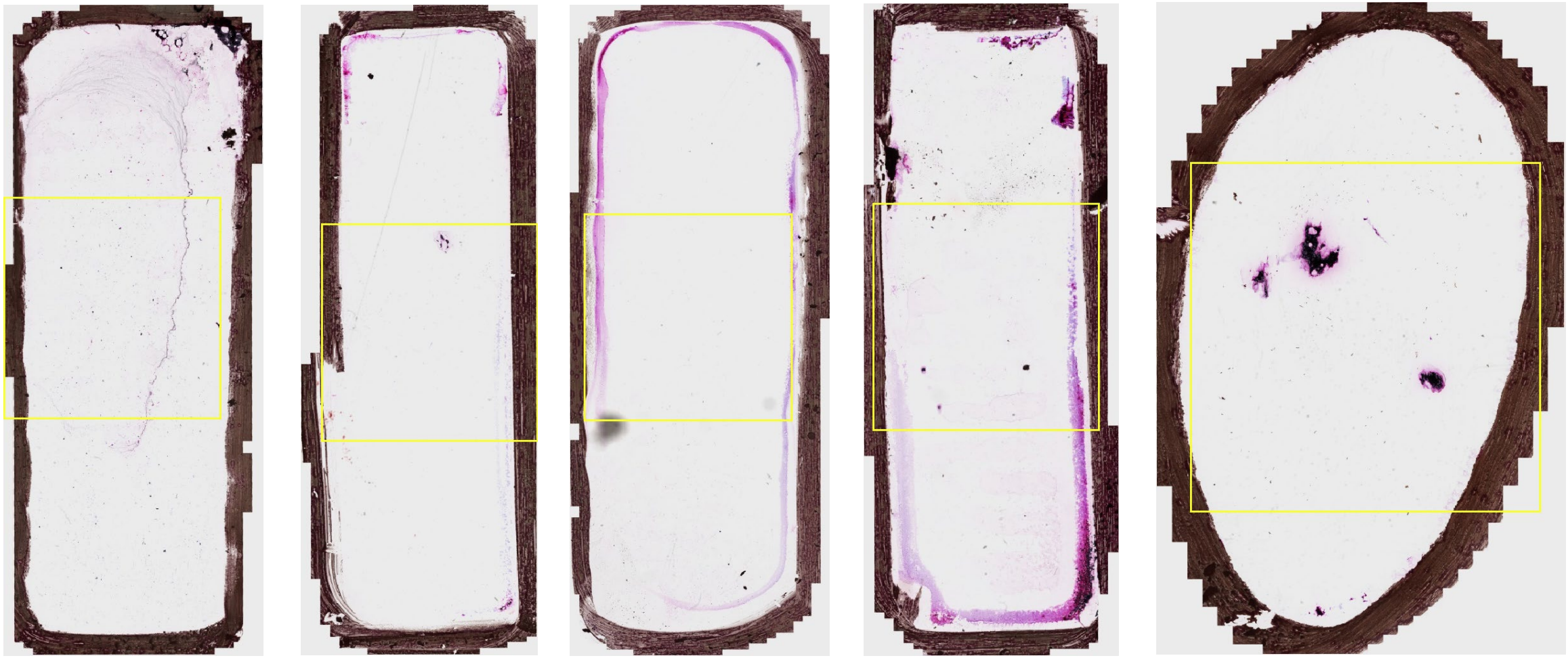
- Overall accuracy: 68% of slides correctly classified
  - » Sensitivity: 79%
  - » Specificity: 63%

- Specimen source
  - » AI performed better on sputum but had more false positives

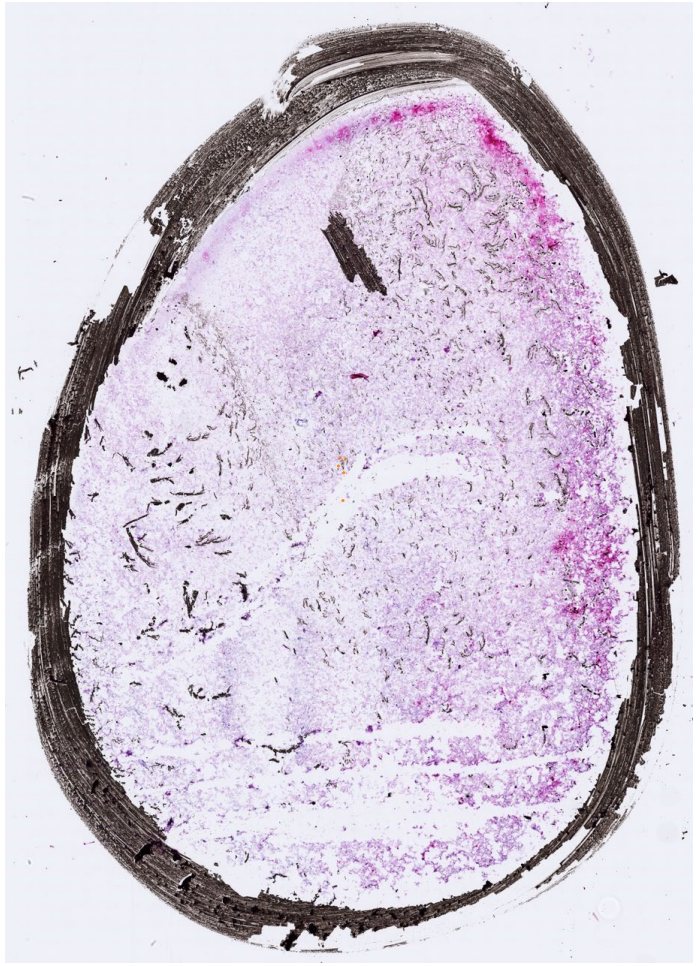
    Sensitivity 92% Specificity of 33%.
  - » Non-sputum samples had lower sensitivity but higher specificity
    - AI Kinyoun vs manual AO: 83% sensitivity, 56% specificity

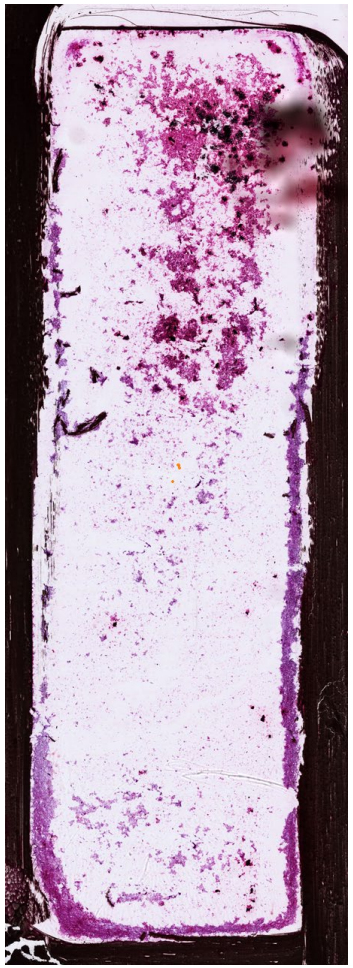
- Manual AO vs culture comparison: 76% sensitivity, 96% specificity

Validation set false negatives



Smear prep and staining variability





Slide courtesy Brandon O'Fallon

Study Conclusions

- Developed a full AI pipeline for AFB detection on brightfield smears
  - » Publicly available dataset of expertly annotated AFB
- Demonstrated a promising proof-of-concept for using AI to detect AFB directly from Kinyoun-stained bright-field slides
- Moderate sensitivity
- Not ready for clinical deployment due to specificity issues
- Slide preparation and specimen thickness matters
- Need larger training dataset for modern deep learning

Key Insights

- AI and machine learning detects AFB well in smears with less background
- High false-positive rate, especially in debris-heavy sputum
  - » "needle-in-a-haystack" problem
- False positives: objects that looked similar to AFB, insufficient annotation of mimics
- Scanner variability affects image quality
- Longer scan times on best-performing scanner (Pramana HT2)
- Brightfield Kinyoun stains inherently challenging for AI
- False negatives may be caused
  - » Uneven smear preparation
  - » Organisms hidden in dense background variations in staining

AI AFB smear implementation considerations

- Ease sample volume burden
- Improving detection and quality of results
- Infrastructure
- Quality of image generated
- Sufficiently trained tools
- IT storage capacity
- Future efficiency gains/cost savings
- Regulatory considerations

From Research Triage to Integrated Diagnostic Decision-Making

PHASE	PRIMARY ROLE OF AI	HUMAN INVOLVEMENT	OPERATIONAL IMPACT
Today (Research)	Triage and validation only	Review all positives	No change to diagnostic workflow
Near-Term	Auto-release clear negatives	Review positives and borderline cases	Faster rule-out, improved efficiency
Future State	First reader in diagnostic algorithm	Confirm positives, oversee Quality	Integrated, streamlined TB diagnosis

Acknowledgement

- ARUP Research and Innovation
  - » Applied AI & Bioinformatic
    - Muir Morrison
    - Paul English
    - Mark Dewey
    - Alexandra Rangel
  - » Medical Directorship
    - Ryan Shean, DO
    - Brendan O'Fallon
    - David Ng, MD
    - Leo Lin, MD
- ARUP Research and Innovation
  - – Research Scientist and Project Specialists
    - Katie Knight
    - Elizabeth Enrico, PhD
    - Deven Rupp
- ARUP AFB Clinical Lab
  - – Jeff Gilivary
  - – Amanda Vance
  - – Blaine Mathison
  - – Haleina Hatch

ARUP LABORATORIES | Institute for Research and Innovation in Diagnostic and Precision Medicine™

ARUP LABORATORIES
A nonprofit enterprise of the University of Utah and its Department of Pathology