

TB LAM URINE ASSAY – A SIMPLE & INEXPENSIVE POINT OF CARE TEST FOR DETECTING TB IN HIV+ & HIV- PATIENTS

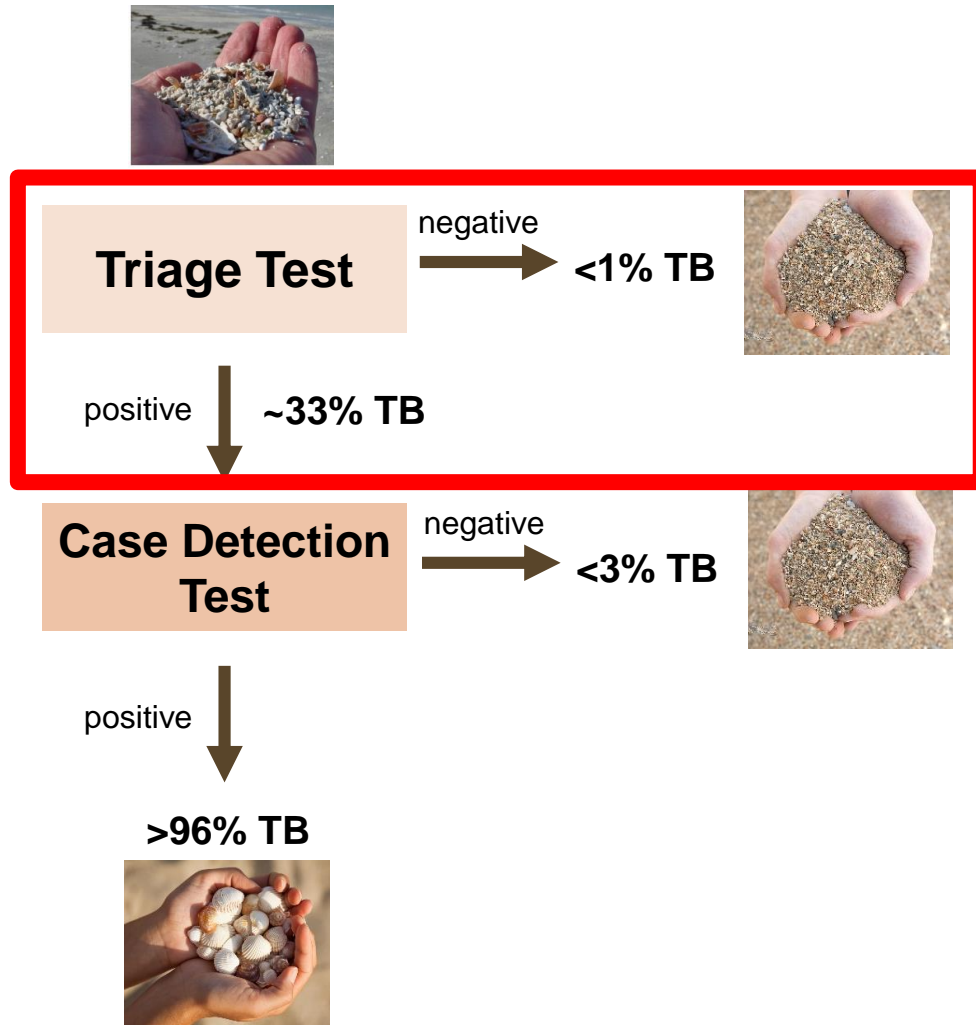
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■ OUTLINE OF THE PRESENTATION

- Using multiple TB tests to maximize TB diagnostics
- Requirements and intended use for the urine-based TB LAM test
- Where are we now?
- How can we improve the TB LAM test so that it may be used for HIV+ AND HIV- patients?
- Creating a “best of the best” TB LAM test
- How should the “best of the best” TB LAM test be used?

TB AND DIAGNOSTICS – MAKING AN IMPACT



- ❑ TB is 1 of the top 10 causes of death worldwide.
- ❑ From a study of 11 countries, it is estimated that ~25% of people with TB are not diagnosed, totaling >2 million.
- ❑ Since TB is easily spread by from person to person through the air, rapid diagnosis, patient entry into healthcare system, and treatment could have a major impact on regional and global health.
- ❑ One strategy to increase TB diagnosis is to use a **combination of tests** that serve different functions:
 - **TRIAGE TEST** – rapidly and inexpensively identify which TB symptomatic patients require a confirmatory test for definitive diagnosis (many of these people will NOT be shown to have TB); for the patients who test negative, very few will actually have TB (few false-negatives).
 - **CASE DETECTION TEST** – more accurately test which patients with a positive triage test actually have TB.

A TALE OF TWO TESTS

LAM IMMUNOASSAY

Triage test
Urine specimen
Inexpensive
No instrumentation
Point of care setting
Very rapid
Rule out TB (high NPV)

Existing lateral flow assays are not sensitive enough to detect LAM in TB+ HIV- patients

Tests are likely only cost-effective in the setting of very high disease prevalence

NUCLEIC ACID TEST (NAT)

Case detection test
Sputum specimen
More expensive
Simple instrumentation
Microscopy center setting
Rapid
TB diagnosis (high PPV & NPV)

Existing nucleic acid tests are too complex and expensive for use in the point of care setting

Sputum is difficult to collect from some patients

Tests are not likely cost-effective for use in triage (e.g. to rule out TB)

TB TRIAGE TEST – WHO 2014 TPP SPECIFICATIONS

Characteristic	Optimal	Minimal
Diagnostic sensitivity	> 95% pulmonary TB	> 90%
Diagnostic specificity	> 80%	> 70%
Time to result	< 5 minutes	< 30 minutes
Price	< \$1.00	< \$2.00
Instrument/capital cost	none	< \$50.00

Most important

Goal: a rapid, inexpensive point-of-care test that can RULE OUT TB (e.g. accurately identify true negatives).

THE INTENDED USE OF THE IMPROVED SENSITIVITY TB LAM ASSAY

- HIV+ or HIV- patients seeking care for TB-related symptoms
- Outpatient setting
- Level 0 or Level 1 care facility
- Test performed by unskilled operator, without an instrument
- Reflex positive patients for definitive diagnosis with case detection test (such as GeneXpert, MolBio TrueNat, culture, etc.)
- Work up negative patients for alternative diagnosis



HOW FAR AWAY ARE WE FROM HAVING A TEST FOR ALL TB PATIENTS?

	Sensitivity	Specificity	HIV-	Triage
WHO optimal (triage)	>95%	>80%	yes	yes
WHO minimal (triage)	>90%	>70%	yes	yes
Alere Determine™ TB LAM*	42%	95%	no	no
Fujifilm SILVAMP TB LAM*	70%	91%	no	no

To get to the goal, we still need:

1. Increased test sensitivity to detect very low levels of LAM
2. Detection of TB in all patients, including HIV- and CD4+ >100 cells/mL
3. Ability to detect all forms of LAM (regardless of LAM levels)



* Broger et al, Lancet Infect Dis, 2019; median CD4+ 86 cells / mL, inpatient, 62% prevalence

THE BIG QUESTION – CAN A LAM URINE ASSAY ACHIEVE A CLINICAL SENSITIVITY TO MEET THE WHO 2014 TPP FOR TB TRIAGE IN TB+ HIV-?

Question: Is the problem due to assay sensitivity?



- Newer LAM urine lateral flow assays top out at 70 – 80% sensitivity
- High sensitivity assays have achieved 80 – 100% (including HIV-) with various improvements

Question: Is the problem due to LAM heterogeneity?



- Even some highly sensitive assays miss 10 – 20% of positives
- Based on studies of epitope mapping and various different LAM antibodies, LAM is heterogeneous
- A recent, unpublished study of patients in Peru suggests that LAM may be detected in all TB+ HIV- patients, however LAM is not universally detectable with a single antibody

INITIAL STEPS TO DEVELOP A NEW TB LAM TEST WITH IMPROVED SENSITIVITY AND ABILITY TO DETECT LAM

- ❑ Convene experts to discuss protocol advancements and brainstorm about optimal assay designs
- ❑ Develop standard positive and negative samples for use by investigators to allow cross-comparison
- ❑ Develop a standard LAM reference assay that may be used as a comparator to assess assay performance and the impact of any assay improvements
- ❑ Test each new improvement, singly and in combination, with an emphasis on pre-analytical steps



CREATING A BEST OF THE BEST TB LAM LATERAL FLOW ASSAY – MAKING INCREMENTAL IMPROVEMENTS TO IMPROVE SENSITIVITY & DETECTABILITY

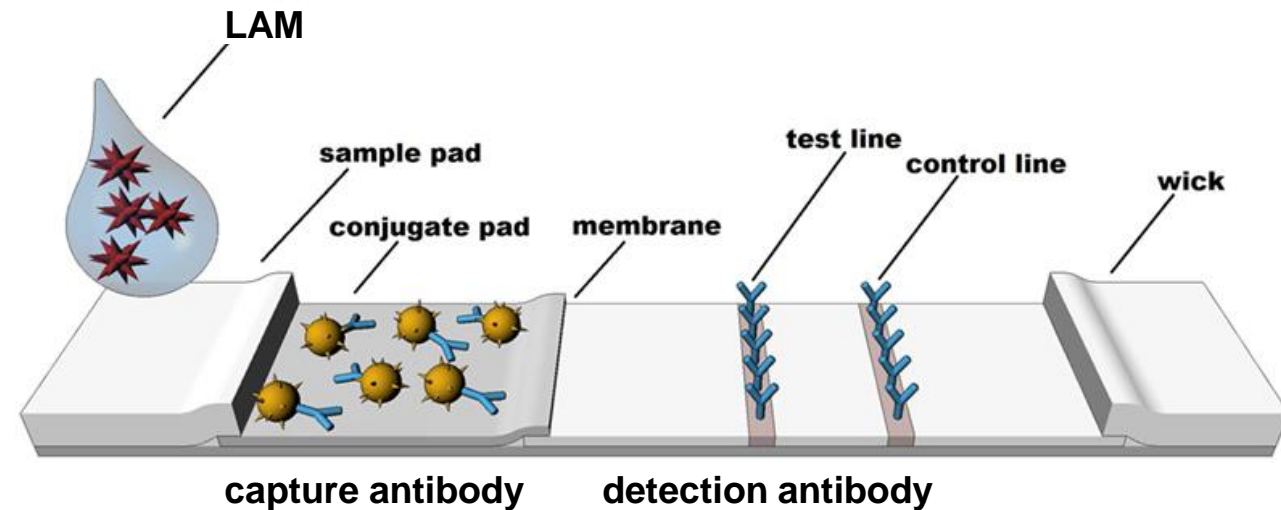
Pre-analytical Processing

LAM Capture on LFA

LAM Detection on LFA



50 – 100X
concentration



TESTING EACH INDIVIDUAL IMPROVEMENT AGAINST THE REFERENCE METHOD USING STANDARD SAMPLES

Preanalytical	LAM liberation (proteinase K) LAM concentration (beads) LAM concentration (nanocages) Novel devices for concentration	Chatterjee lab (CSU) Salus Discovery, Global Good Liotta lab (GMU) Salus Discovery, Global Good
LAM capture	Human antibodies from TB patients Non-human antibodies Engineered antibodies/fragments	Pinter lab (Rutgers) Commercial labs Commercial LFA companies
LAM detection	Human antibodies from TB patients Non-human antibodies Engineered antibodies/fragments Signal amplification	Pinter lab (Rutgers) Commercial labs Commercial LFA companies

THE CHINESE MENU APPROACH TO BUILDING A BEST OF THE BEST TB LAM LATERAL FLOW ASSAY

Pre-analytical Processing

LAM Capture

LAM Detection

Yan Yan
Chinese Cuisine

11:00am – 3:00pm

LUNCH MENU
LUNCH SPECIAL \$7.75

Served with **Steamed Rice** or Pork Fried Rice

CHOICE OF ONE
FROM EACH BOX BELOW:

PICK ONE

- Crab Puff - Egg Foo Young - **Egg Roll**
- Sweet & Sour Chicken - Sweet & Sour Pork
- BBQ Pork - Fried Shrimp (\$0.50 Extra)

Add one additional item \$2.50

PICK ONE

- Black Bean Sauce Beef <small>(or Chicken)</small>	- Mandarin Chicken
- Broccoli w/Beef <small>(Chicken or Pork)</small>	- Mongolian Beef <small>(or Chicken)</small>
- Cashew Nut Chicken	- Orange Chicken <small>(or Lemon Chicken)</small>
- Curry Beef <small>(or Chicken)</small>	- Pea Pod Chow Yuk with Beef <small>(Chicken, or Pork)</small>
- Deep Fried Sesame Beef	- Sesame Beef w/Vegetables <small>(or Sesame Chicken w/Vegetables)</small>
- Sesame Chicken	- Spicy Green Bean Beef <small>(or Chicken)</small>
- General Tao's Chicken	- Spicy Mushroom Beef <small>(or Chicken)</small>
- Ginger Beef <small>(or Chicken)</small>	- Szechuan Beef <small>(or Chicken)</small>
- Hawaiian Chicken	- Teriyaki Chicken
- Kung Pao Beef <small>(or Chicken)</small>	

■ HOW SHOULD THE TB LAM POINT OF CARE TEST BE USED?

1. *For TB screening or case finding?*

~1% prevalence

2. *To prioritize TB symptomatic patients for further TB diagnostic testing?*

~10% prevalence

3. *In a hospitalized setting?*

~30% prevalence



SCREENING APPLICATION FOR TB LAM IMMUNOASSAY?

	Screening		Triage		In-patient	
	1% prevalence		10% prevalence		30% prevalence	
	PPV	NPV	PPV	NPV	PPV	NPV
WHO 2014 TPP – Optimal	4.5%	99.9%	34.5%	99.3%	67.1%	97.4%

1000 people

207 positive calls

793 negative calls

9 true positives

198 false positives

< 1 false negative

792 true negatives

No, too many false-positives!!!!

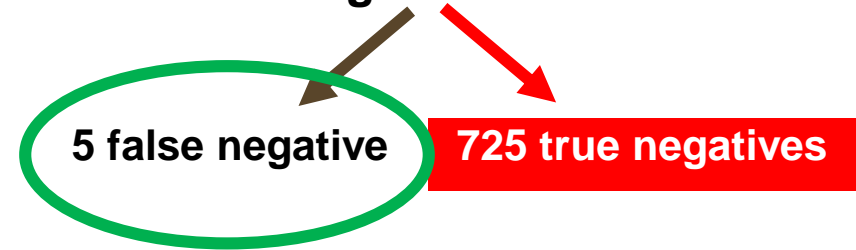
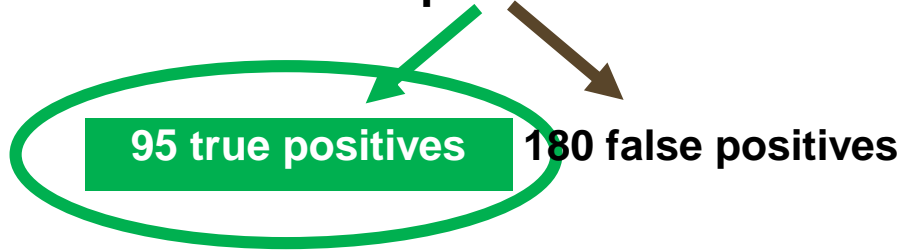
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	Screening		Triage		In-patient	
	1% prevalence		10% prevalence		30% prevalence	
	PPV	NPV	PPV	NPV	PPV	NPV
WHO 2014 TPP – Optimal	4.5%	99.9%	34.5%	99.3%	67.1%	97.4%

1000 people

275 positive calls

725 negative calls

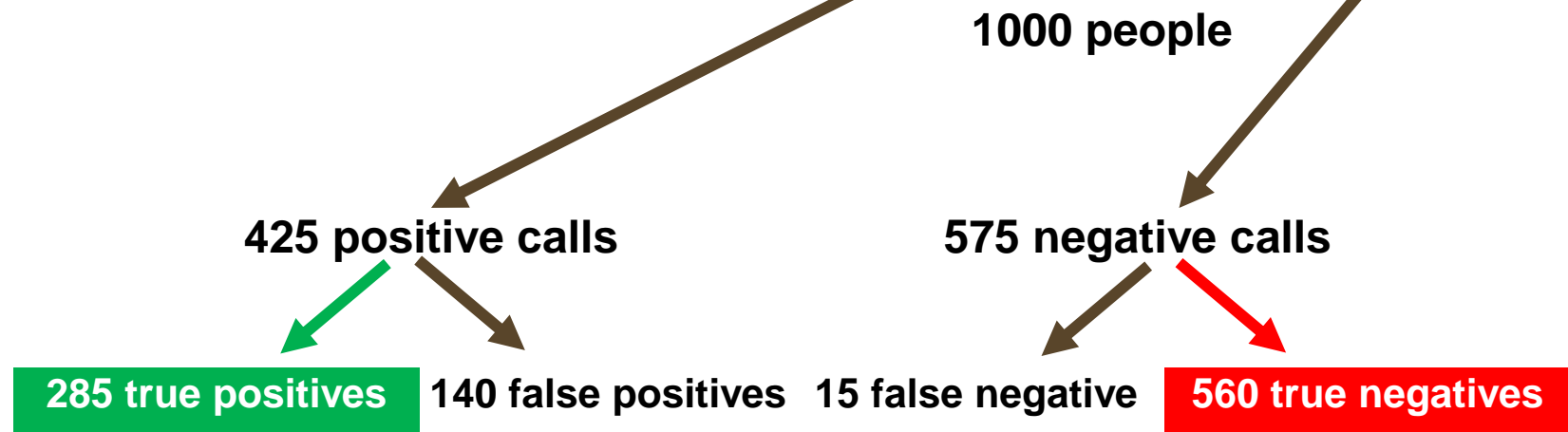


Yes, good enrichment for positives

Yes, few false-negatives

INPATIENT APPLICATION FOR TB LAM IMMUNOASSAY?

	Screening		Triage		In-patient	
	1% prevalence		10% prevalence		30% prevalence	
	PPV	NPV	PPV	NPV	PPV	NPV
WHO 2014 TPP – Optimal	4.5%	99.9%	34.5%	99.3%	67.1%	97.4%



Yes, for patients who cannot produce sputum for Xpert

CONCLUSIONS

- It may be possible to develop a urine-based TB LAM test with improved sensitivity if we combine methods and reagents from multiple groups.
- A urine TB LAM test may be capable of detecting TB LAM in HIV+ and HIV- patients, as well as extra-pulmonary TB.
- Even a TB LAM test with performance that meets the WHO 2014 TPP requirements would not be appropriate for use in a screening setting.
- A urine-based TB LAM test is probably best used in the setting of triage, whereby patients present with TB symptoms, and can be referred for definitive diagnosis with a second, more complex test.

