# Tuberculosis Diagnostics:

## *Moving towards the point-of-care*

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# DISCLOSURE

The following planner/speaker has reported a relevant financial relationship with a commercial interest:

- None.

# Outline

- Overview of POC Diagnostics
- TB Pathogen Biomarkers
- Host Biomarkers for TB
- Conclusion

# POC Diagnostics - History

1957 – Urine dipstick for albumin, blood, and acetone

1962 – First rapid test to measure blood glucose[1]

1993 – Small portable devices measure multiple serum electrolytes[2,3]

2002 –"Medical test conducted at or near the site of patient care"[4]

2012 – First POC test for a human genetic allele[5]

1. Clark LC Jr. Ann N Y Acad Sci, 1962; 2. Woo J. Am J Clin Pathol, 1993; 3. Erickson KA. Clin Chem 1993;
4. Kost GJ. Lippincott Williams & Wilkins; 2002; 5. Roberts JD. Lancet, 2012.

# POC Diagnostics – Scope & Settings

| Disease or Specialty | Diagnostic Point-of-care Test |
|---|---|
| Cardiology | Creatine Kinase-MB; Troponin I; Troponin T; Brain Natriuretic Peptide; N-Terminal Prohormone of Brain Natriuretic Peptide; Human-type Fatty Acid Biding Protein; Myosin Light Chain-1; Myoglobin |
| Endocrinology | Glucose; Hemoglobin A1c; Urine Microalbumin Cholesterol; C-reactive Protein; Lactate |
| Gastroenterology | Fecal Occult Blood; Liver Function Tests |
| Hematology | Hemoglobin; Prothrombin time; D-dimer |
| HIV/AIDS | HIV Antigen; HIV Antibody; CD4 T cell count |
| Infectious Diseases (non-HIV) | Group A Strep; Influenza A & B; Parainfluenza; Respiratory Syncytial Virus; Syphilis; Chlamydia; Falciparum-Malaria; Hepatitis C; Tetanus; Tuberculosis; Cryptococcus; Visceral Leishmaniasis; African Trypanosomiasis |
| Nephrology | Urinalysis; Urine Microalbumin; Serum Creatinine |
| Neurology | Nerve Conduction Device |
| Obstetrics | Pregnancy and Ovulation Prediction Tests |
| Pulmonology | Airflow Meters |
| **Medical Settings** | **Commonly Used Point-of-care Test** |
| Emergency Room | Serum Electrolytes; Medication Levels; Drugs of Abuse; Blood Alcohol Level; Troponin-I; Troponin-T; Lactate; Arterial Blood Gas |
| Intensive Care Unit | Serum Electrolytes; Ionized Calcium; Magnesium; Arterial Blood Gas; Blood pH; Glucose; Lactate; Hemoglobin; Prothrombin Time |
| Primary Care Clinic | Urinalysis; Pregnancy Test; Group A Strep; HIV Antibody; Fecal Occult Blood |

Drain PK et al., Lancet Infectious Diseases, 2013.

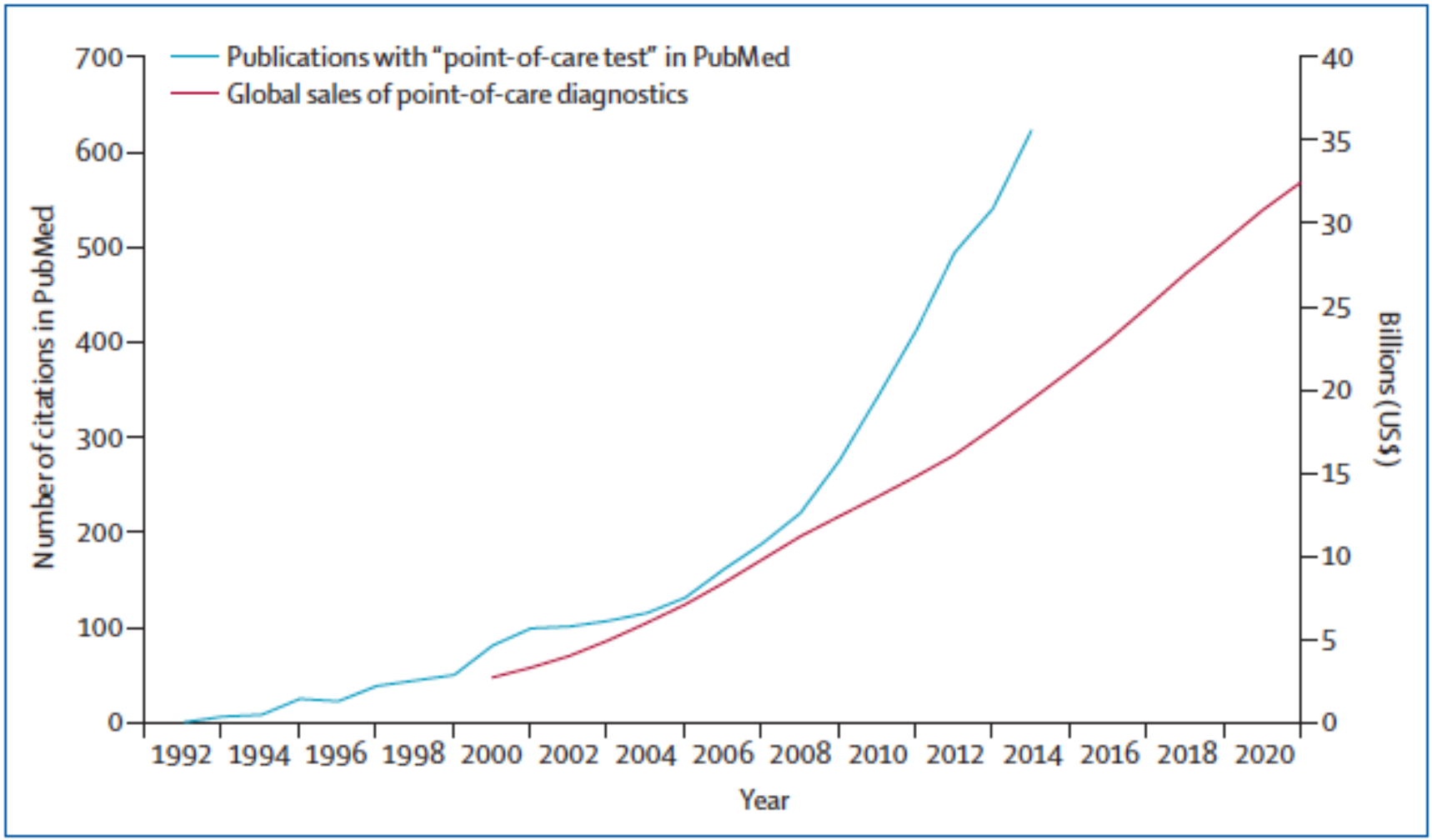



***Figure:*** **Estimated annual research and global market for point-of-care diagnostics**
The annual number of citations was determined by a customised search in the PubMed database for the term "point-of-care test". The estimated global market data were provided by Visiongain.[11]

Drain PK et al., Lancet Global Health, 2015.

# Rapid Diagnostic Test for HIV/AIDS

2002 – First rapid HIV test using finger prick

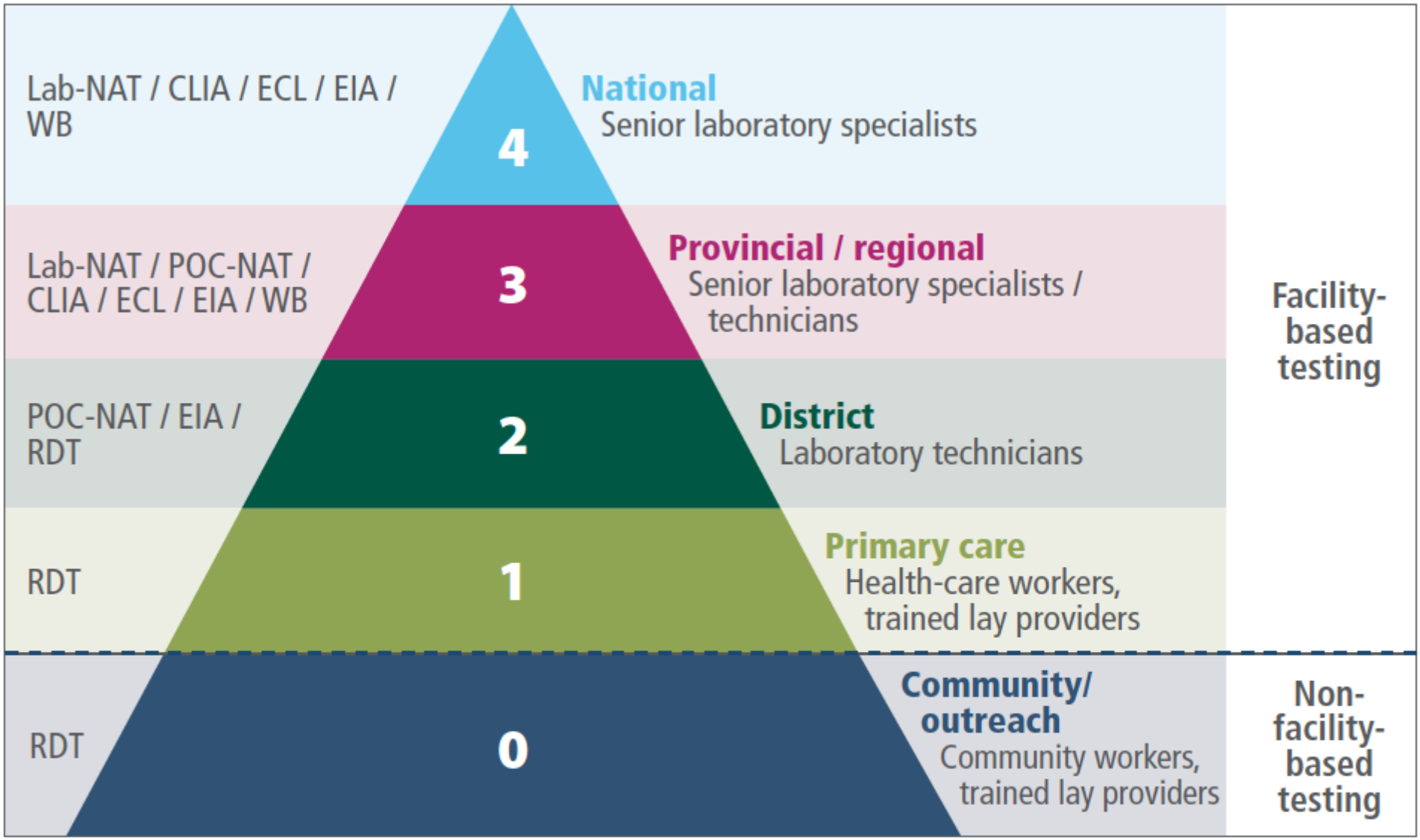
2006 – CDC recommends routine HIV screening in US health care settings

2007 – WHO/UNAIDS recommend routine HIV screening in health care settings

2012 – First rapid HIV test for oral fluid home test

*From 2010 to 2014, used to test **600 million adults** in 122 low- and middle-income countries*

Kaiser Family Foundation, HIV testing in the US, 2014.
World Health Organization. HIV Testing Services. WHO. July 2015.

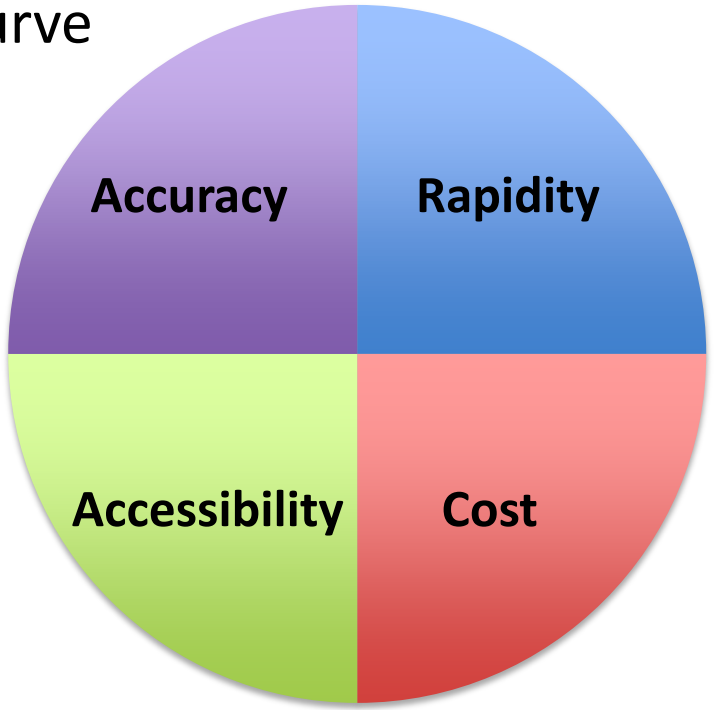



Lab-NAT: laboratory-based nucleic acid testing; POC-NAT: nucleic acid testing at point-of-care; CLIA: chemiluminescence immunoassay; ECL: electrochemiluminescence immunoassay; EIA: enzyme immunoassay; WB: Western blot; RDT: rapid diagnostic test.

WHO. Consolidated Guidelines on HIV Testing Services, 2015.

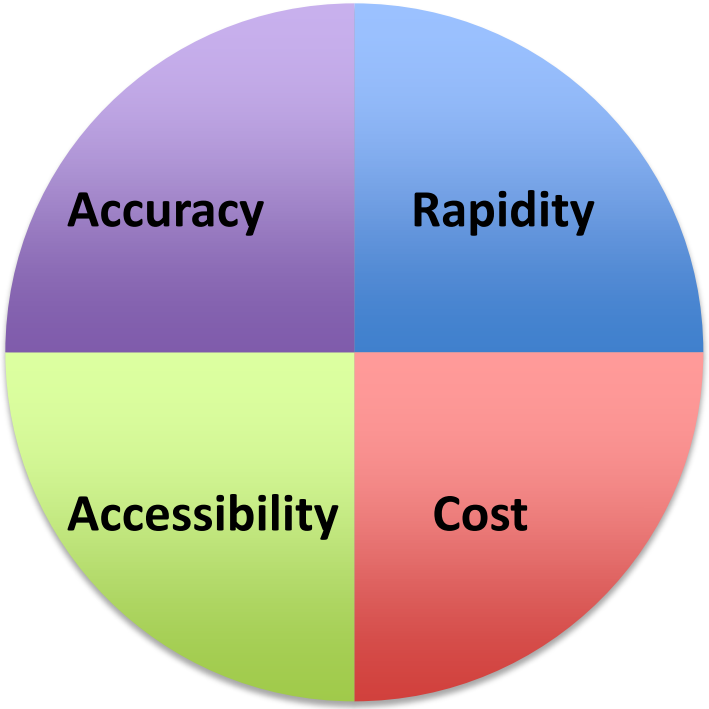
# Evaluation of POC Diagnostics

1. Diagnostic Accuracy
   - Sensitivity/Specificity
   - Likelihood ratio
   - Area under receiver operating curve
2. Clinical Effectiveness
   - Time to therapy
   - Retention in care
   - Survival
3. Cost Analyses
4. Cost-Effectiveness Analysis




Drain PK et al., Lancet Infectious Diseases, 2013.

# Rapid Diagnostic Test for HIV/AIDS




***Accuracy*** – ~98% sensitive/specific

***Rapidity*** – 20 minutes

***Accessibility*** – Lateral flow assay;
Finger prick whole blood

***Cost*** – ~$2/test

Drain PK et al., Lancet Infectious Diseases, 2013.

# POC Diagnostics – Summary

- POC diagnostics are rapidly emerging and evolving
- Potential for real clinical impact, particularly in primary care clinics and community/outreach
- Trade-offs with Accuracy, Rapidity, Accessibility, Cost
- Adoption of a POC test will not always translate to clinical impact or cost-effective results

# Outline

- Overview of POC Diagnostics
- **TB Pathogen Biomarkers**
  - **Xpert MTB/Rif**
  - **Urine LAM**
- Host Biomarkers for TB
- Conclusion

# History of TB Diagnostics

1821 – Laennec invented **stethoscope** and described utility in diagnosing TB

1882 – Koch presented TB bacilli as the infectious agent of TB on March 24

1895 – Roentgen invented **chest X-ray** and used to track TB progression

1890s – Franz Ziehl/Friedrich Neelson developed **acid-fast stain** for TB

1908 – Mantoux developed **tuberculin skin test** for latent TB

1936 – **Solid culture** introduced to grow and identify TB

Robert Koch,
Nobel Prize in 1905

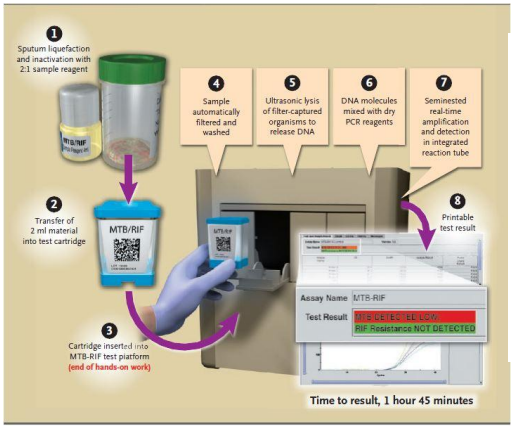
**In 2010, ~53% of clinics in Africa had access to Mycobacterial culture***

1980 – Liquid culture

2008 – Line probe assay

2010 – **Xpert MTB/RIF assay**

2011 – **Rapid LAM assay**




Xpert MTB/RIF assay

Rapid LAM assay

* Saito S, et al. J AIDS 2012.

# Xpert MTB/RIF assay

**Accuracy**

- Cochrane Review (27 studies, 9,557 people)[7]
  - Pooled - sensitivity 89%; specificity 99%
  - HIV+ - sensitivity 79%
  - Smear-neg - sensitivity 67%

**Rapidity** – ~2 hours

**Accessibility** – Unprocessed sputum,
Requires electricity,
WHO endorsed

**Cost** – $5,000-20,000/machine,
$10-15/cartridge (subsidized)

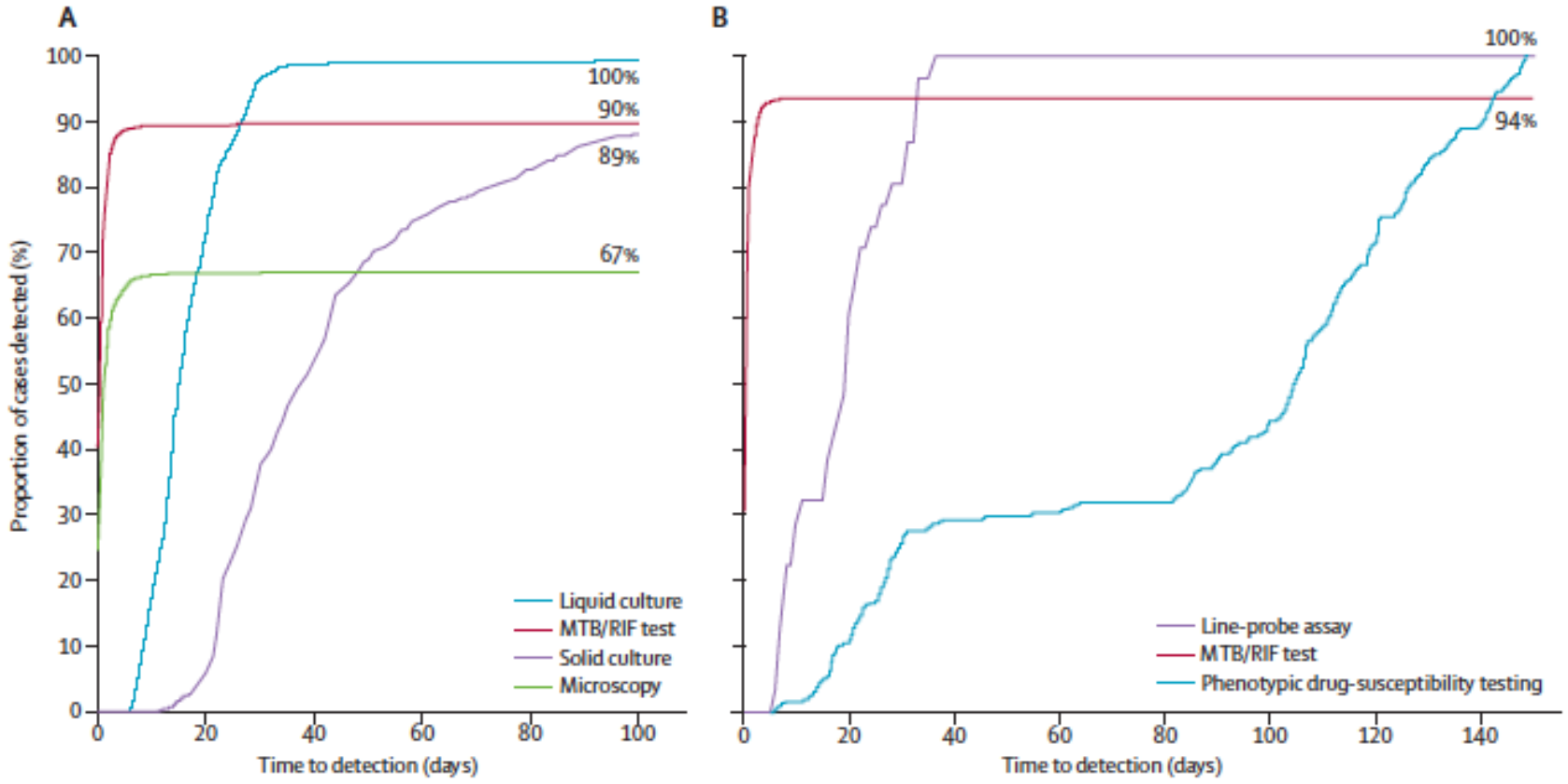

***By July 2015:***

- Over 4,000 GeneXpert Systems in use worldwide
- 13 million Xpert MTB/RIF cartridges shipped

7. Steingart KR. Xpert MTB/RIF Assay. Cochrane Review, 2014.

# Xpert MTB/RIF assay

- FIND (Foundation for Innovative New Diagnostics) Study[2]
  - 6,648 patients with suspected TB in 6 countries (2009/10)
  - Performed same-day Xpert, smear microscopy, and TB culture




2. Boehme CC, et al. Lancet 2011.

# Real-world TB Diagnostics in Durban (n=414)

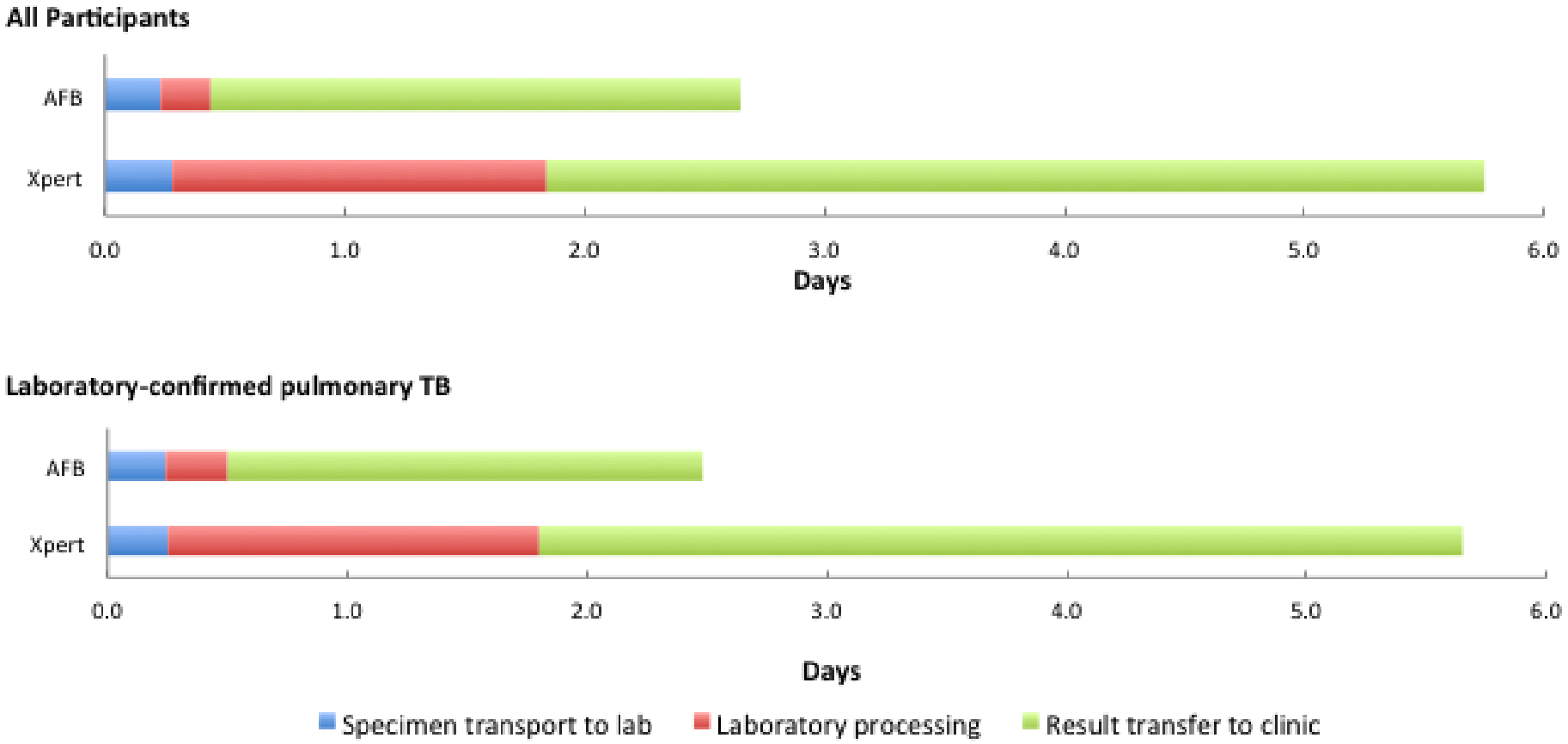
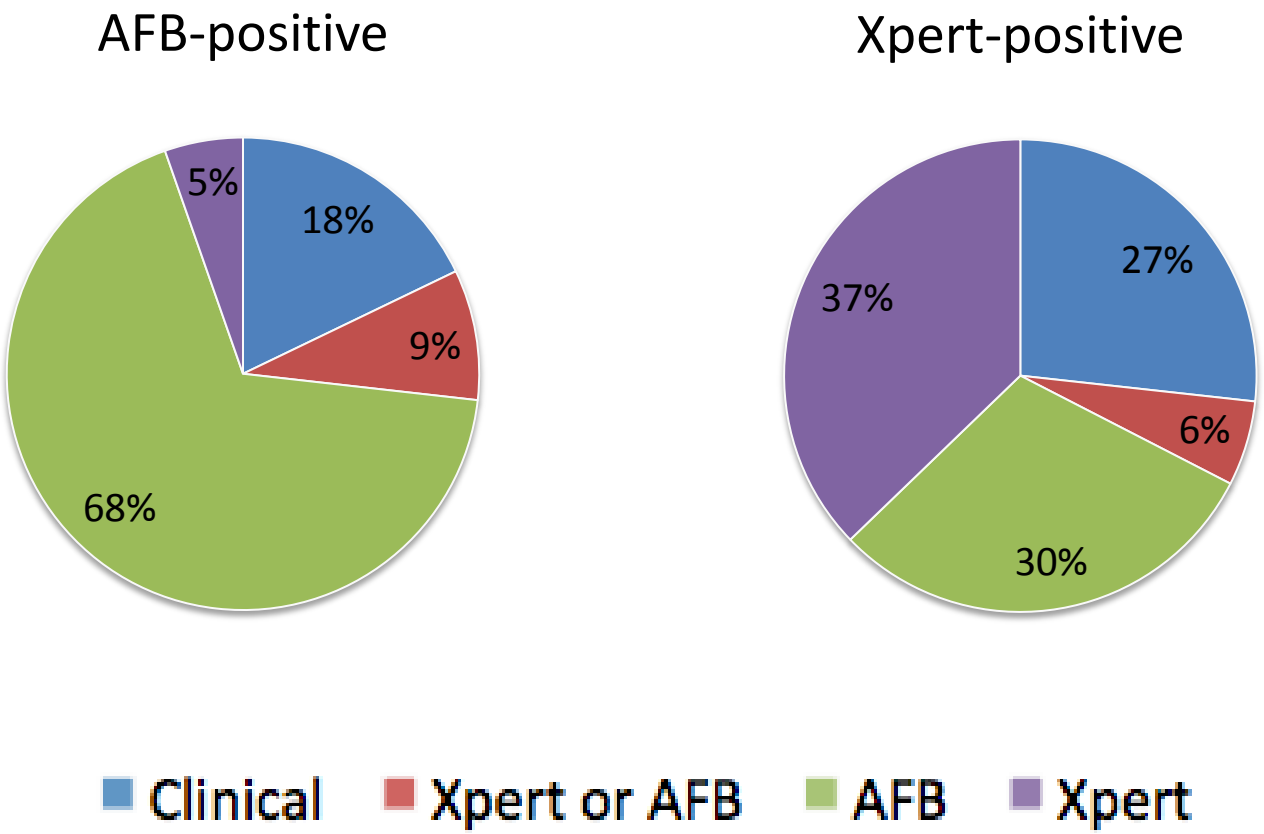



**Figure 1.** Median time between consecutive events from sputum specimen collection to a clinician's receipt of test results, for AFB and Xpert tests.

Cohen G, Drain PK, et al. J AIDS, 2014.

# Reason for starting TB therapy (N=414)




Cohen G, Drain PK, et al. J AIDS, 2014.

# Xpert in South Africa

- EXTEND Trial[8]
  - 4,656 patients (62% HIV+) with suspected TB in South Africa
  - Randomized to central lab-based testing with 1 Xpert vs. 2 smear microscopy tests (40 clinics, 20 labs)
  - Primary Outcome – Treatment Initiation
  - Results
    - No difference in rate of Treatment Initiation
    - Mortality was same between study arms
  - Study Conclusion:
    - Xpert in central lab did not improve clinical diagnosis
    - Scale up of a new diagnostic tool requires a strong health system

- A real-word implementation of Xpert based on empiric data from Western Cape, South Africa was not cost-effective[9]

8. Churchyard G. Xpert for TB: Evaluating a New Diagnostic Trial (EXTEND), CROI 2014;
9. Naidoo P. Union TB conference, 2013.

# Xpert in South Africa

- TB-NEAT Study
  - Randomized, "pragmatic" clinical trial in 4 African countries
  - 1,502 patients presenting with TB-related symptoms
  - Nurse-led diagnosis of Xpert vs. sputum-smear microscopy
  - Xpert testing done a clinical point-of-care
  - Primary Outcome – patient morbidity at 2- and 6- months
  - Results:
    - Xpert had greater diagnostic sensitivity (83% vs. 50%)
    - Xpert led to more same-day Rx initiation (23% vs. 15%)
    - By 2-months – Rx rate was same in both groups (43% vs. 42%)
    - Primary outcome (morbidity) had **no difference** b/n study arms

***Conclusion: Too much empirical treatment among smear-neg (i.e. didn't trust negative smear microscopy result)***

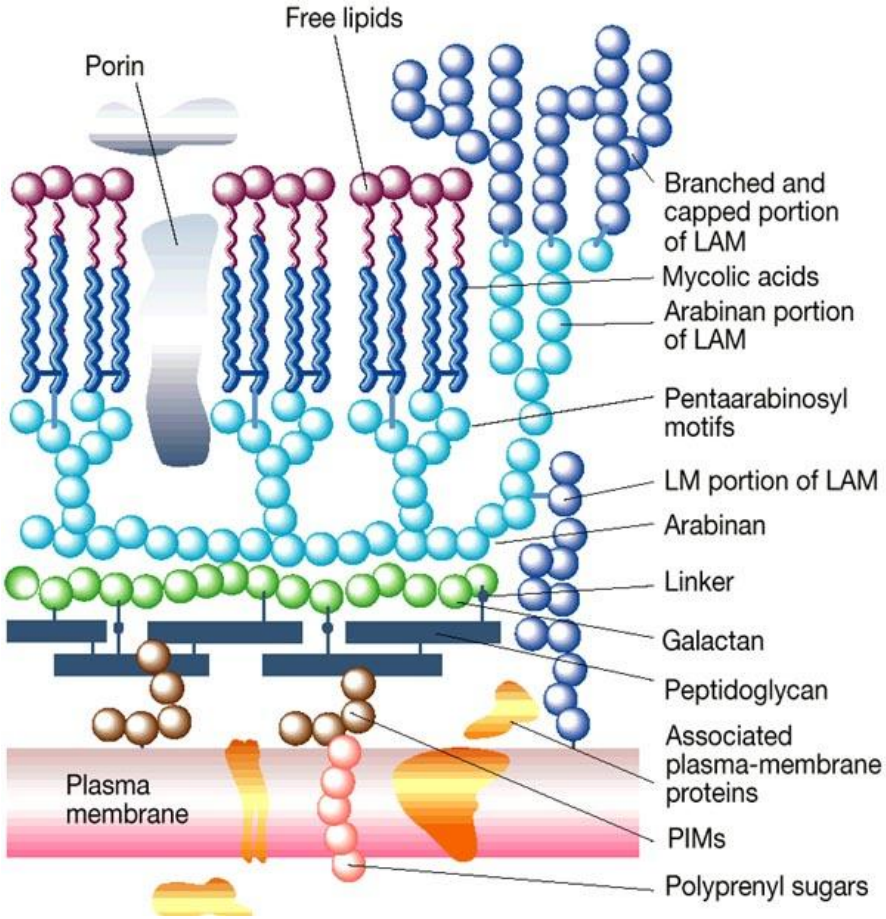
Theron G, et al. Lancet, 2013. Theron G, et al. Lancet ID, 2014.

# Lessons from Xpert

- Adoption of a POC test may not always translate to clinical impact or cost-effective results
- Location and Comparison for a POC test matters
- But, how do we assure quality control and oversight of clinic-based POC testing?

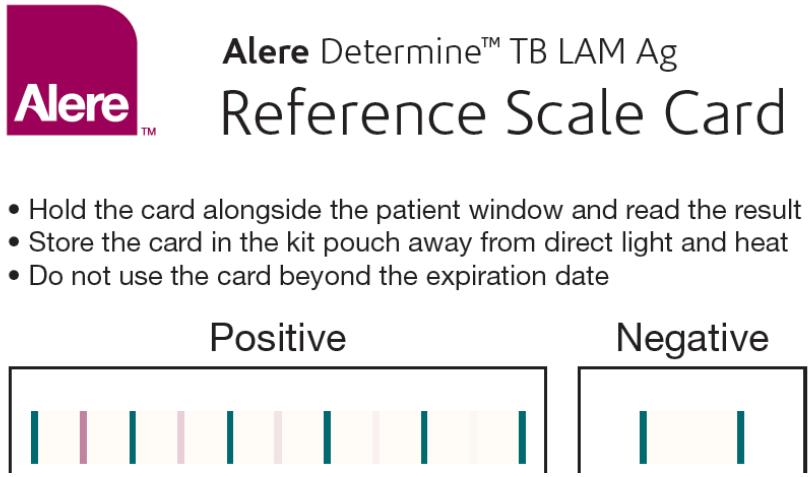
# Lipoarabinomannan (LAM)

- Molecular weight is 17.3 KDa, comprises ~60-70% of the *M. tuberculosis* cell wall
- Released from metabolically active or degenerating bacteria, and secreted from infected alveolar macrophages
- LAM can be recovered from *in vitro* cultures of *M. tuberculosis*
- Detectable in serum and excreted in urine of people with active TB disease




Lawn SD, BMC ID, 2012; Wood R, BMC ID, 2012.

# Arrival of Urine LF-LAM Assay




***Format*** *– rapid immunochromatographic assay*

***Volume*** *– 60 microliters of urine*

***Time*** *– 25 minutes*

***Accessibility*** *– Not sputum-based, no electricity, no machine*

***Cost*** *– $3.00/test*

# Urine LAM Study #1

*Study Design:*

- Prospective clinic-based study in Durban
- LAM test performed by nurses at clinical POC
- Gold standard: Sputum TB culture

*Cohort:*

- 360 newly-diagnosed HIV+ (med. CD4 182/mm$^3$)

*Results:*

| | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|
| Urine LAM | 28% (18-41) | 90% (86-93) |
| Sputum AFB | 18% (10-30) | 95% (92-98) |

Drain PK et al. BMC Infect Dis, 2014.

# Urine LAM Study #2

*Study Design:*

- Prospective clinic-based study in Durban
- LAM test performed by nurses at clinical POC
- Gold standard: Sputum TB culture

*Cohort:*

- 320 newly-diagnosed HIV+ (med. CD4 248/mm$^3$)

*Results:*

| | Sensitivity % | Specificity % |
|---|---|---|
| **Sputum AFB Smear** | 15 | 99 |
| **Urine LAM – Test #1** | | |
| ≥"faint" | 41 | 90 |
| 5+ | 6 | 98 |

| | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|
| CD4 >100 | 24% (9-45%) | 95% (91-98%) |
| CD4 <100 | 56% (35-75%) | 80% (64-91%) |

Drain PK et al. J AIDS, 2015.

# Urine LAM Study #3

*Study Design:*

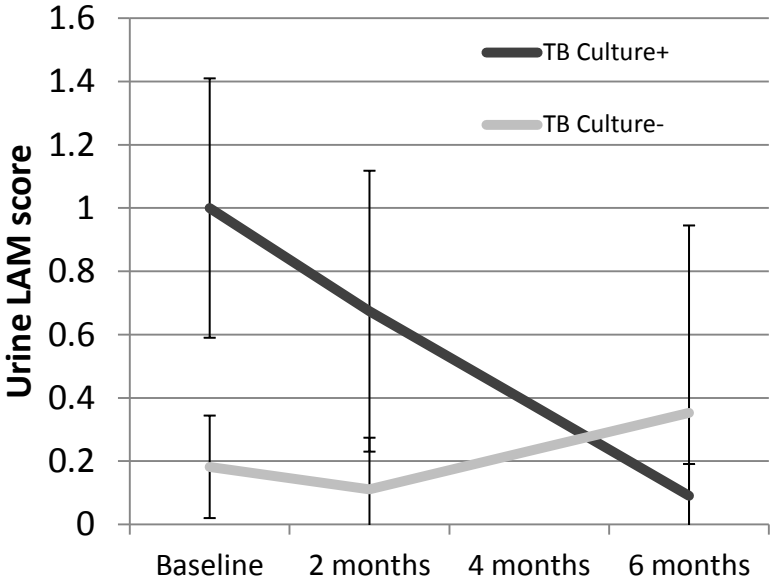
- Prospective hospital-based study in Durban
- Gold standard: Sputum TB culture

*Cohort:*

- 90 TB suspects (93% were HIV+; med. CD4 182/mm$^3$)
- All patients started on anti-TB therapy for 6 months
- Urine LAM testing at baseline, 2-months, and 6-months
- All patients followed for ≥3 years to assess mortality

*Results:*

| | Sensitivity % (95% CI) | Specificity % (95% CI) |
|---|---|---|
| **Sputum AFB Smear** | 21 (11-34) | 94 (80-99) |
| **Rapid Urine LAM** | | |
| ≥1+ score | 42 (29-56) | 85 (68-95) |
| ≥2+ score | 23 (13-36) | 97 (84-100) |
| ≥3+ score | 16 (8-28) | 100 (89-100) |
| ≥4+ score | 12 (5-24) | 100 (89-100) |
| 5+ score | 7 (2-17) | 100 (89-100) |




Drain PK et al. BMJ, 2014.

# Comparison of Urine LF-LAM Studies

| | Sensitivity % (95% CI) | Specificity % (95% CI) |
|---|---|---|
| Clinic-based Studies (asymptomatic screening) | | |
| Lawn, Retrosp., New HIV+, Cape Town | 28 (19-39) | 99 (97-100) |
| **Drain, Prosp., New HIV+, Durban – study #1** | **28 (18-41)** | **90 (86-93)** |
| **Drain, Prosp., New HIV+, Durban – study #2** | **41 (28-55)** | **92 (89-95)** |
| Hospital-based Studies (symptompatic diagnostic) | | |
| Peter, Retrosp., HIV+ TB suspects, Cape Town | 66 (57-74) | 66 (57-73)* |
| Dorman, Prosp., HIV+ TB suspects, SA/Uganda | 62 (57-67) | 78 (75-81) |
| Van Rie, HIV+, extrapulm TB suspects, Jo-burg | 69 (56-82) | 92 (88-96) |
| Shah, Retrosp., HIV+ TB suspects, Uganda | 63 (53-72) | 88 (80-93) |
| **Drain, Retrosp., HIV+ TB suspects, Durban** | **42 (29-56)** | **85 (68-95)** |

* Specificity increased to 90% (82-95%) when using a non-TB control group.

# WHO Recommendation on Urine LF-LAM Assay

1. LF-LAM may be used to **assist in the diagnosis of TB** in **HIV-positive adult inpatients** with **signs or symptoms of TB (pulmonary and/or extrapulmonary)** who have a **CD4 cell count ≤100 cells/µL**, or **HIV-positive patients** who are **seriously ill* regardless of CD4 count** or with unknown CD4 count *(conditional recommendation; low quality of evidence).*

   - *This recommendation **also applies to HIV-positive adult outpatients** with signs and symptoms of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count less than or equal to 100 cells/µL, or HIV-positive patients who are seriously ill regardless of CD4 count or with unknown CD4 count, based on the generalization of data from inpatients.*
   - *This recommendation **also applies to children**, based on the generalization of data from adults while acknowledging very limited data and concern regarding the low specificity of the LF-LAM assay in children.*

2. LF-LAM should **not be used as a screening test** for TB
   *(strong recommendation; low quality of evidence)*

*** "seriously ill" is defined based on four danger signs: respiratory rate > 30/min, temperature >39 C, heart rate >120/min and unable to walk unaided.**

# Hospital-based LAM Implementation

**Effect on mortality of point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group, multicountry, open-label, randomised controlled trial**

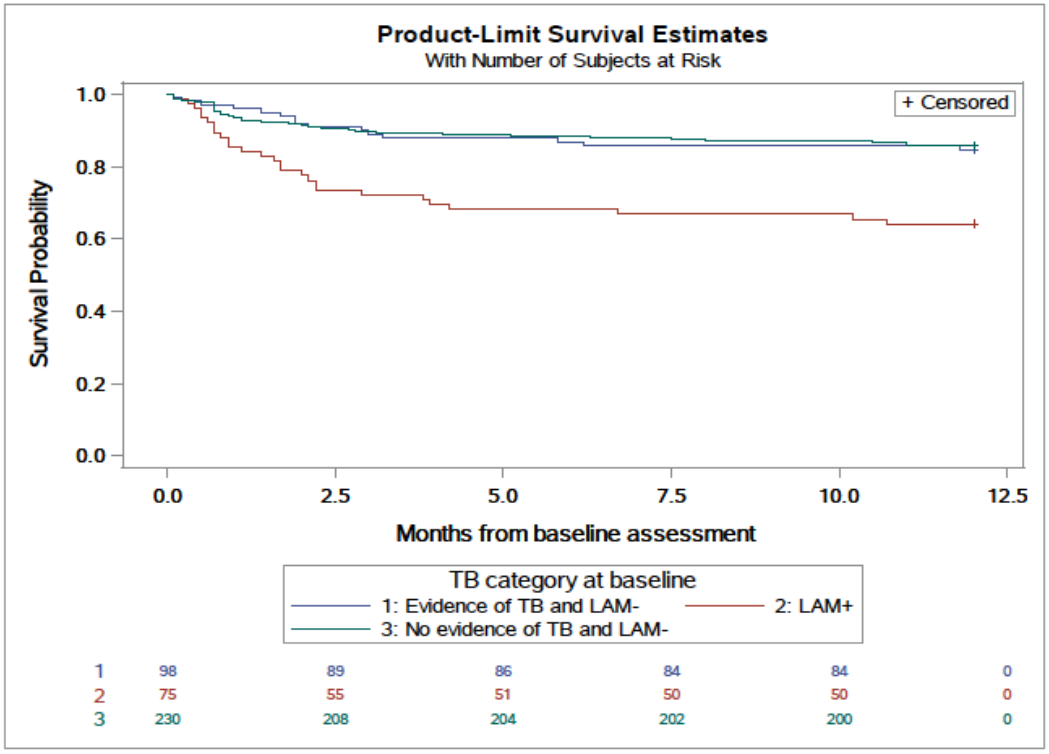
*Jonny G Peter*, Lynn S Zijenah*, Duncan Chanda*, Petra Clowes*, Maia Lesosky, Phindile Gina, Nirja Mehta, Greg Calligaro, Carl J Lombard, Gerard Kadzirange, Tsitsi Bandason, Abidan Chansa, Namakando Liusha, Chacha Mangu, Bariki Mtafya, Henry Msila, Andrea Rachow, Michael Hoelscher, Peter Mwaba, Grant Theron, Keertan Dheda*

- Randomized, pragmatic trial
  - 2,659 HIV+, hospitalized patients with suspected TB
  - Randomized to urine LAM plus routine TB testing (AFB smear, Xpert, culture) versus routine TB testing (10 hospitals)
  - Primary Outcome – 8-week all-cause mortality
  - Results
    - LAM group – 21% mortality (261 patients)
    - No LAM group – 25% mortality (317 patients)
  - Study Conclusion:
    - LAM testing had an absolute mortality reduction of 4%
    - Likely to benefit patients presenting with severe illness

Peter JG, et al. Lancet, 2016.

# Clinic-based LAM Implementation

- Clinic-based urine LAM screening at HIV diagnosis predicts mortality in a TB-endemic region




Drain PK, et al. under review

# Summary of Urine LAM

- Advantages
  - Can be conducted at clinical POC by nurses
  - Non-sputum based (safer for HCWs)
  - Simple LFA with no machinery/electricity
  - Diagnose extrapulmonary TB
  - Applicable for diagnosing children
  - Marker for treatment response
  - Inexpensive
  - Good Diagnostic Specificity
  - Better among TB-suspects, high bacillary load, sicker pts
- Disadvantages
  - Low/Moderate Diagnostic Sensitivity

# Outline

- Overview of POC Diagnostics
- TB Pathogen Biomarkers
- **Host Biomarkers for TB**
  - **C-reactive protein**
  - **Transcriptional Signature**
- Conclusion

# Rapid TB Diagnostics

**Diagnostic Tests**

"Rule IN" test
(high specificity)

- *AFB smear microscopy*
- **Urine LAM**
- **Xpert MTB/RIF**
- other nucleic acid tests

**Screening Tests**

"Rule OUT" test
(high sensitivity)

- *Symptom screening*
- **C-reactive protein**
- D-dimer, haptoglobin
- Many cytokines, others

# Rapid C-reactive Protein (CRP)

***Accuracy*** *– sensitivity ~90%; spec ~70%*

***Rapidity*** *– 10 minutes*

***Accessibility*** *– Finger prick whole blood assay with a small portable device*

***Cost*** *– $3.50/test*

# Hospital-based CRP Study

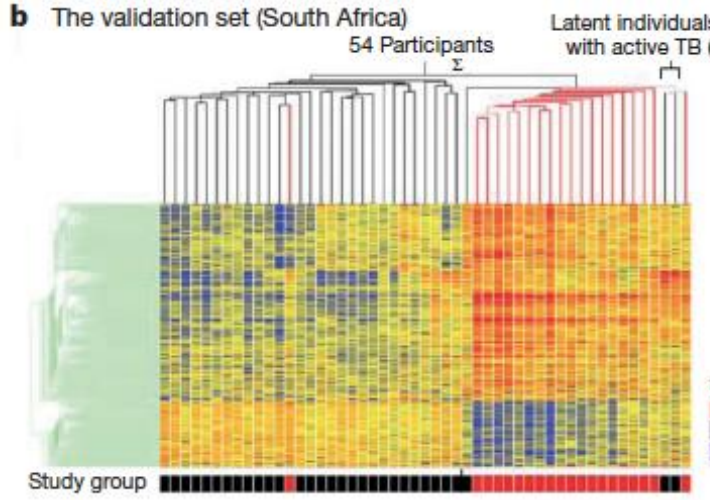
- Prospective study at Edendale Hosp., Pietermaritzburg
- 90 TB-suspects;  All HIV+ (med. CD4 177/$mm^3$)
- Nurses performed rapid CRP on finger prick whole blood; obtained lab-based CRP test
- All patients received independent nurse and physician assessments

| | Sensitivity % (95% CI) | Specificity % (95% CI) |
|---|---|---|
| **Rapid C-reactive protein** | | |
| CRP ≥10 mg/l | 95 (83-99) | 51 (36-66) |
| CRP ≥25 mg/l | 77 (61-89) | 73 (58-85) |
| CRP ≥50 mg/l | 59 (42-74) | 87 (73-95) |

# Host Transcriptional Signature

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

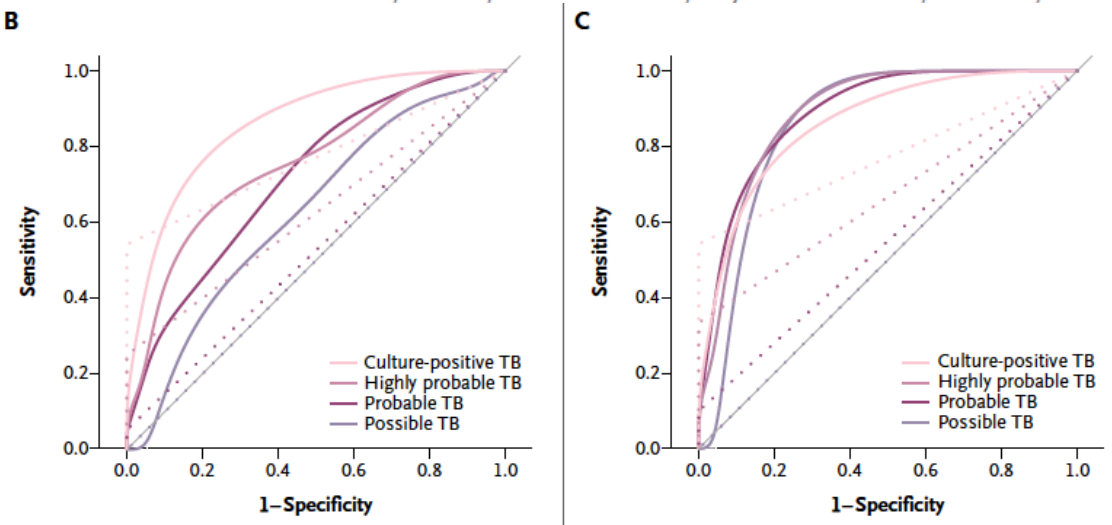
Matthew P. R. Berry[1], Christine M. Graham[1]*, Finlay W. McNab[1]*, Zhaohui Xu[6], Susannah A. A. Bloch[3], Tolu Oni[4,5], Katalin A. Wilkinson[2,4], Romain Banchereau[9], Jason Skinner[6], Robert J. Wilkinson[2,4,5], Charles Quinn[6], Derek Blankenship[7], Ranju Dhawan[8], John J. Cush[6], Asuncion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




ORIGINAL ARTICLE

## Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa

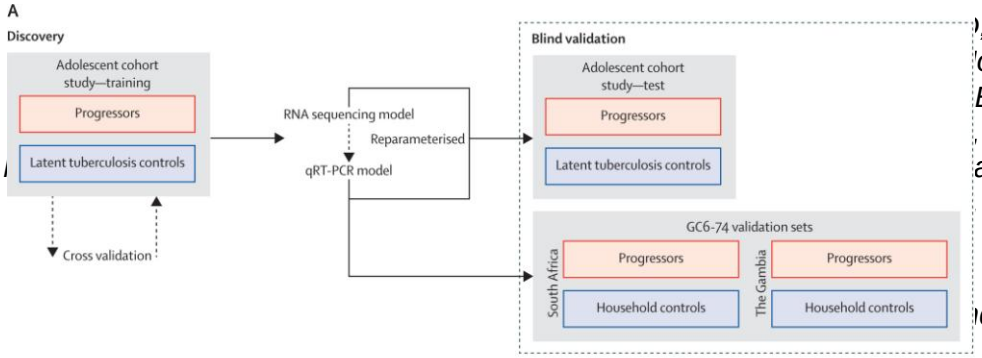
Suzanne T. Anderson, Ph.D., M.R.C.P.C.H., Myrsini Kaforou, M.Phil.,

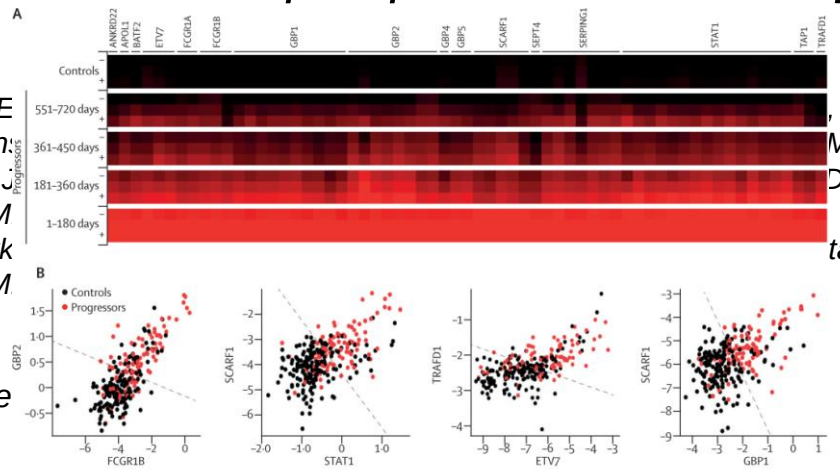


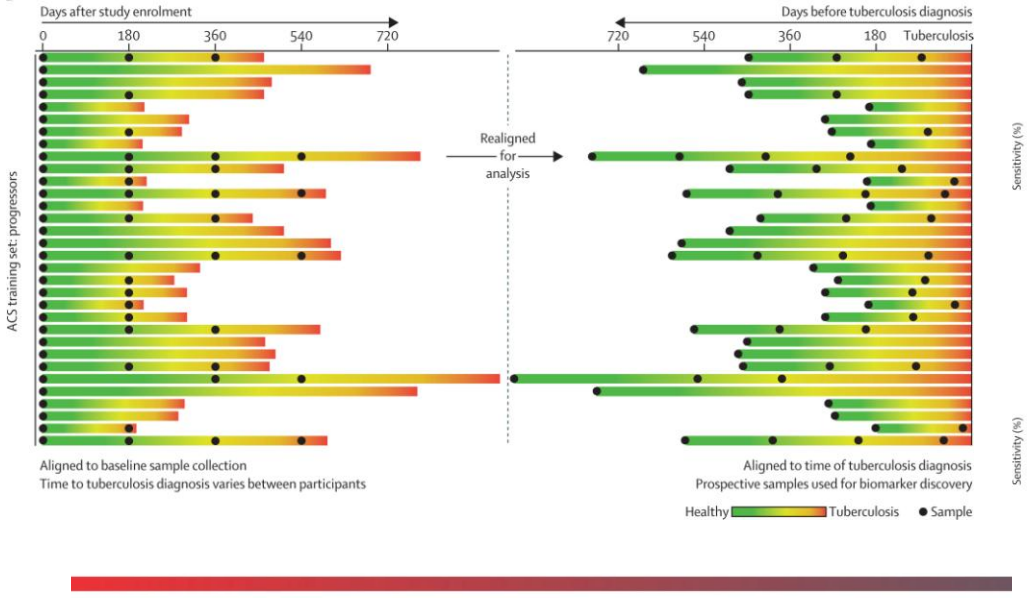

Berry MPR, et al. Nature, 2010; Anderson ST, et al. NEJM, 2013.

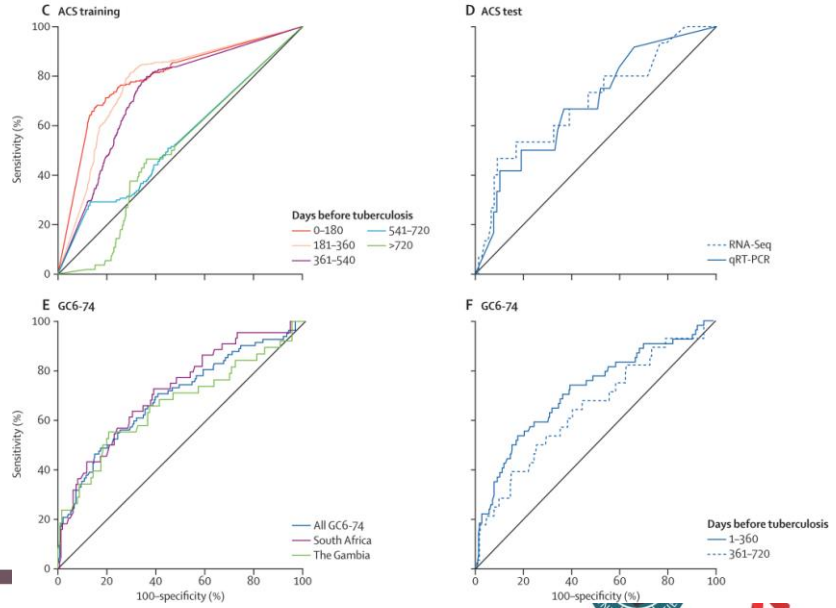
# Host Transcriptional Signature

*A blood RNA signature for tuberculosis disease risk: a prospective cohort study*




Zak DE, et al. Lancet, 2016

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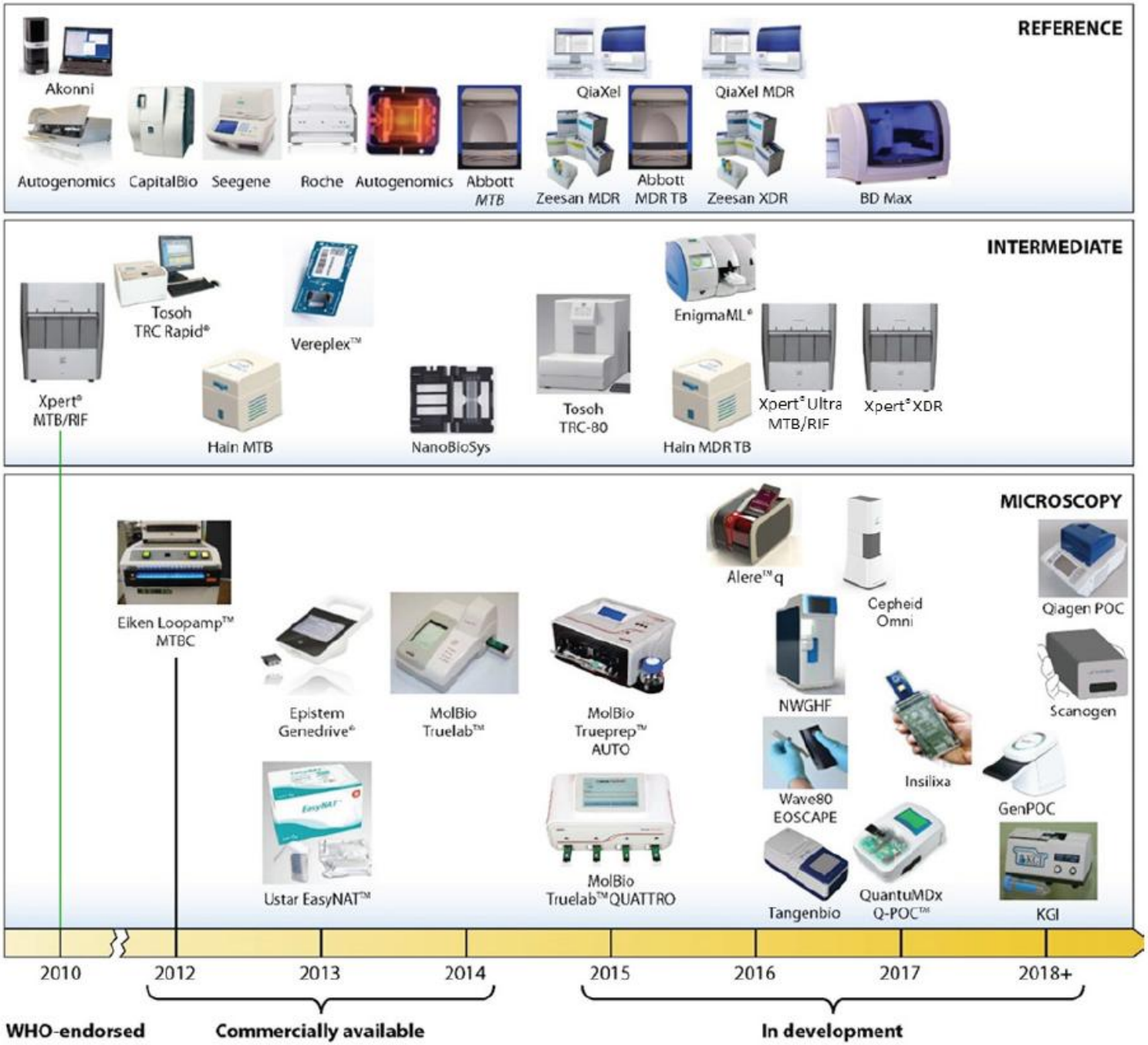
- Host Biomarkers for TB
- **Conclusion**




UNITAID, TB Diagnostic Landscape, 2015.

# GeneXpert® Omni

- Developed by Cepheid and FIND
- Announced July 28, 2015
- Small (23 cm tall)
- Lightweight (1 kilogram)
- Easy to use
- Powered by a rechargeable battery
- Wireless connectivity


Drain PK et al., Lancet Global Health, 2015.

# The arrival of a true point-of-care molecular assay—ready for global implementation?

Comment

LANGLH-D-15-00669

S2214-109X(15)00186-2

Gold Open Access

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1. Agree on regulatory assurances and QC measures to ensure oversight for maintaining the accuracy of diagnostic testing
2. Understand whether clinic-based testing might place additional strain on laboratory system, or whether POC testing could help offload the burden on laboratory workers
3. Develop clear guidance on the adoption of novel point-of-care tests

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