URINE LAM

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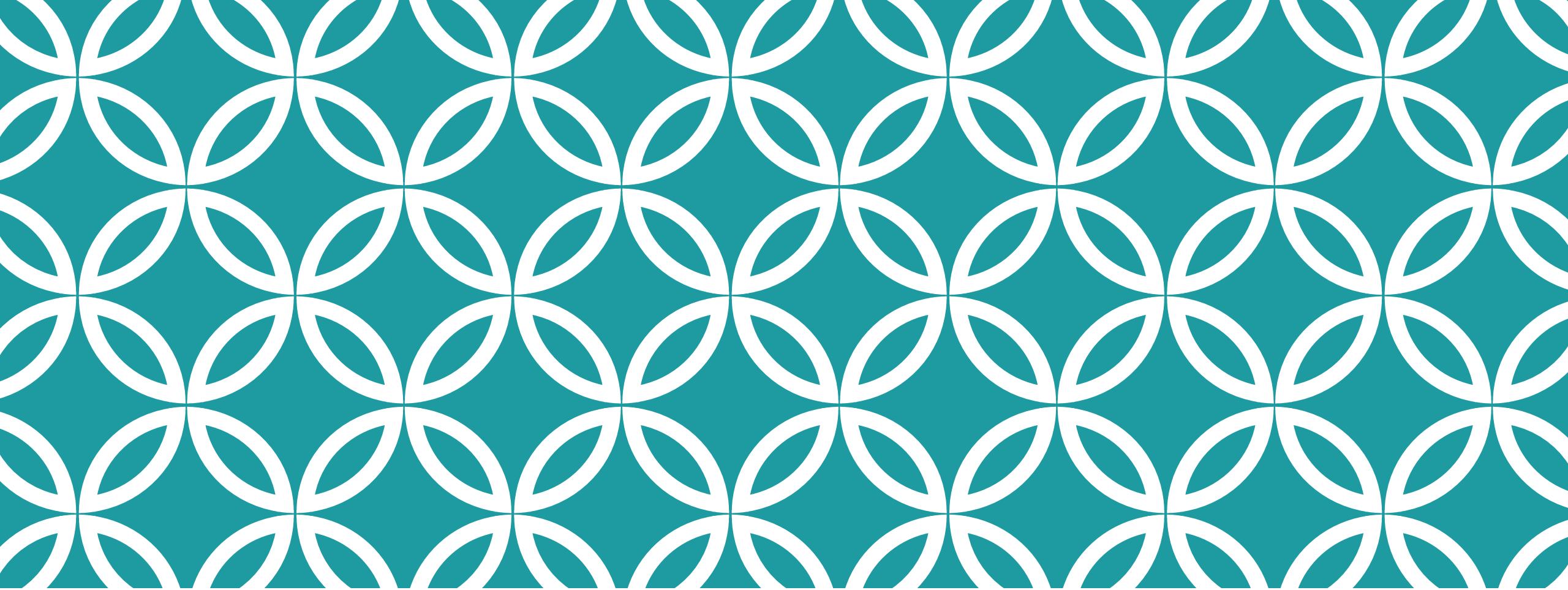
DISCLOSURES

Speaker fees for public academic lectures on Infectious Diseases topics from Abbvie, MSD, Cepheid.

Research grants from Oxford University and Novo Nordisk Foundation.



WHAT IS LAM?

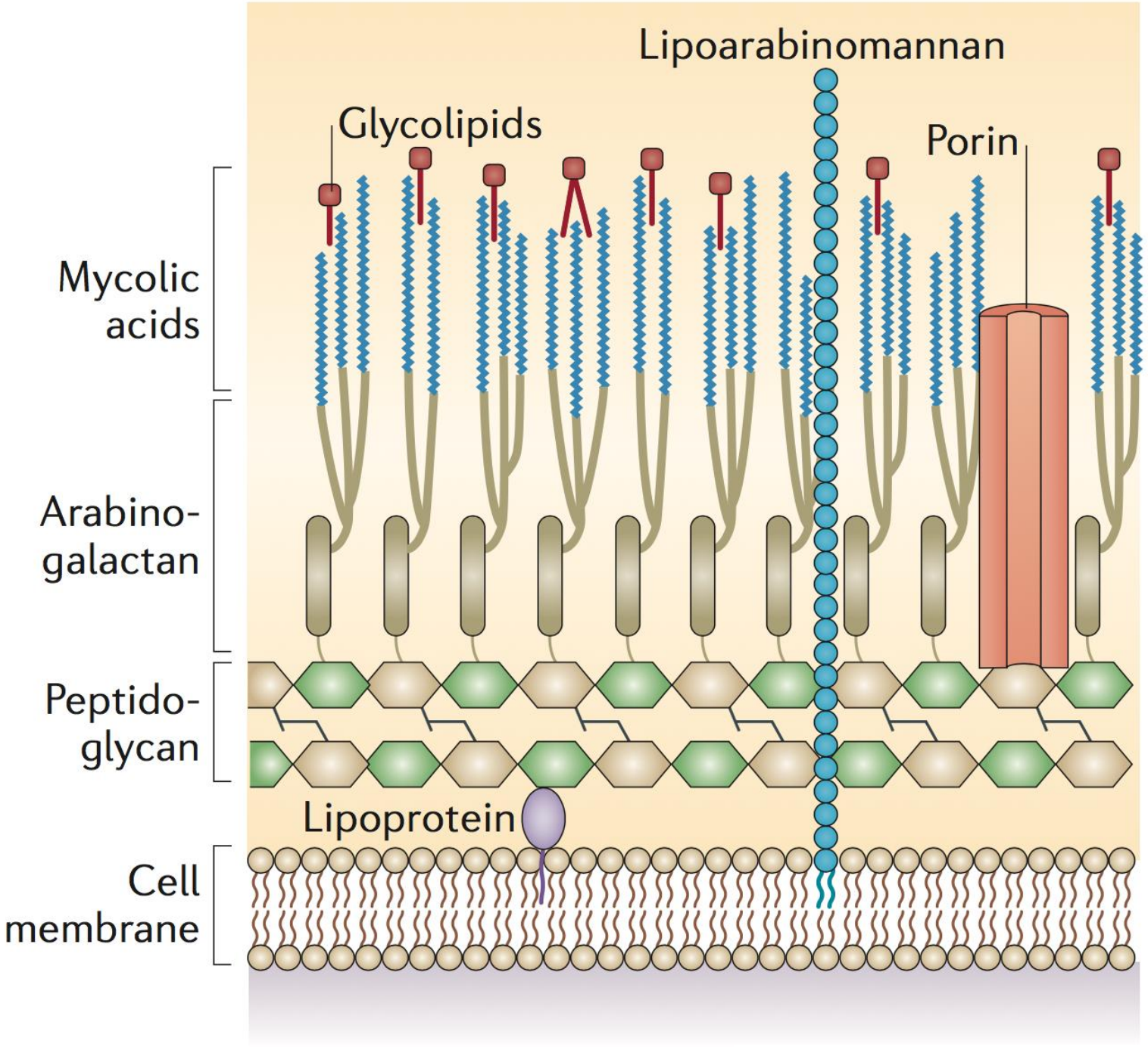
MYCOBACTERIA

Aerobic

Non-motile

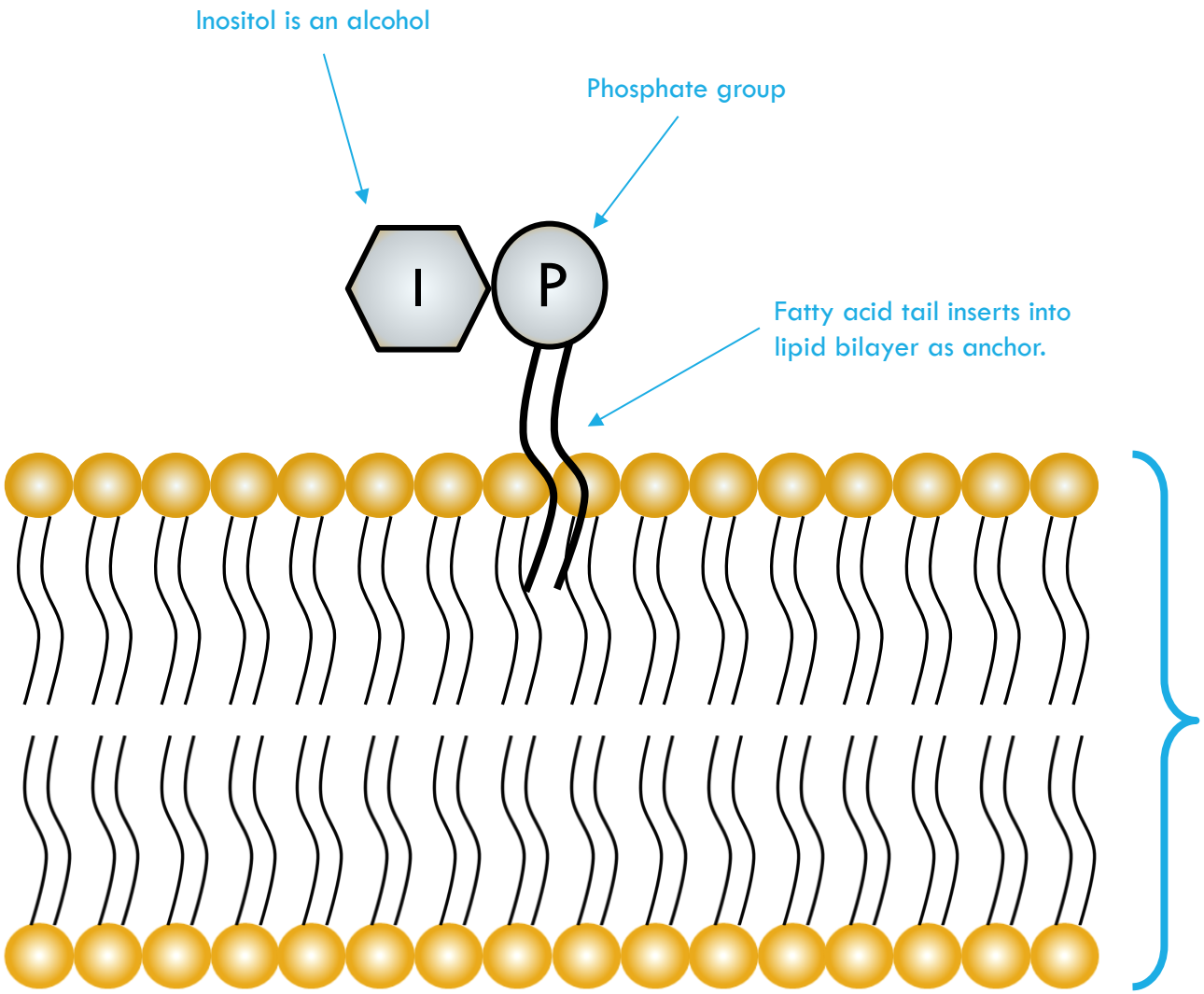
No capsules

Cell wall has thick, waxy mycolic acid component



Nature Reviews Microbiology **13**, 620–630 (2015)

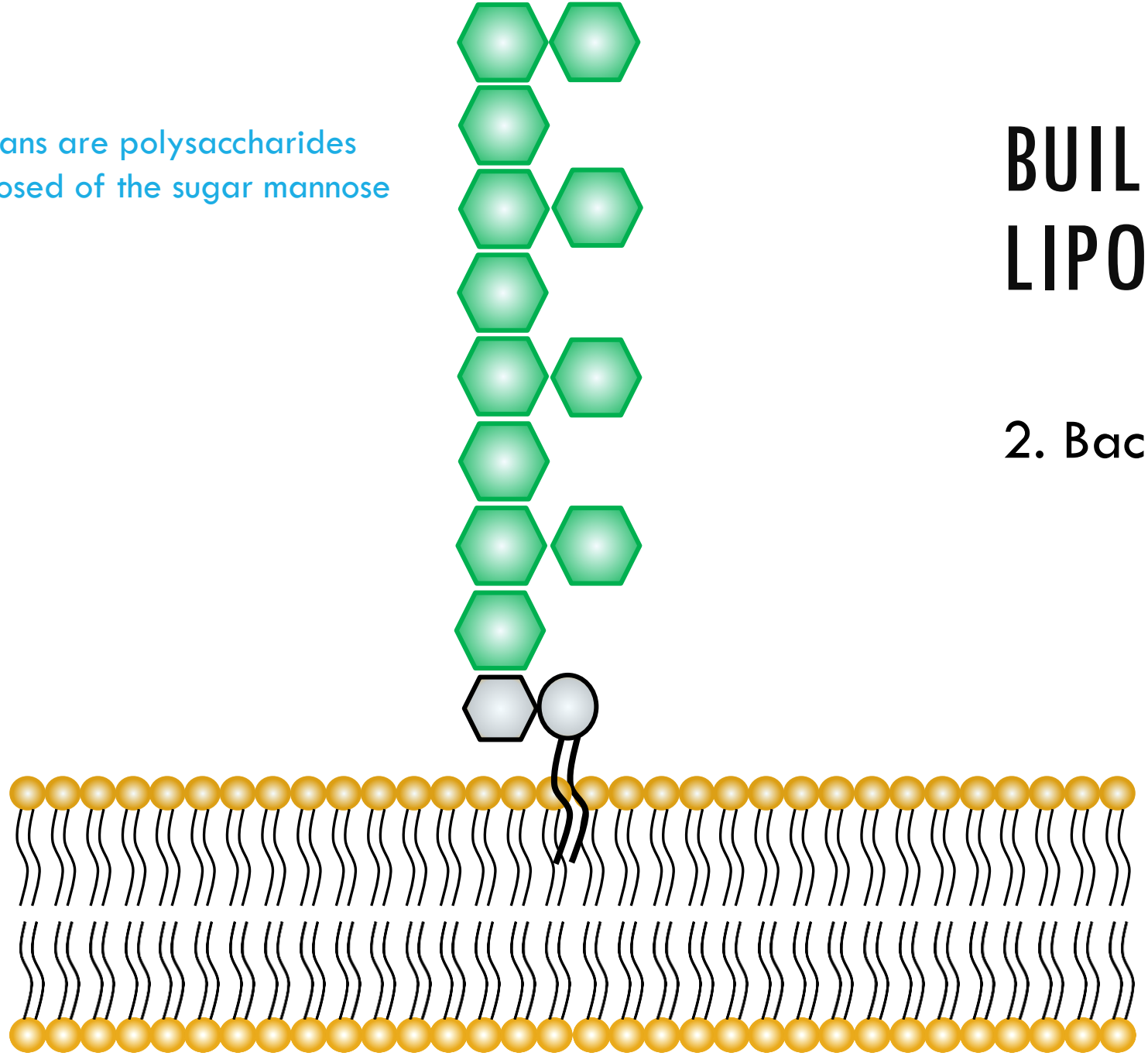
BUILDING LIPOARABINOMANNAN



1. Anchor: phosphatidylinositol

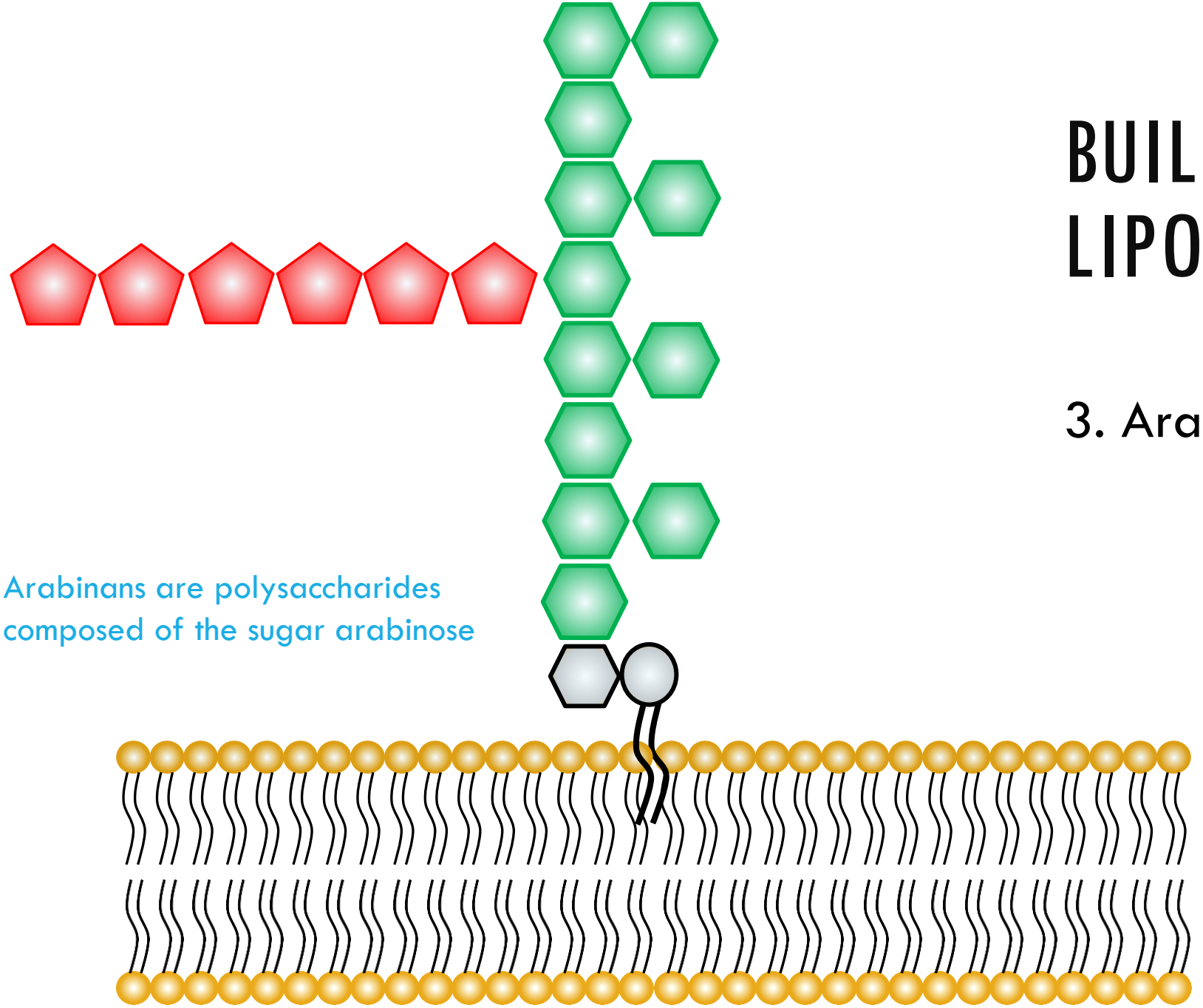
BUILDING LIPOARABINOMANNAN

Mannans are polysaccharides composed of the sugar mannose



2. Backbone: mannan

Together = “lipomannan”

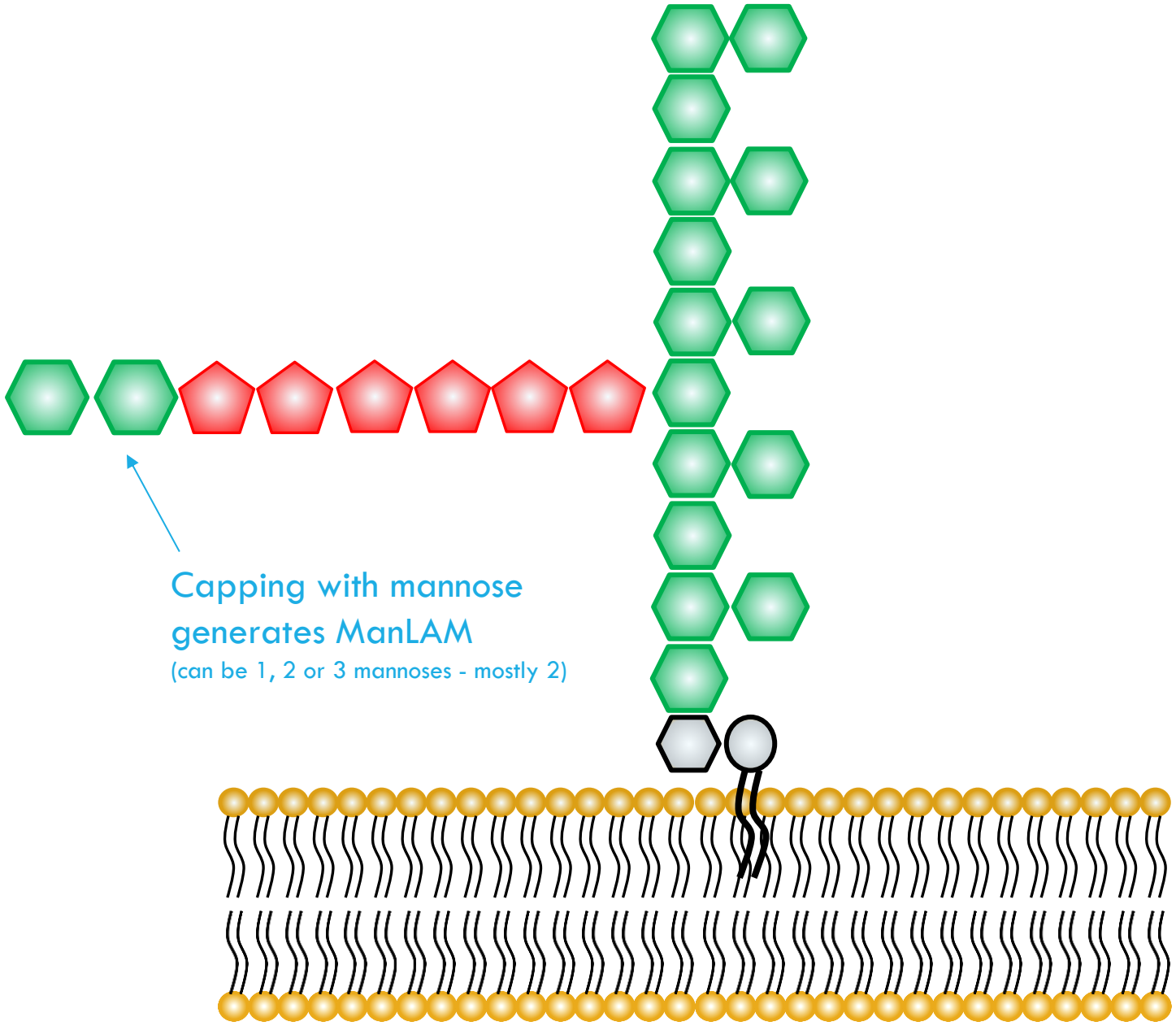


BUILDING LIPOARABINOMANNAN

3. Arabinosyl side-chains

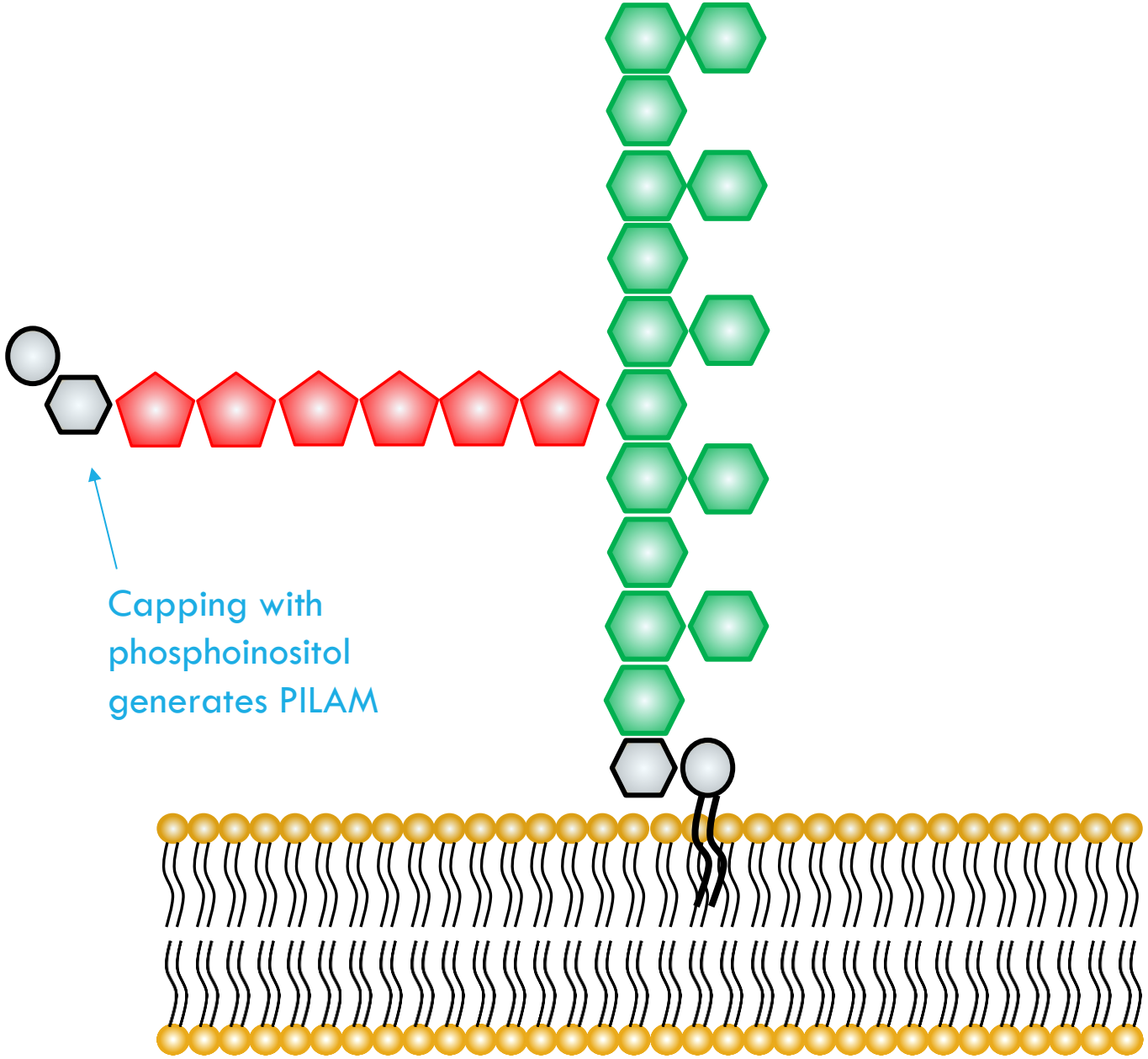
Arabinans are polysaccharides composed of the sugar arabinose

Together = “lipoarabinomannan”



BUILDING LIPOARABINOMANNAN

4. Side-chain capping

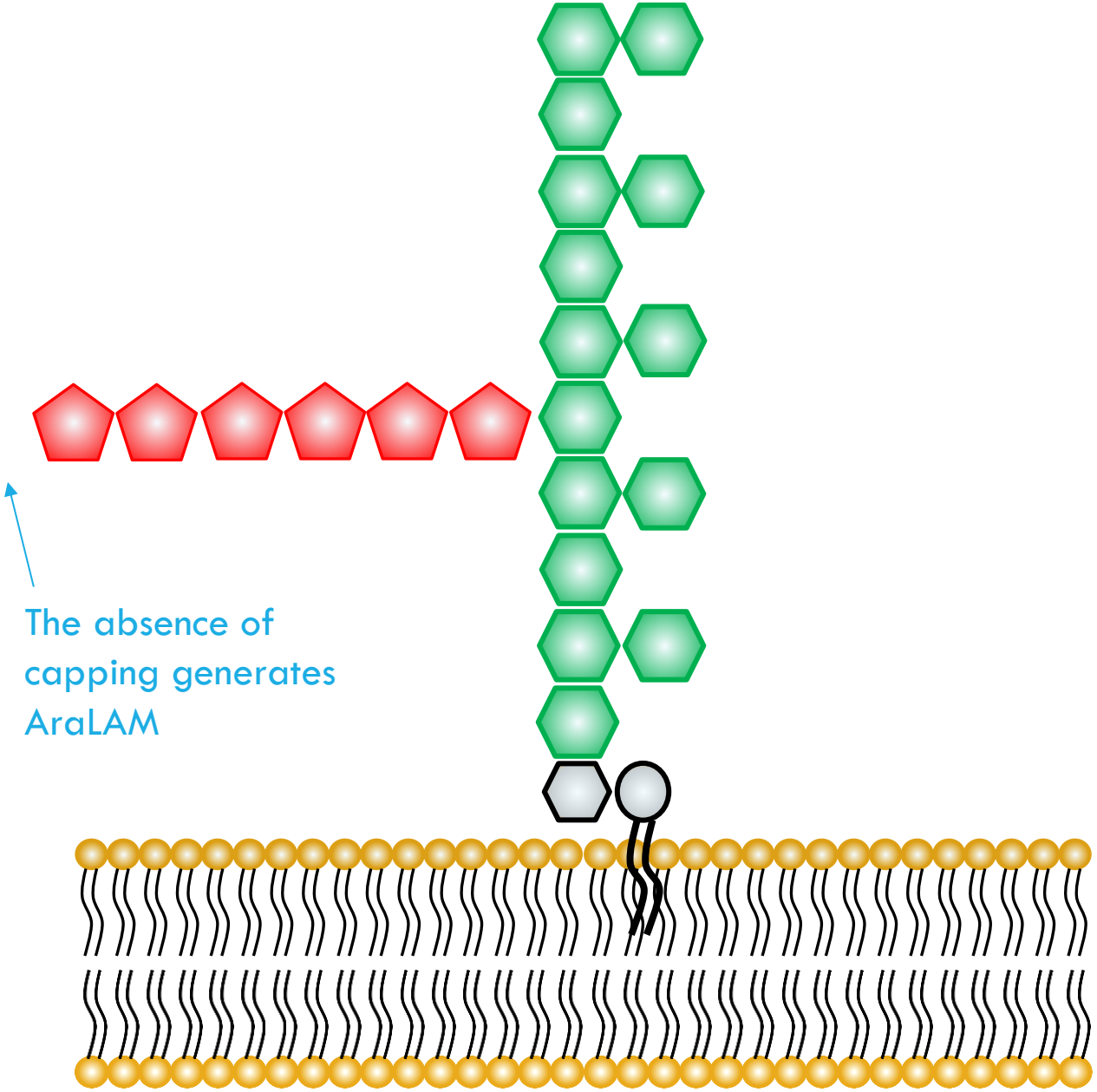


BUILDING LIPOARABINOMANNAN

4. Side-chain capping

BUILDING LIPOARABINOMANNAN

4. Side-chain capping



LAM SUBTYPES

Type	Species	Human immune response
ManLAM	*M. tuberculosis* *M. leprae* *M. avium-intracellulare* *M. kansasii*	Anti-inflammatory Allows mycobacteria to bind mannose receptors on macrophages and enter them, but doesn't trigger the inflammatory response.
PILAM	*M. smegmatis* *M. fortuitum*	Proinflammatory Binds to TLR2
AraLAM	*M. chelonae*	?

LAM AND MYCOBACTERIA

LAM is found in *all* species of mycobacteria.

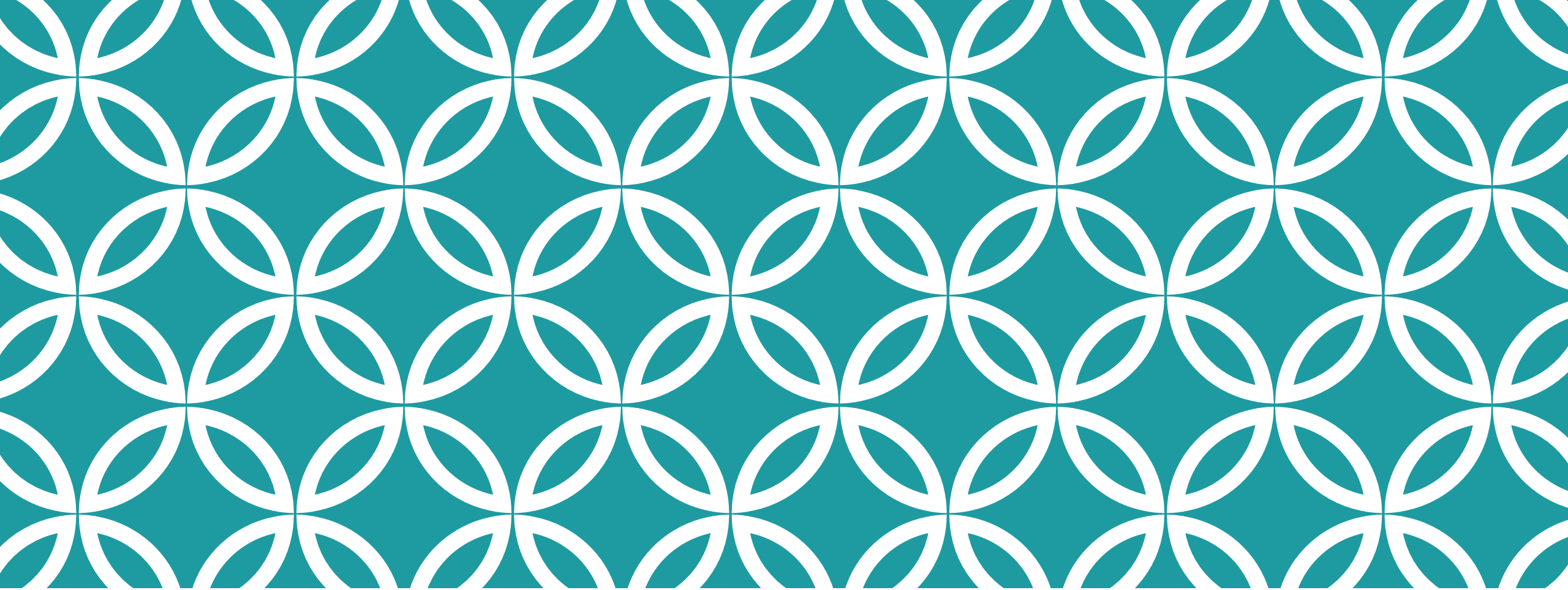
- The phosphatidyl-inositol anchor is conserved throughout the genus.
- The size and degree of branching of the mannose backbone is species dependent.

LAM from any mycobacterium is also probably heterogeneous:

- Slightly different size, branching patterns, etc.

Compared to ManLAM, PILAM:

- Increases IL-8, IL-12, TNF-α secretion from macrophages, as well as macrophage apoptosis.



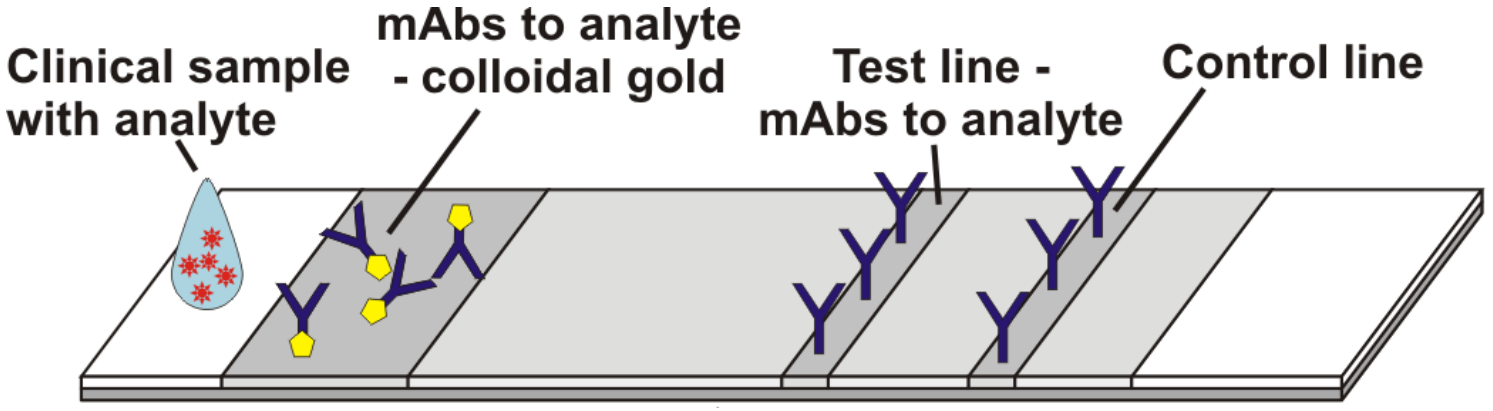
LAM – THE TEST

ASSAY TYPES

Research assays

- In-house, required extensive processing. Not commercially available

DETERMINE TB-LAM MECHANISM



Lateral flow assay = immunochromatographic assay.

Lateral flow is the movement of a fluid parallel to the slope of the surface

Detection antibodies are labelled by conjugation to colloidal gold.

"The gold particles are red in colour due to localised surface plasmon resonance" - Wikipedia

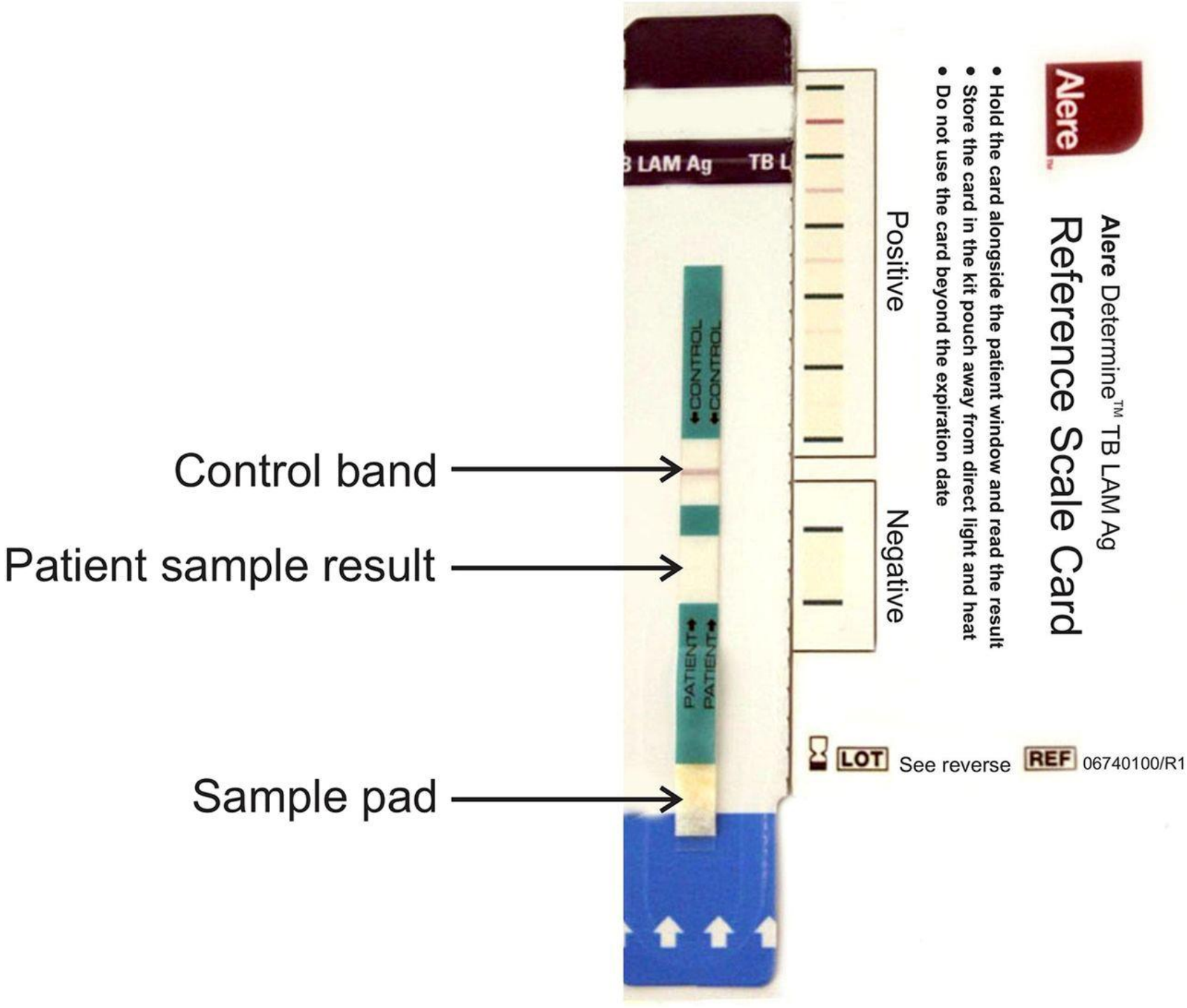
Capture antibodies are stuck onto the nitrocellulose membrane of the test strip.

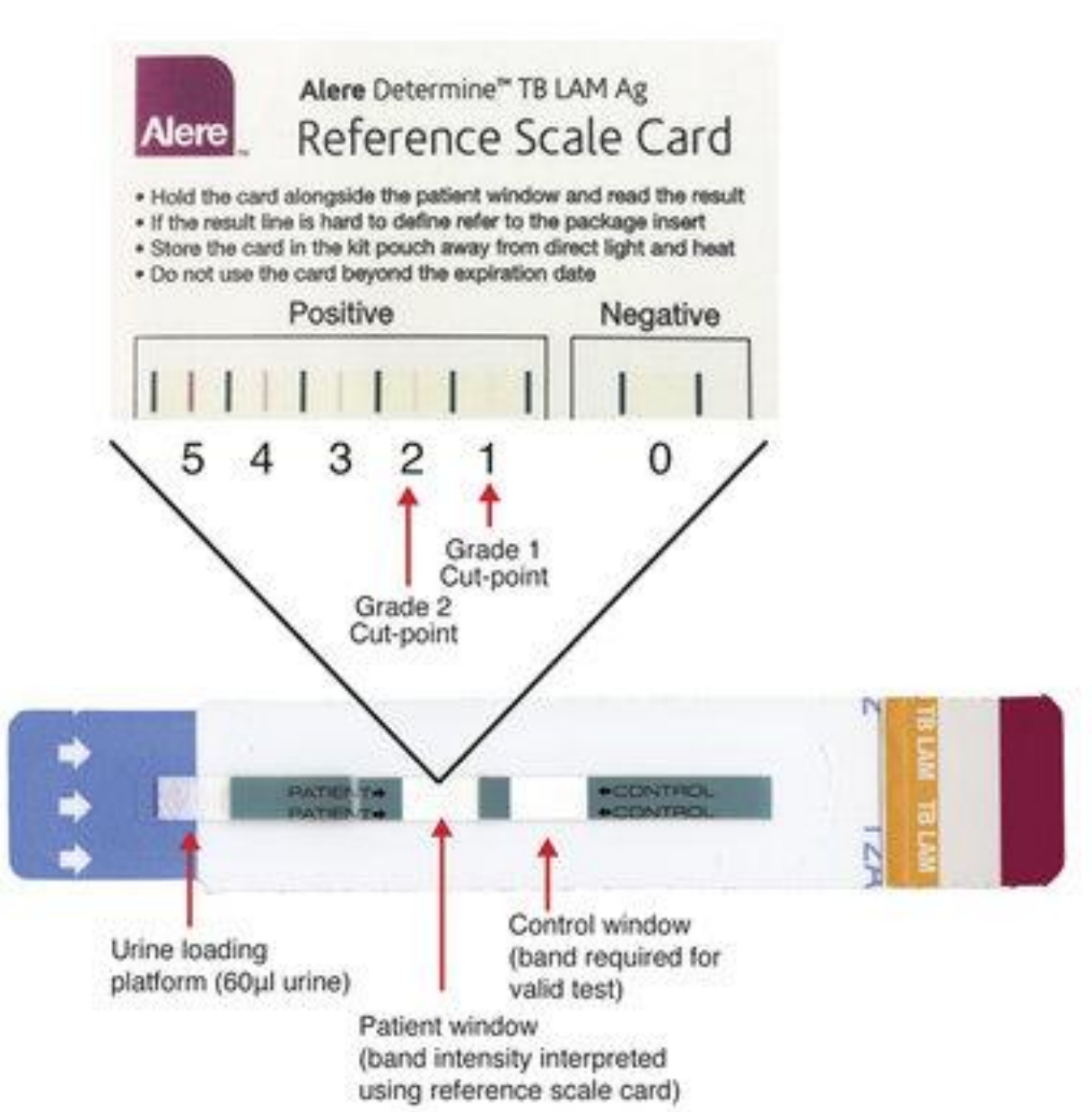
READING THE STRIP

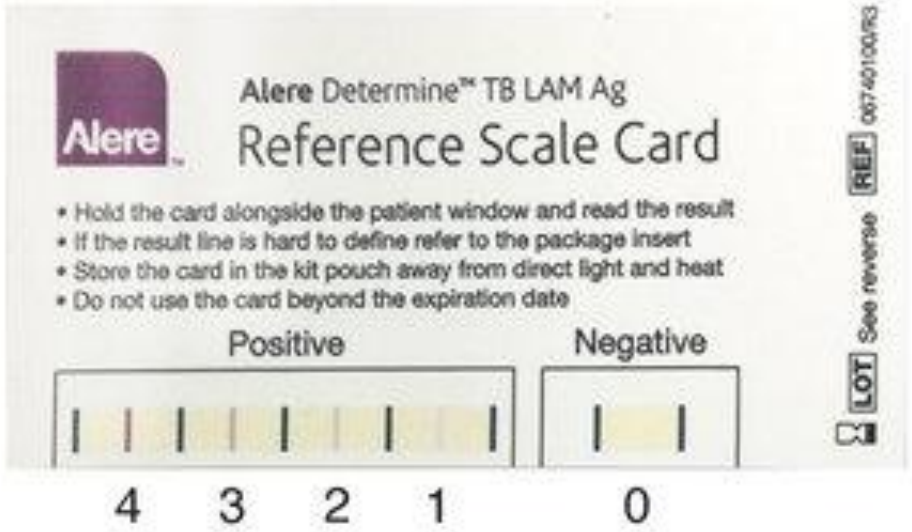
60 µL of urine added to sample pad.

Read test strip between 25-35 mins later

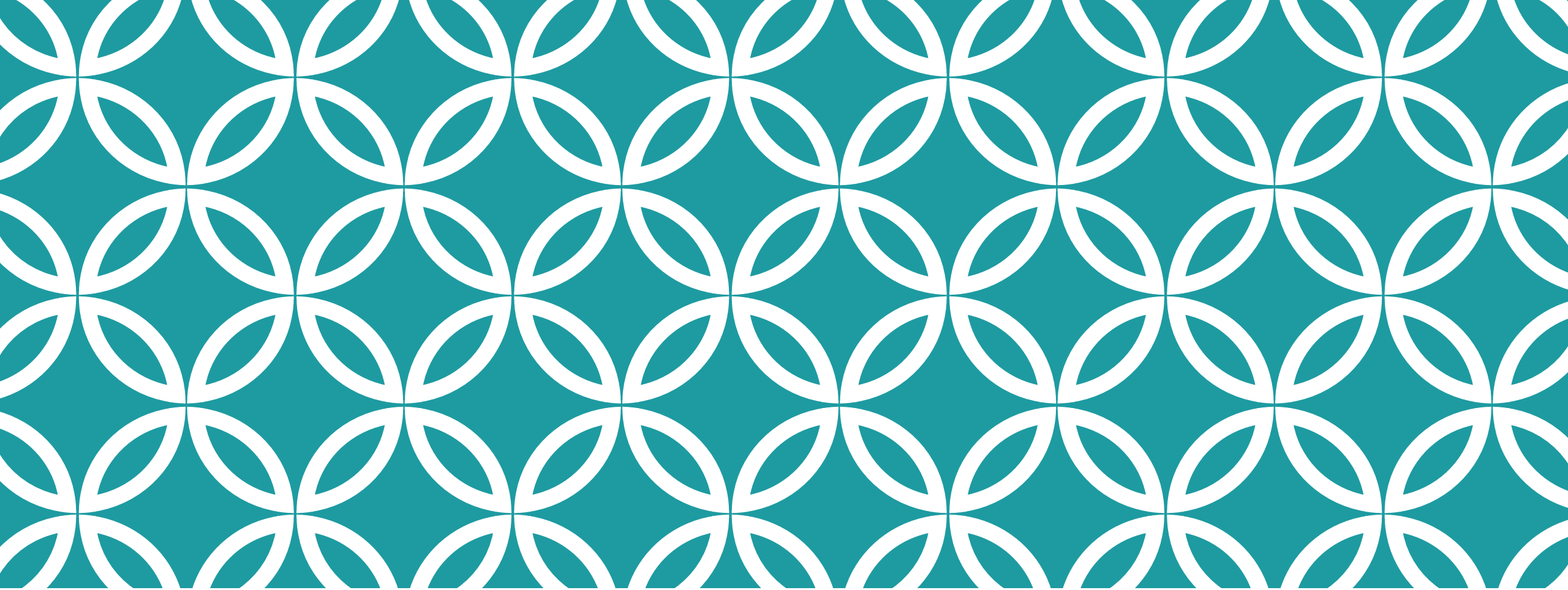
Compare with reference card – sample result must be at least as faint as the faintest line on reference card







Post-Jan 2014, the old grade 1 line was removed (poor specificity), and so the new grade 1 line now corresponds to the old grade 2 line.



TEST PERFORMANCE

Cochrane Library

Cochrane Database of Systematic Reviews

Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in people living with HIV (Review)

Bjerrum S, Schiller I, Dendukuri N, Kohli M, Nathavitharana RR, Zwerling AA, Denkinger CM, Steingart KR, Shah M

2019

REMINDER:

Sensitivity = % of patients who have TB who test LAM positive

Specificity = % of patients who <u>don't</u> have TB who test negative

OVERALL TEST PERFORMANCE - HIV PATIENTS

Symptomatic participants

Sensitivity 42% (31-55%)
Specificity 91% (85-95%)

Unselected participants (irrespective of signs/symptoms)

Sensitivity 35% (22-50%)
Specificity 95% (89-98%)

* Microbiological reference standard used

SYMPTOMATIC PEOPLE LIVING WITH HIV

Pooled sensitivity (95%CrI): 42% (31% to 55%); pooled specificity (95% CrI): 91% (85% to 95%)

Test result	Number of results per 1000 participants tested (95% CrI)			Number of participants (studies)	Certainty of the evidence (GRADE)
	Prevalence 1% **Typically seen in asymptomatic persons in outpatient settings**	**Prevalence 10%** **Typically seen in symptomatic persons in all settings**	**Prevalence 30%** **Typically seen in seriously ill persons in inpatient settings**		
True positives	**4** (3 to 6)	**42** (31 to 55)	**126** (93 to 165)	1277 (8)	⨁⨁⨁◯ **MODERATE**[a,b,c,d]
False negatives	**6** (4 to 7)	**58** (45 to 69)	**174** (135 to 207)		
True negatives	**901** (842 to 941)	**819** (765 to 855)	**637** (595 to 665)	2172 (8)	⨁◯◯◯ **VERY LOW**[c,e,f]
False positives	**89** (49 to 148)	**81** (45 to 135)	**63** (35 to 105)		

Abbreviations: CrI: credible interval.

INPATIENTS VS OUTPATIENTS

Inpatients

Sensitivity 52% (40-64%)
Specificity 87% (78-93%)

Outpatients

Sensitivity 29% (17-47%)
Specificity 96% (91-99%)

LF-LAM Diagnostic Accuracy for Symptomatic TB

Stratified by CD4 count | Symptomatic HIV-positive adults | Microbiological reference standard

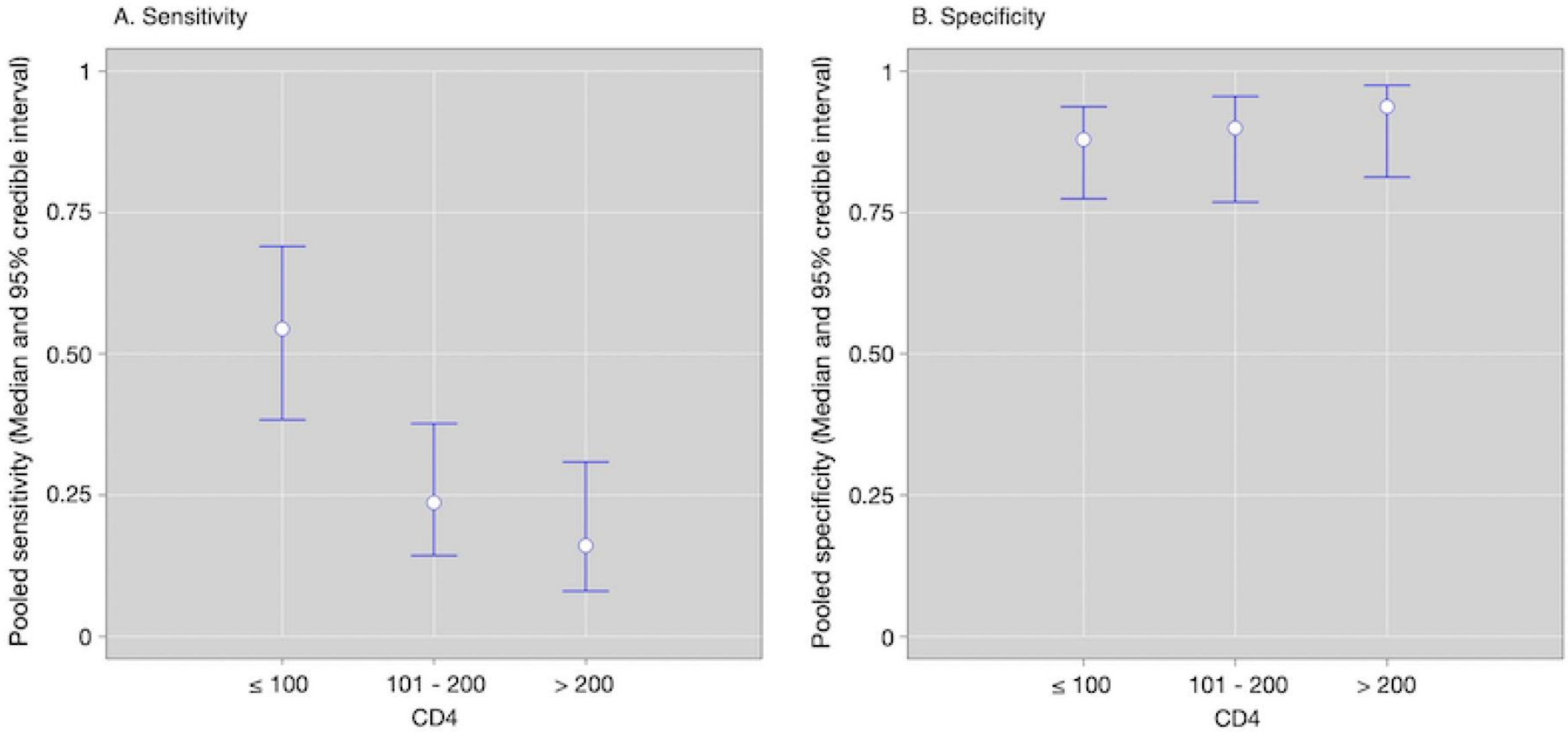
2019 Cochrane Database of Systematic Reviews (Bjerrum et al.)

CD4 Threshold	Studies (participants, % with TB)	Pooled Sensitivity % (95% CrI)	Pooled Specificity % (95% CrI)
CD4 > 200	3 studies (738 participants, 22% TB)	**16% (8 – 31)**	**94% (81 – 97)**
CD4 ≤ 200	4 studies (1,825 participants, 40% TB)	**45% (31 – 61)**	**89% (77 – 94)**
CD4 101-200	4 studies (586 participants, 41% TB)	**24% (14 – 38)**	**90% (77 – 94)**
CD4 ≤ 100	4 studies (1,239 participants, 41% TB)	**54% (38 – 69)**	**88% (77 – 94)**

Sensitivity of LF-LAM increases substantially at lower CD4 counts (advanced HIV), while specificity remains high but slightly lower.

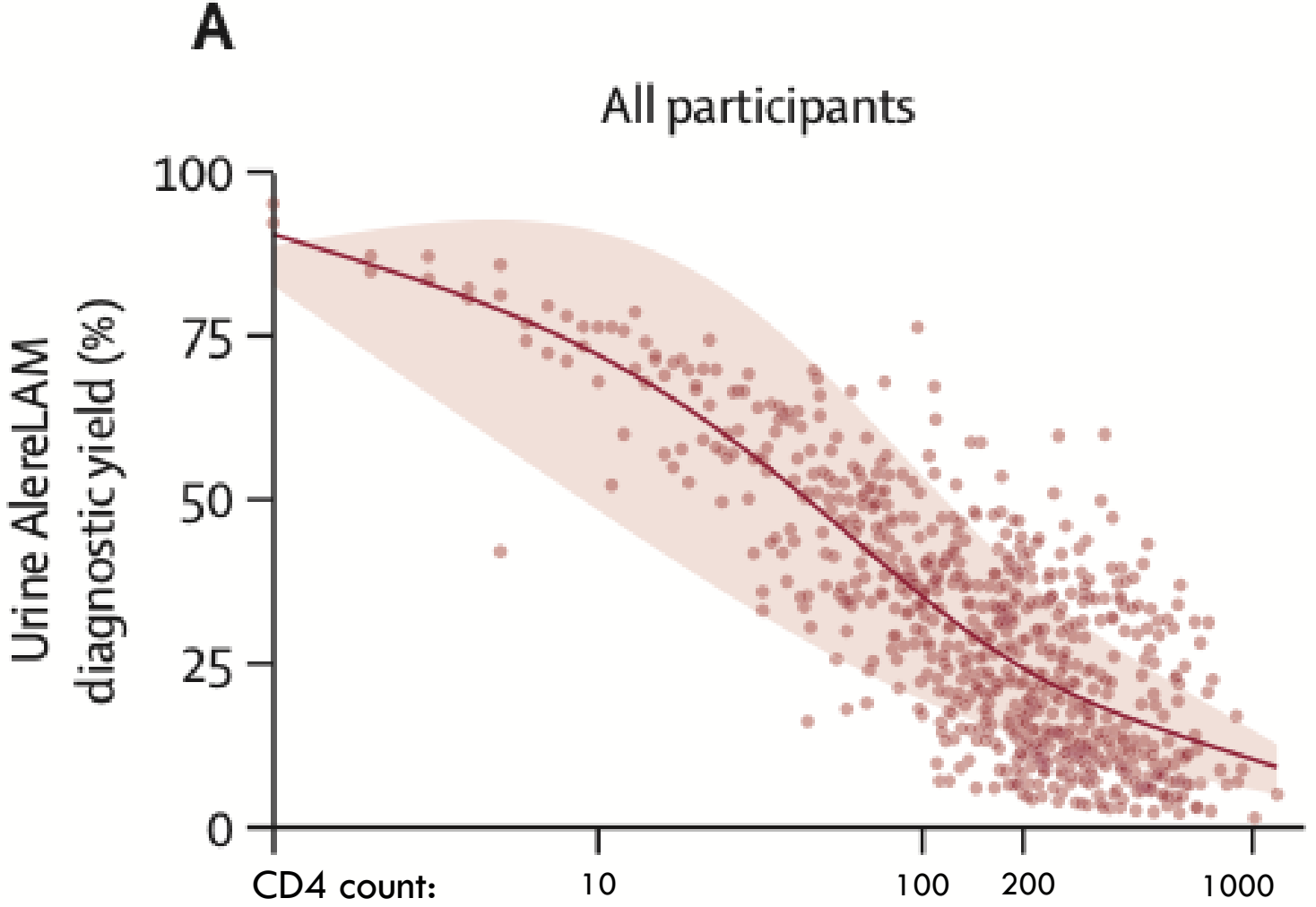
Note: Not all studies contributed to every CD4 stratum.

Figure 7. Plot by CD4 of diagnostic accuracy in adults with signs and symptoms of tuberculosis. (A) Sensitivity by CD4 strata; (B) Specificity by CD4 strata. The circle represents the pooled estimates (median), with bars representing 95% credible intervals.



META-ANALYSIS: LAM

Lancet Glob Health . 2023 Jun;11(6):e903-e916



INPATIENTS STRATIFIED BY CD4 COUNT

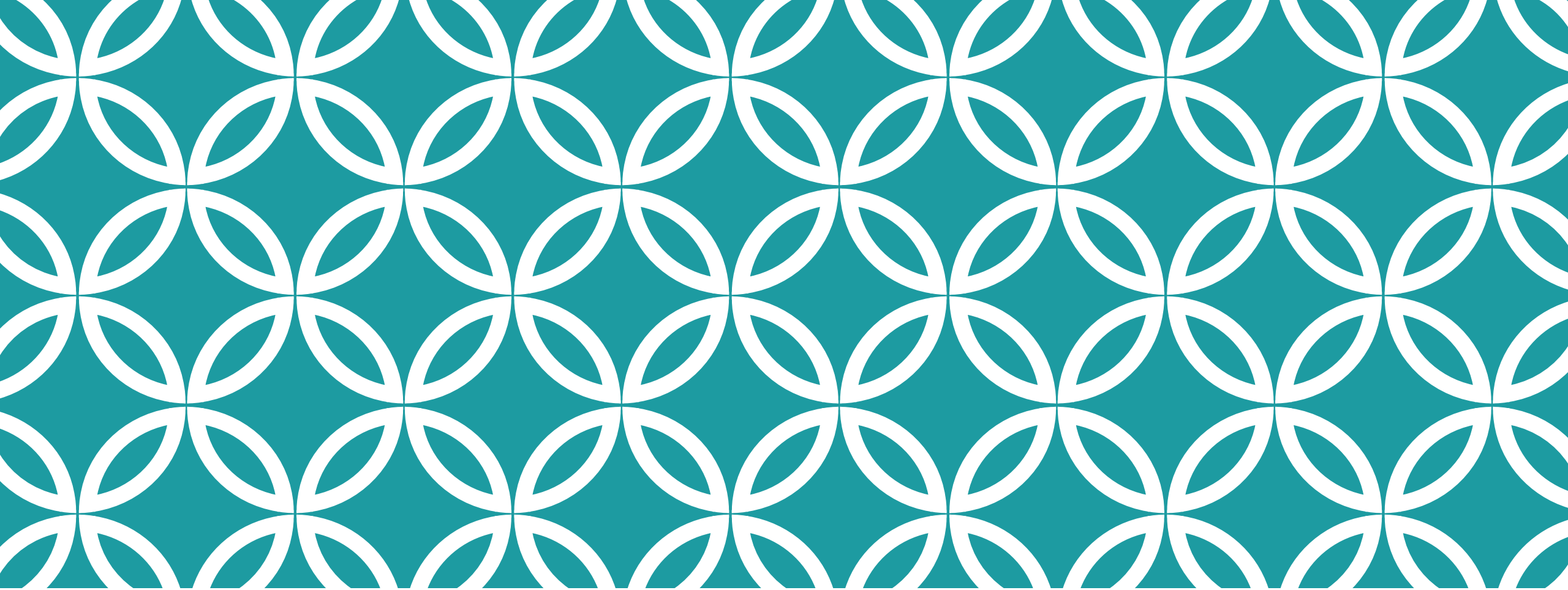
Inpatients with CD4 ≤ 100

Sensitivity 61% (40-78%)
Specificity 81% (61-91%)

Inpatients with CD4 101-200

Sensitivity 32% (16-57%)
Specificity 81% (55-92%)

2019 Cochrane Database of Systematic Reviews (Bjerrum et al.)



LAM ALONGSIDE OTHER TESTS

LAM AND XPERT MTB/RIF

Xpert MTB/RIF better

Xpert – sens & spec	LAM – sens & spec
61% & 97%	36% & 96%

Better together

Sensitivity 75%

Specificity 93%

Patients unable to produce sputum classified as negatives

LAM AND MYCOBACTERIAL BLOOD CULTURES

Strong correlation:

Of positive mycobacterial blood cultures: **65-73% LAM positive**

Lawn et al. BMC Medicine (2017) 15:67, Nakiyingi et al. BMC Infectious Diseases 2015; 15: 62, Nakiyingi et al. *J Acquir Immune Defic Syndr*. 2014 July 1; 66(3),

LAM AND URINE XPERT MTB/RIF

Urine concentrated by centrifugation first

3 studies:

Study	Of the positive Xperts, percent LAM positive	Of the positive LAMs, percent Xpert positive
Lawn et al. J Acquir Immune Defic Syndr. 2012 July 1; 60(3)	**75** (50.5-90.0)	**50** (31.4-68.6)
Peter et al. PLoS ONE 7(7): e39966, 2013.	**75** (40.9-92.9)	**50** (25.4-74.6)
Lawn et al. BMC Medicine (2017) 15:67	**52** (41.1-63.6)	**83** (70.8-90.8)

PUTTING IT ALL TOGETHER

Lawn *et al. BMC Medicine* (2015) 13:192
Lawn *et al. BMC Medicine* (2017) 15:67

High-burden TB/HIV setting, with *routine* screening of all HIV-infected inpatients

427 patients, 1745 tests (mean 5.6 per patient)

TB diagnosed in 139 patients – 1:3 patients overall (!)

- Urine Xpert (concentrated): 59%
- Sputum Xpert (spot + induced): 42%
- Sputum culture: 42%
- Urine LAM: 38%
- Blood cultures: 30%

Combinations:
Urine culture or Xpert: 66%
Sputum culture or Xpert: 54%

PUTTING IT ALL TOGETHER

Lawn *et al. BMC Medicine* (2015) 13:192
Lawn *et al. BMC Medicine* (2017) 15:67

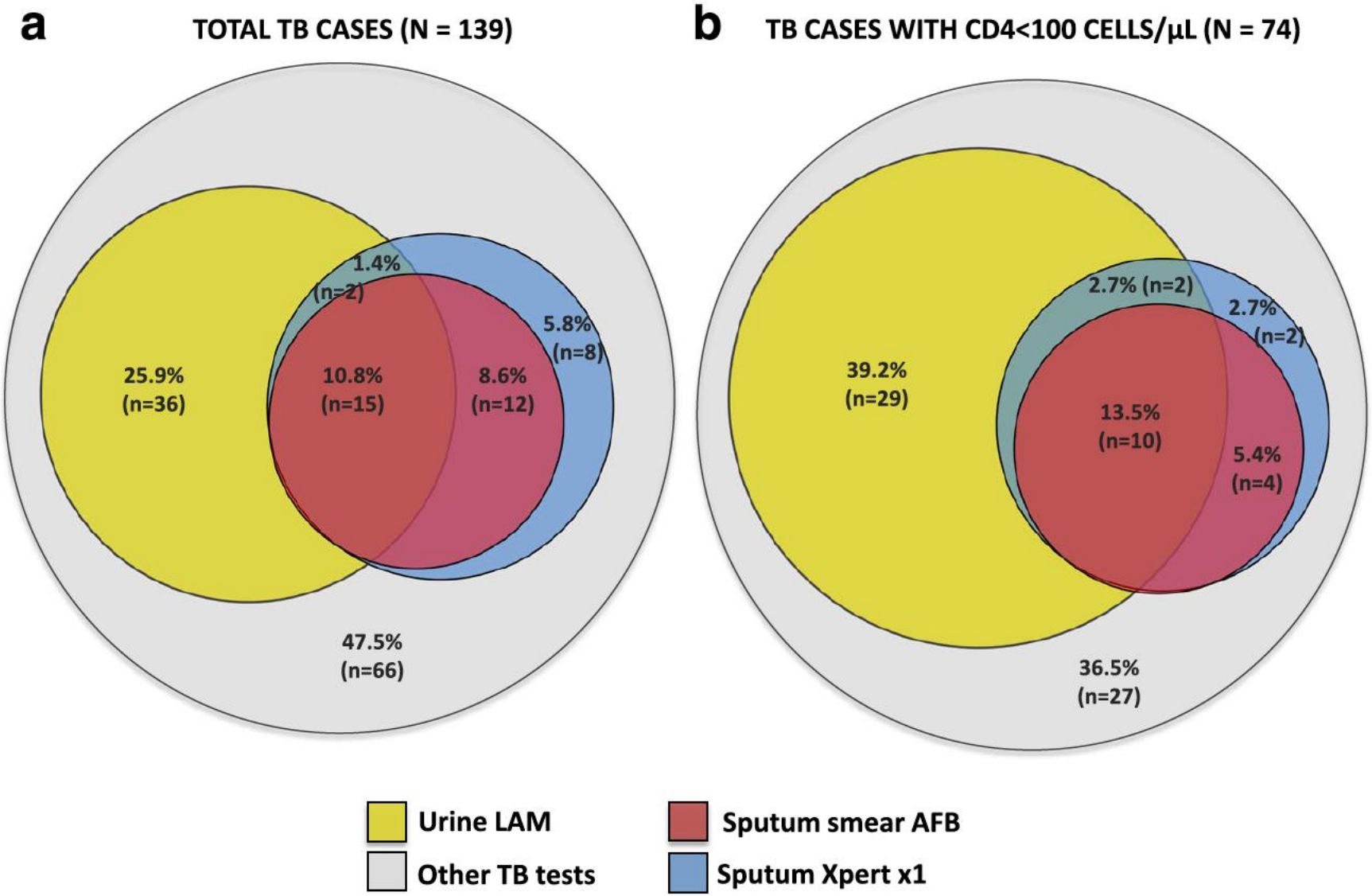
In the first 24 hours, 33% could produce sputum (spot or induced) and 99.5% could produce urine for testing.

<u>Yield:</u>

- Urine Xpert (concentrated) 59%
- Urine LAM: 38%
- Sputum Xpert (spot + induced): 28%

Combinations:
- Urine and sputum Xpert: 78%
- Urine and sputum Xpert, and urine LAM: 84%

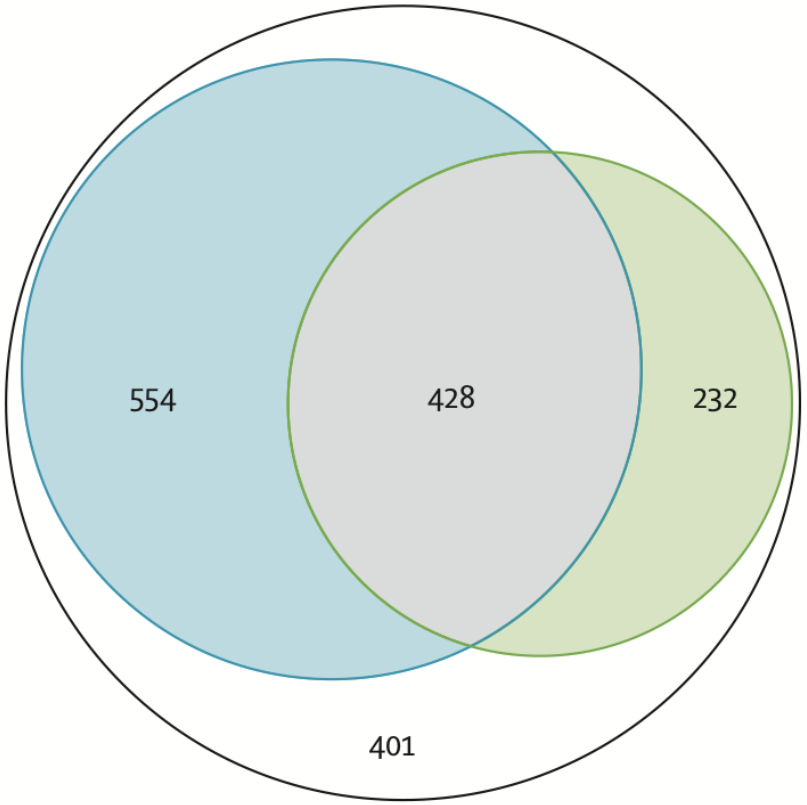
FIRST 24 HOURS:



Diagnostic yield of urine lipoarabinomannan and sputum tuberculosis tests in people living with HIV: a systematic review and meta-analysis of individual participant data

Lancet Glob Health 2023;
11: e903–16

B 2-day diagnostic yield using the MAD (n=1615)

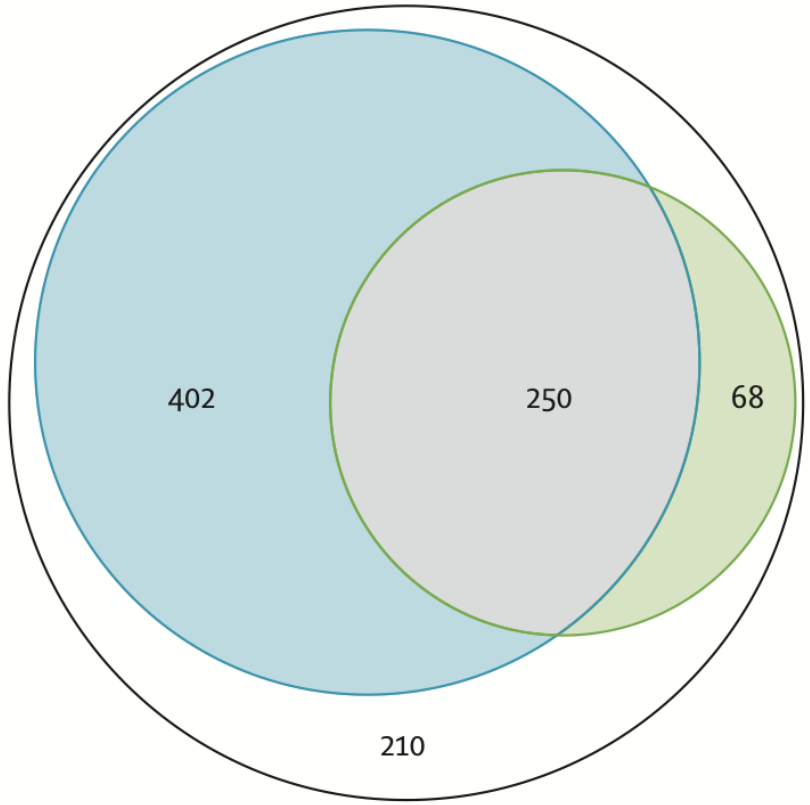


Urine AlereLAM=41% (660/1615)
Sputum Xpert=61% (982/1615)
Urine AlereLAM and sputum Xpert=75% (1214/1615)
Tuberculosis cases missed=23% (401/1615)

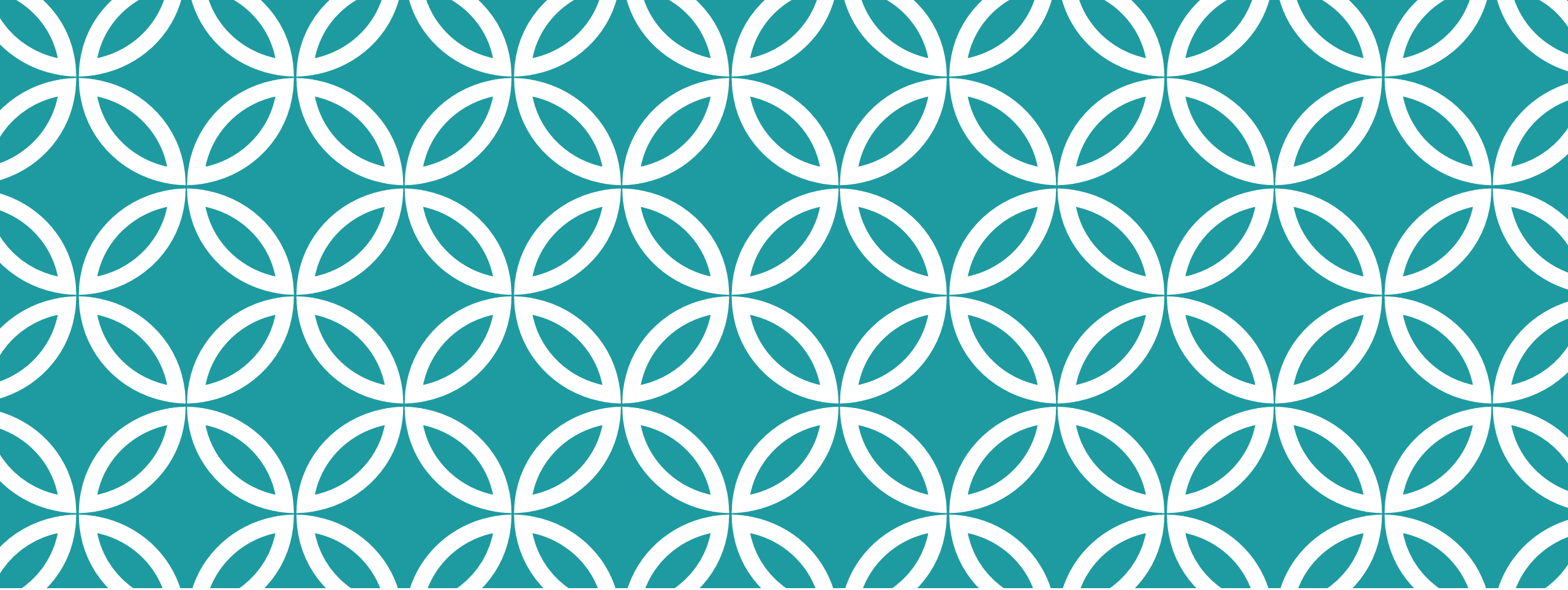
C 2-day diagnostic yield in inpatients using the MAD (n=685)

Urine AlereLAM=50% (342/685)
Sputum Xpert=48% (330/685)
Urine AlereLAM and sputum Xpert=72% (494/685)
Tuberculosis cases missed=28% (191/685)

D 2-day diagnostic yield in outpatients using the MAD (n=930)



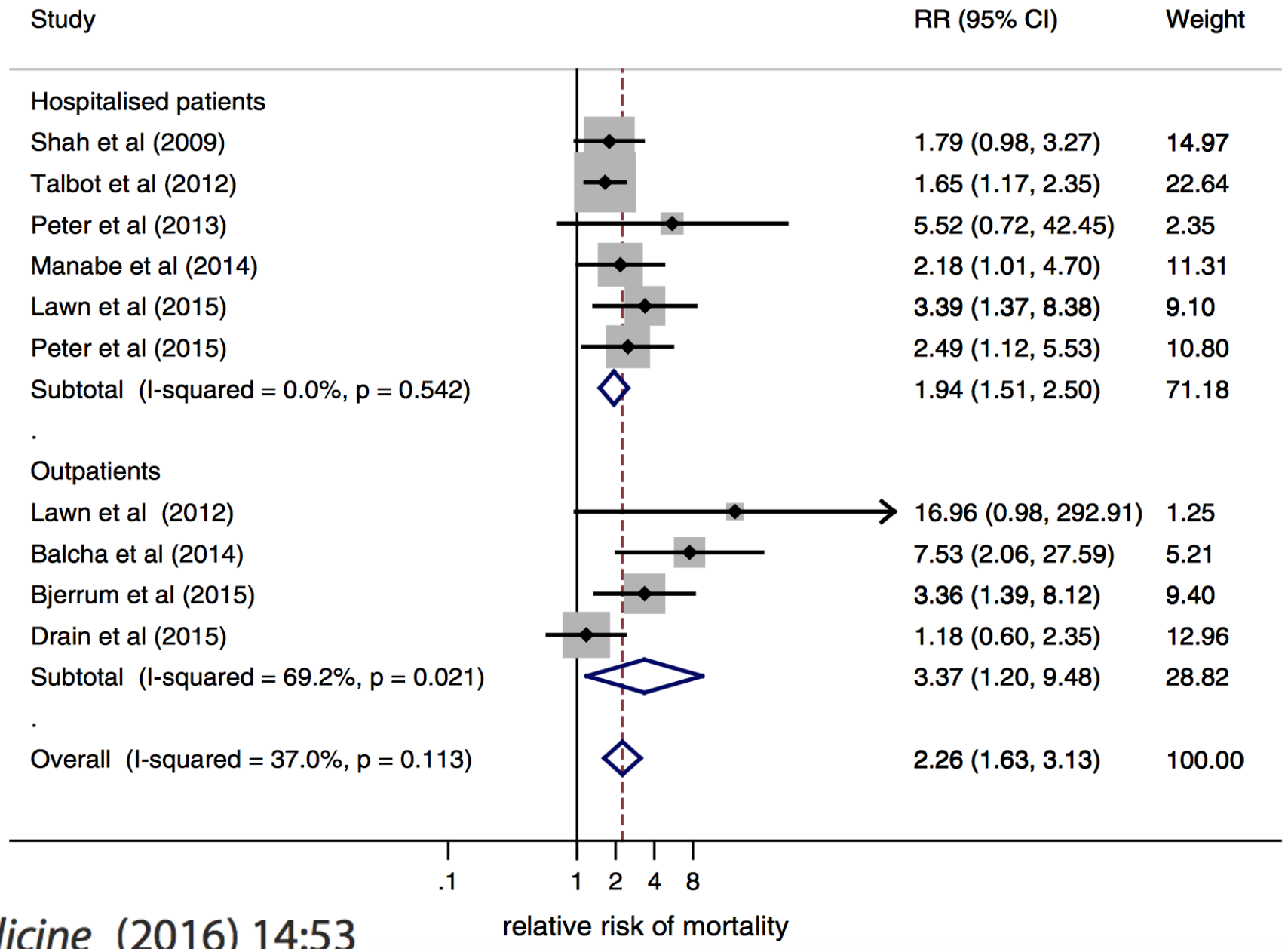
Urine AlereLAM=34% (318/930)
Sputum Xpert=70% (652/930)
Urine AlereLAM and sputum Xpert=77% (720/930)
Tuberculosis cases missed=23% (210/930)



LAM AND MORTALITY

LAM AS A MARKER FOR INCREASED MORTALITY

Two-fold increased morality:
RR 2.3 (1.6-3.1)



Gupta-Wright *et al. BMC Medicine* (2016) 14:53

LAM AS A MARKER FOR INCREASED MORTALITY

Why?

1. Marker of higher mycobacterial burden
2. Proxy for low CD4 count
   - But difference persists when controlled for this.
3. LAM itself is immunosuppressive → poorer immunity against TB and other OIs
4. Identifies patients too sick to produce sputum.

Gupta-Wright *et al. BMC Medicine* (2016) 14:53

Impact of LF-LAM on Mortality & TB Treatment Initiation

Nathavitharana et al. Cochrane Database Syst Rev 2021; CD014641 | People living with HIV

INPATIENT SETTINGS

2 RCTs • 5,102 participants

Mortality at 8 weeks

RR 0.85 (0.76 – 0.94)

34 fewer deaths per 1,000
(14 to 55 fewer) • MODERATE certainty

Started on TB Treatment

Increased initiation rate

More patients started on treatment
with LAM-inclusive strategy

OUTPATIENT SETTINGS

1 RCT • 2,972 participants

Mortality at 6 months

RR 0.89 (0.71 – 1.11)

11 fewer deaths per 1,000
(low certainty – direction favors LAM)

Started on TB Treatment

Increased in some analyses

Evidence less consistent than
in inpatient settings

Main takeaway: In inpatient settings, LAM likely reduces 8-week all-cause mortality. In outpatient settings, there is a possible mortality reduction at 6 months, but the evidence is less certain.

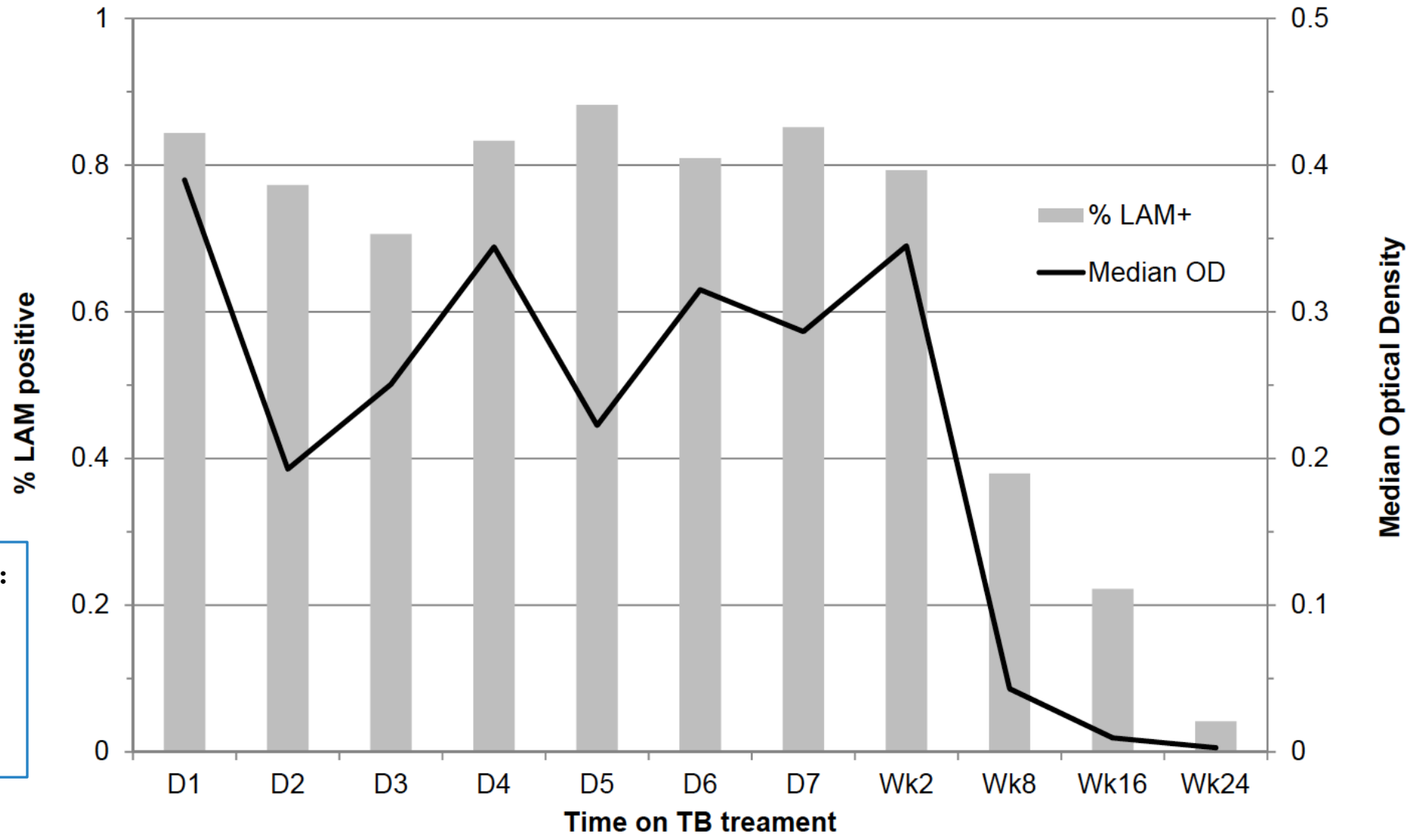
Source: Nathavitharana RR et al. Impact of diagnostic strategies for tuberculosis using lateral flow urine lipoarabinomannan assay in people living with HIV. Cochrane Database Syst Rev. 2021;10:CD014641. doi:10.1002/14651858.CD014641.pub2

Note: Results from RCTs where LF-LAM was used to guide clinical decisions (not just diagnostic accuracy). Benefit appears larger in inpatients and those with CD4 ≤200.

WHAT HAPPENS TO LAM ON TREATMENT?

Wood *et al. BMC Infectious Diseases* 2012, **12**:47

- Stable for 2 weeks
- Declines sharply by 8 weeks
- Gone by 6 months



Of those LAM positive at baseline:

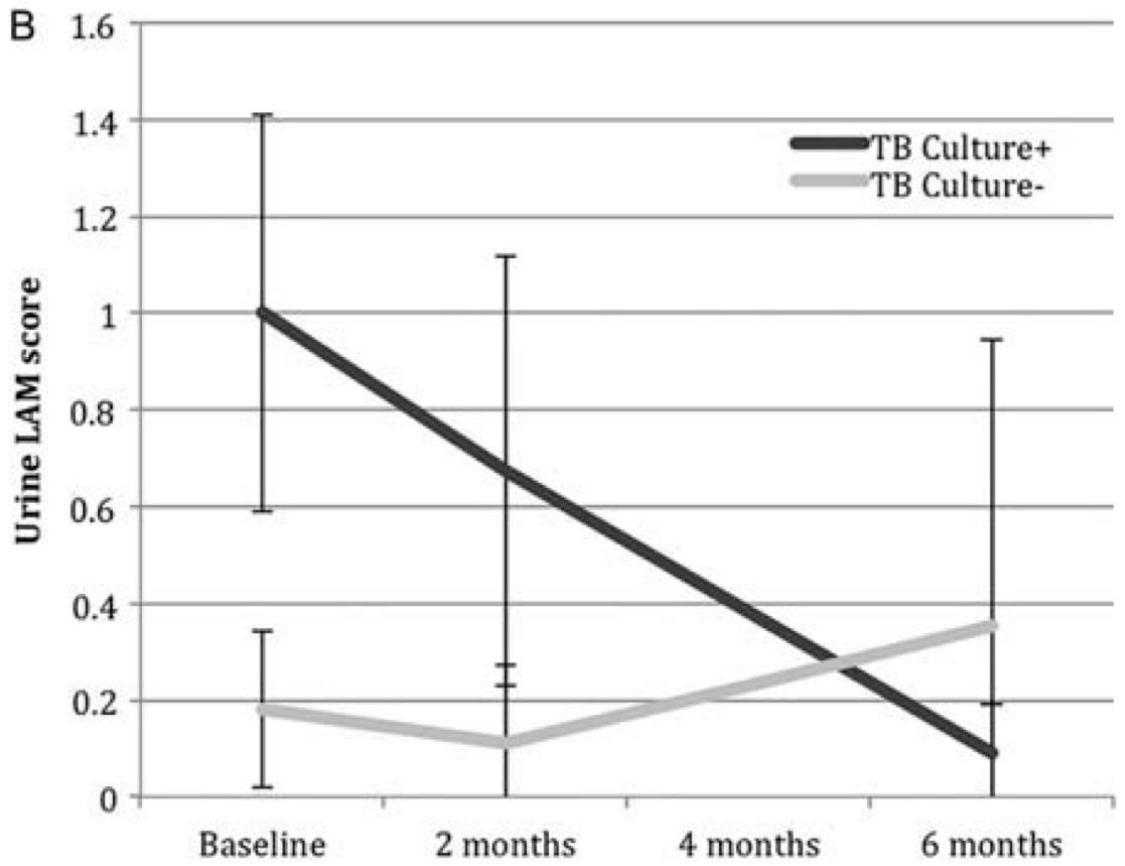
- ~70% positive at 2 weeks
- ~33% positive at 8 weeks
- ~20% positive at 4 months
- 3% positive at 6 months

LAM Pos Tests	27	17	12	15	15	17	23	23	11	6	1
Total Tests	32	22	17	18	17	21	27	29	29	27	24

BMJ Open Urine lipoarabinomannan to monitor antituberculosis therapy response and predict mortality in an HIV-endemic region: a prospective cohort study

LAM positive among TB culture positive:

27% at baseline
16% at 2 months
6% at 6 months

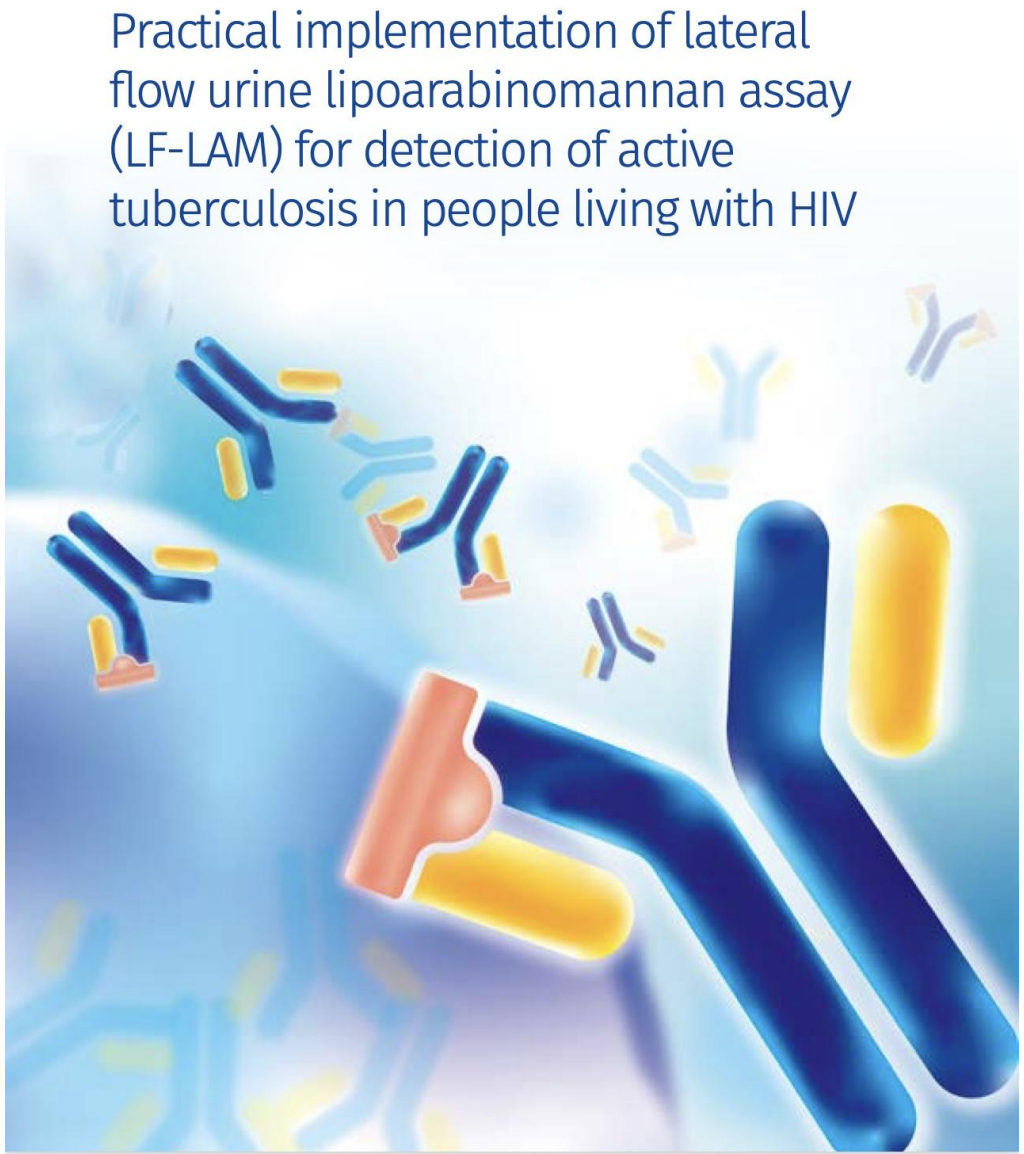


LAM positivity at 6 months was associated with increased mortality over 3-year follow-up:

aHR 42 (2-950), p=0.02

Drain PK, *et al. BMJ Open* 2015;**5**:e006833. doi:10.1136/bmjopen-2014-006833

HOW TO DO A URINE LAM TEST



Global Laboratory Initiative (GLI)

Developed under coordination of the GLI Working Group Secretariate, WHO Global TB Programme / Stop TB Partnership, 2021

https://internationalbiosafety.org/wp-content/uploads/2021/03/practical-implementation-lf-lam.pdf

URINE SPECIMEN

Patient should clean and wipe dry the urogenital area before collecting urine. Collect midstream urine.

Ideally test a fresh urine sample.

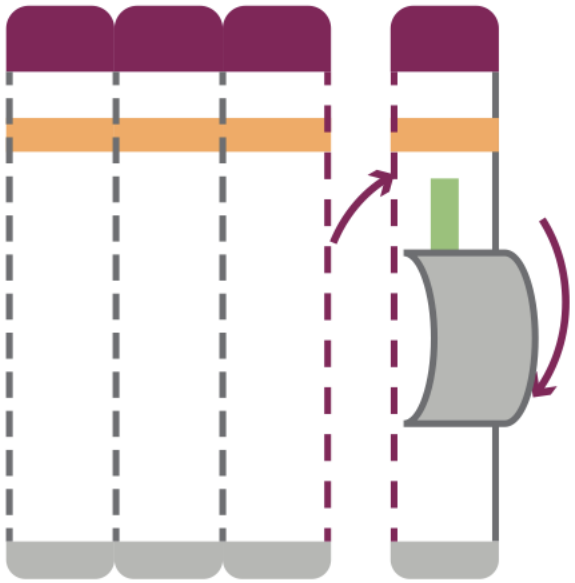
If you can't test immediately, can store urine at room temperature for max 8 hours, or at fridge temperature for maximum 3 days.

- if refrigerated, wait to get to room temperature before testing.

Ideally test **early morning urine** (higher sensitivity).

1 Prepare test

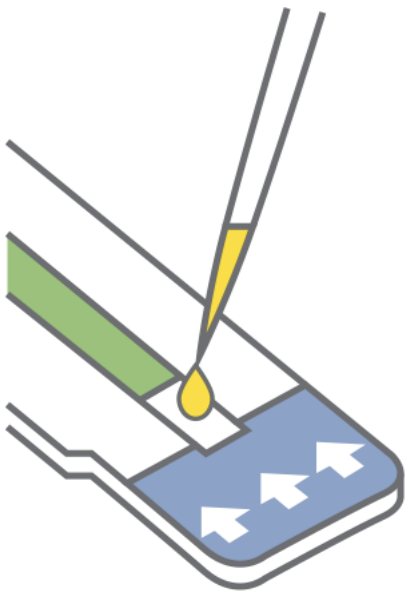
Tear one strip from the right side of the test card and remove the foil cover.

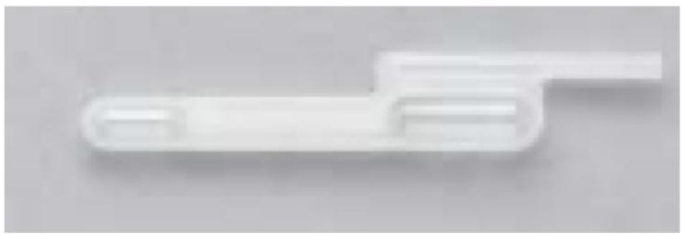


Kits should be stored at 2-30°C
Check expiry date

2 Add sample

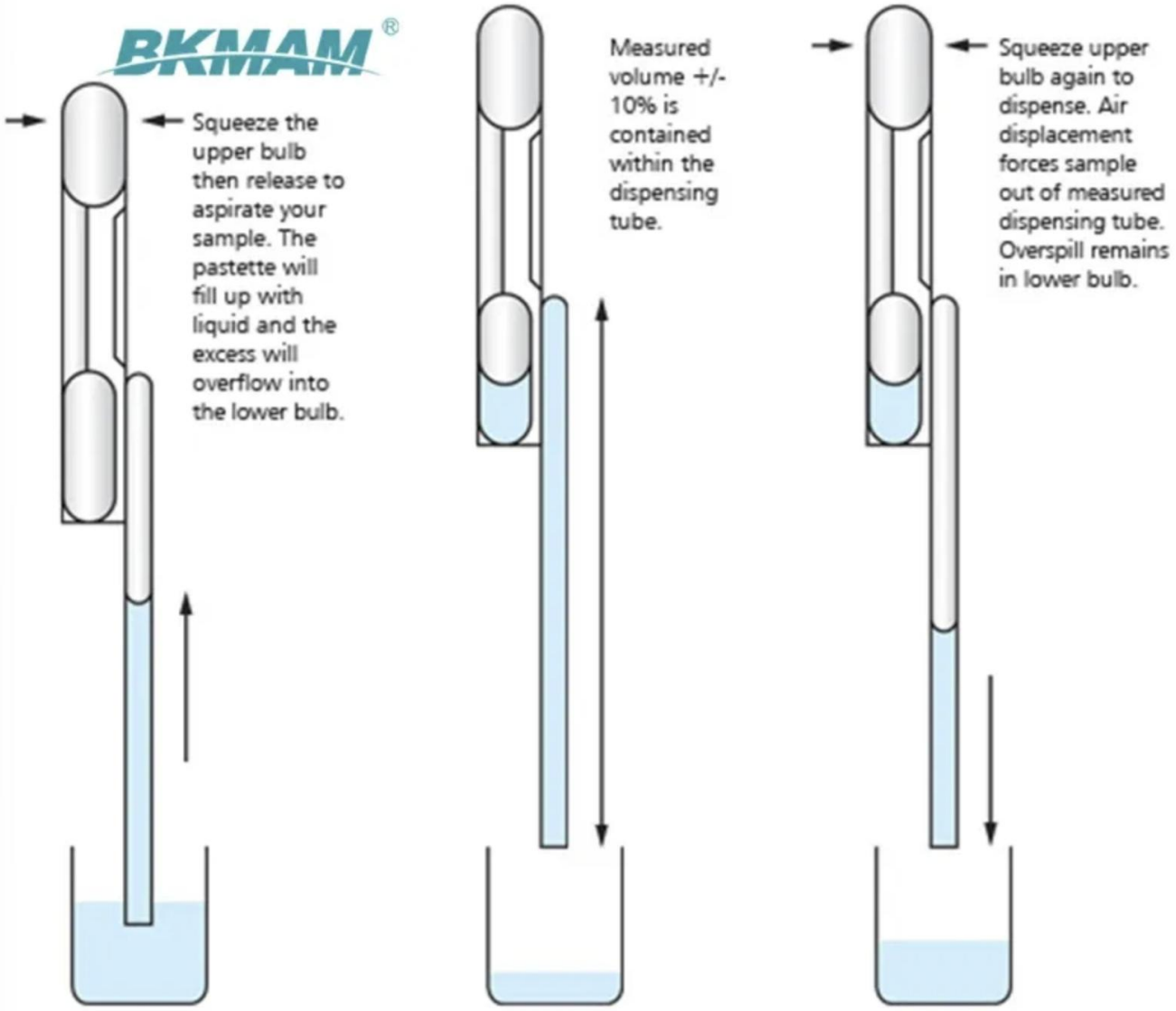
Add 60 µL of urine to the sample pad.





Dual-bulb 60 µL micropipette

How to use double bulb pipette?



Attention: when use the double bulb pipette, please make sure it is vertically straight.

https://bkmbio.en.made-in-china.com/product/mnJrQxOoWukE/China-60UL-100UL-Fixed-Volume-Pipette-Sterile-LDPE-Dual-Bulb-Pipet-for-Laboratory-Liquid-Transfer.html

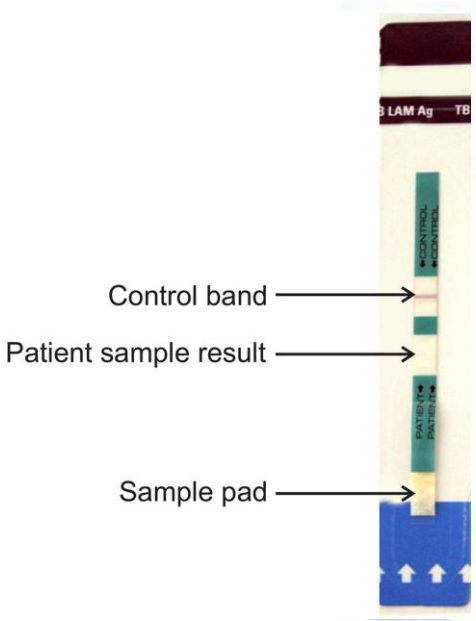
WHAT HAPPENS IF YOU PUT THE WRONG AMOUNT OF URINE ON THE STRIP?

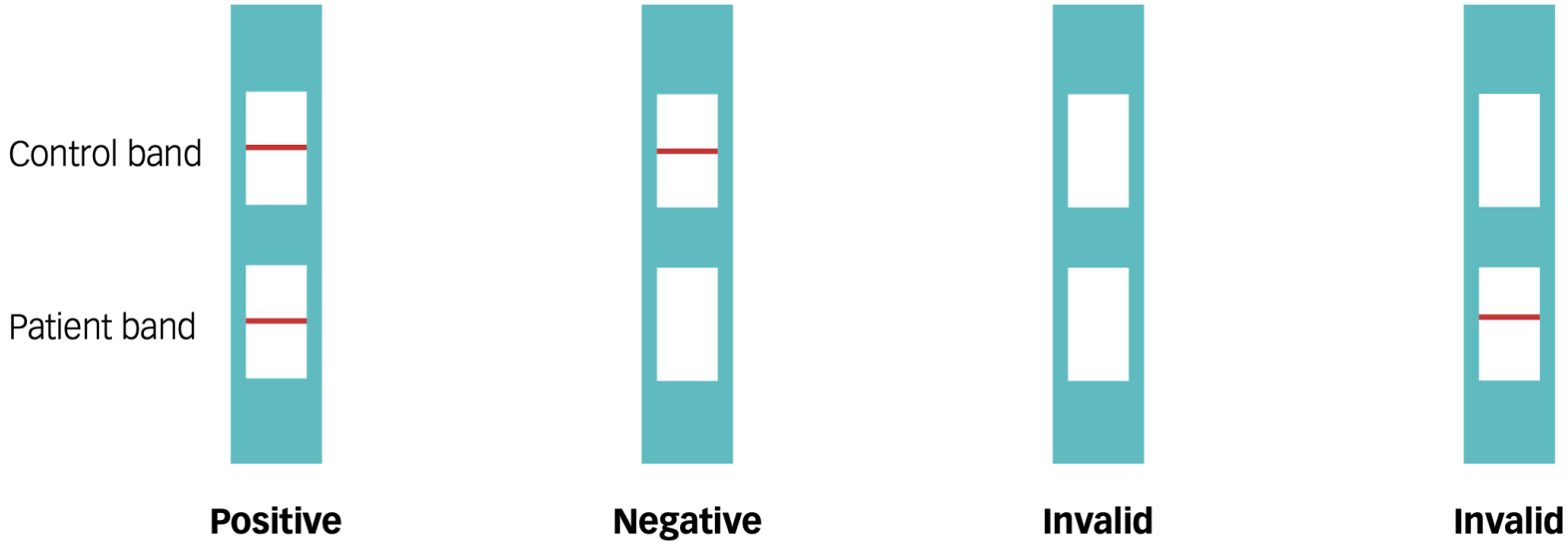
More than 60 µL: increased background staining, can cause the test to fail in unpredictable ways (mostly either false positive or invalid results)

Less than 60 µL: Can cause test to be invalid (weak/absent control line) or false negative.

3 Read results

Wait 25 minutes and then read the results.





What Happens If You Read Too Early (< 25 minutes)

Effect	Consequence	Risk Level
Reaction not complete	Test line may be weak or not yet visible	High
Control line may still be developing	Can appear faint	Medium
Band intensity lower than final	True positive may be read as negative or lower grade	High
Overall result	**False negative** or under-graded result	High

What Happens If You Read Too Late (> 35 minutes)

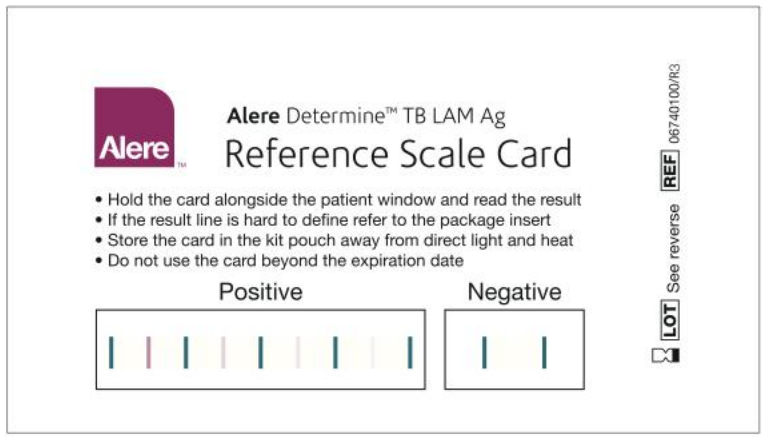
Effect	Consequence	Risk Level
Continued development / background increase	Test line can become darker or smear	High
Non-specific binding	False-positive lines can appear	High
Control line may weaken or bleed	Can make interpretation difficult	Medium
Overall result	**False positive** or over-graded result	High

The test result is positive even if the patient bar appears lighter or darker than the control bar.

As long as there is a control line present, it is a valid test even if control line is lighter than the patient line.

4 Check results

Compare the results with the Reference Scale Card.



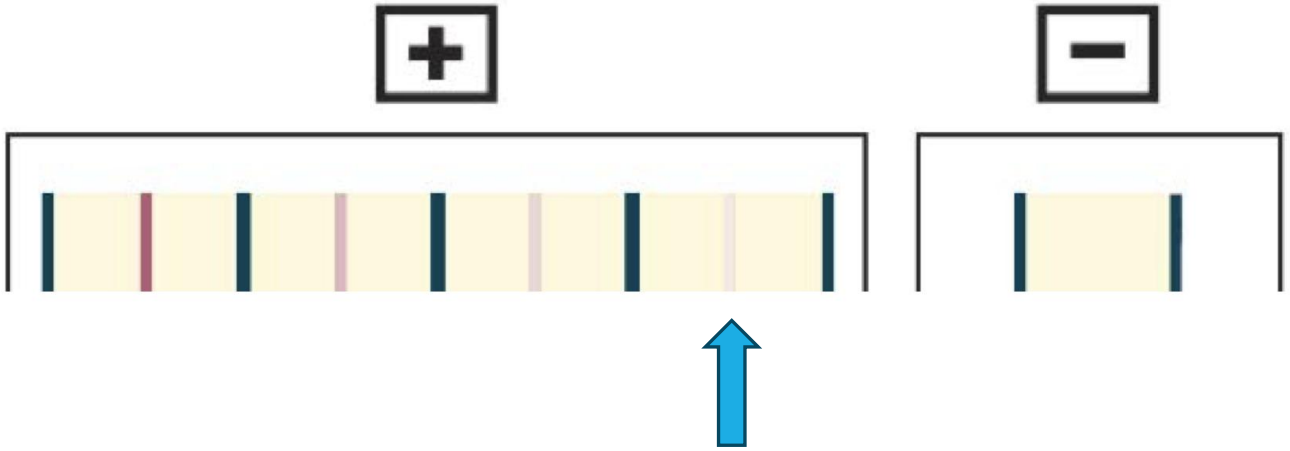
DETERMINE™

TB LAM Ag

Abbott

REFERENCE SCALE CARD

SCALE CARD



REF 06740101/R1

1+ band

A positive result is at one least as strongly positive as the 1+ band. A line less faint than the 1+ band is indeterminate (not positive!); ideally repeat on another sample (early morning).

13. Recording of U-LAM Test Data

- Record the date for LF-LAM specimen collection and results (Positive / Negative) in the patient file. This is the responsibility of the nurse who collects the specimen and it must be done daily.

- Record the date for LF-LAM specimen collection and results (Positive / Negative) in the TB Identification register (GW/13 version 2020) under non-bacteriological investigations conducted section. This is the responsibility of the nurse and it must be done daily.

- LF-LAM test data must be captured into the TB Module in Tier.NET (TB Identification module), using the TB Identification register as a source document for capturing. This is the responsibility of the data capturer and it must be done daily.

- At the end of the month, the clinician must collate data for the LF-LAM tests done with their respective results and complete the monthly summary sheet attached at the back of the TB ID register.

Please Note: For more detailed information, refer to the developed TB Monitoring and Evaluation Standard Operating Procedures.

National Tuberculosis Programme. Guidance document 2021.

WHO SHOULD GET A URINE LAM?

South African Guidelines: Who Should Get Urine LF-LAM?

National Department of Health Guidance (2021)

INPATIENTS

All people living with HIV who are admitted to hospital

- ✓ Irrespective of TB signs/symptoms
- ✓ Irrespective of CD4 count
- ✓ Irrespective of advanced HIV disease status

Broadest eligibility — use in all admitted PLHIV

OUTPATIENTS

With signs & symptoms of TB (PTB and/or EPTB)

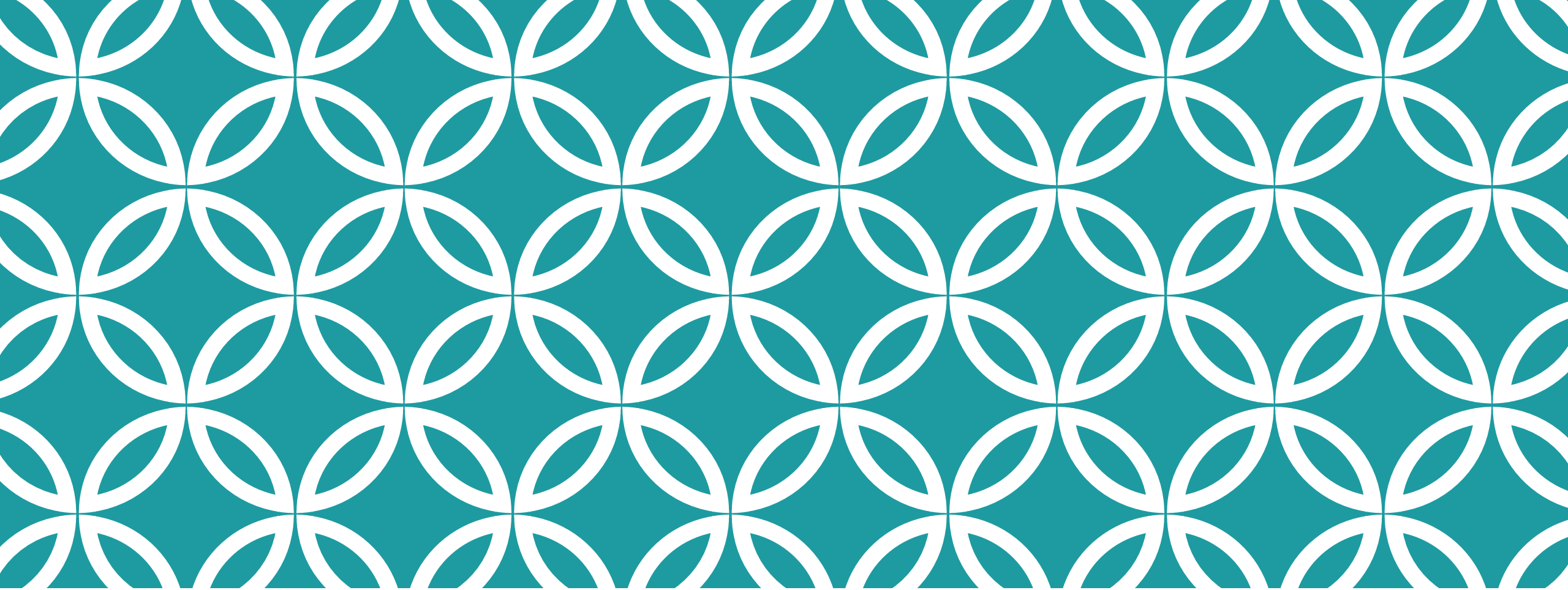
AND one of the following:

- CD4 count < 200 cells/μL
- Advanced HIV disease (WHO stage 4)
- Seriously ill

Targeted to higher-risk symptomatic outpatients

South Africa has broader inpatient eligibility than the 2019 WHO recommendations

Source: National Department of Health, South Africa. Guidance on the use of the Lateral Flow Urine Lipoarabinomannan assay (LF-LAM) for the Diagnosis and Management of Active Tuberculosis in PLHIV. 2021. (Adapted from WHO 2019 policy update)



BARRIERS TO IMPLEMENTATION

1. HEALTH SYSTEM & LOGISTICS

Supply chain and stockouts

Weak integration into existing workflows

- Seen as an "extra" test with own algorithm

Slow/uneven guideline uptake

- Facility-level implementation can lag

2. HEALTHCARE WORKER KNOWLEDGE

Poor awareness of eligibility criteria

Interpretation difficulties

- Faint bands, control vs patient band.
- Hesitation to start TB treatment, esp. if sputum results negative and no clear TB symptoms.

Operational mistakes

- 25-35 mins, expired kits, too much/little urine added

3. OPERATIONAL BARRIERS

Workflow disruption

- Can feel burdensome in busy clinics/wards

Linkage to treatment

Staff turnover - incomplete training

SUMMARY QUESTIONS:

1. LAM performs best in:

A. Inpatients with low CD4s
B. Outpatients with low CD4s
C. Inpatients with high CD4s
D. Outpatients with high CDs

SUMMARY QUESTIONS:

2. All the following patients are eligible for urine LAM testing EXCEPT (i.e. which is wrong):

A. Inpatients regardless of CD4 status
B. Outpatients with CD4 <200 who have symptoms of TB
C. Inpatients regardless of HIV status
D. Outpatients who with CD4 <200 who are seriously ill

SUMMARY QUESTIONS:

3. When should you read a urine LAM result?

A. 10-20 mins
B. 25-35 mins
C. Immediately
D. After an hour

SUMMARY QUESTIONS

4. Which of the following is/are a positive result?