



Recent Advances in Diagnostics for MDR/XDR TB and Biomarkers: Potential Implications for Women and Children

Pediatric MDR TB: Emerging Global Challenge
Nov 14-15, 2011, Moscow, Russia,
Catharina Boehme

Partnering for better diagnosis for all

Conflict of interest

- ❖ **FIND is a non-profit foundation devoted to developing and rolling out diagnostic tools for poverty-related diseases.**
- ❖ **In this role, FIND has development partnerships with industry.**
- ❖ **FIND has no financial benefits in any form.**

A changing landscape: WHO recommendations 2006 - 2010



2006	<ul style="list-style-type: none"> • Smear-positive case definition from 2 to 1 positive smears • Screening for TB with 2 instead of 3 smears • Conventional FM
2007	<ul style="list-style-type: none"> • Commercial liquid culture / DST • Rapid speciation (MPT64)
2008	<ul style="list-style-type: none"> • Line probe assay (Rif & INH)
2009	<ul style="list-style-type: none"> • LED-based FM • Non-commercial culture (MODS, CRI, NRA)
2010	<ul style="list-style-type: none"> • Cartridge-based Automated NAAT (Xpert MTB/RIF)

Planned review 2012:

-2nd line LPA

-2nd generation LPA

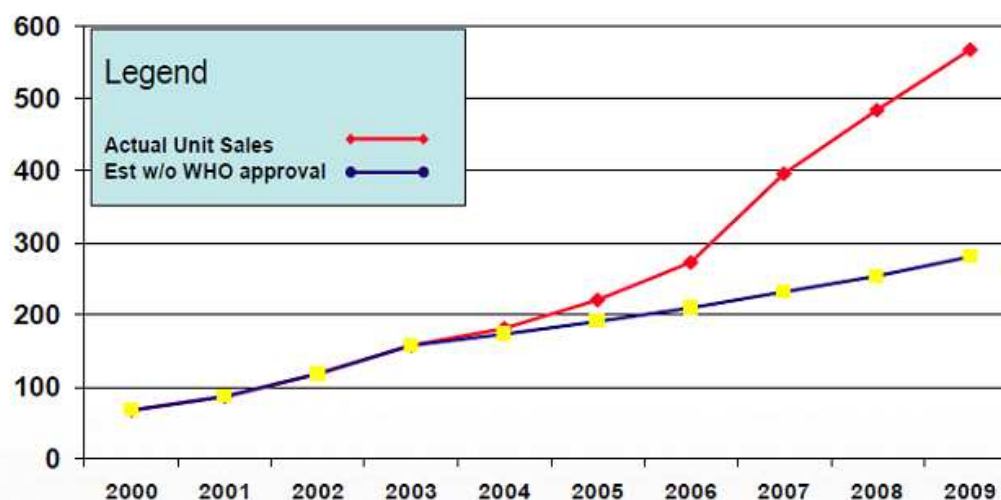
Reducing time to diagnosis of MDR

	Micro- scopy	Specimen transport or patient transfer	Test 1	MDR Treatment decision	Test 2	Total time to MDR diagnosis
Solid Culture / 1st line DST	24 h	Yes	SC 6-8 w	No	1 st line DST 3– 4 w	9-12 w
Liquid Culture / 1st line DST	24 h	Yes	LC 2-3 w	No	1 st line DST 1– 3 w	3-6 w
Line Probe Assay / Liquid Culture DST	24 h	Yes	Sm+ LPA 24 h	Yes	Full DST where required	2 d
			Sm- LC 2-3 w	No	1 st line DST LPA/LC 24 h – 3 w	2-6 w
Single step NAAT / Liquid Culture DST	No	No	NAAT 2 h	Yes	Full DST where required	2 h

Uptake of diagnostics at the example of liquid culture

MGIT 960 Growth

Trend with WHO endorsement vs estimate w/o endorsement



Started TB in
select
Emerging
Markets

Demonstration
sites started

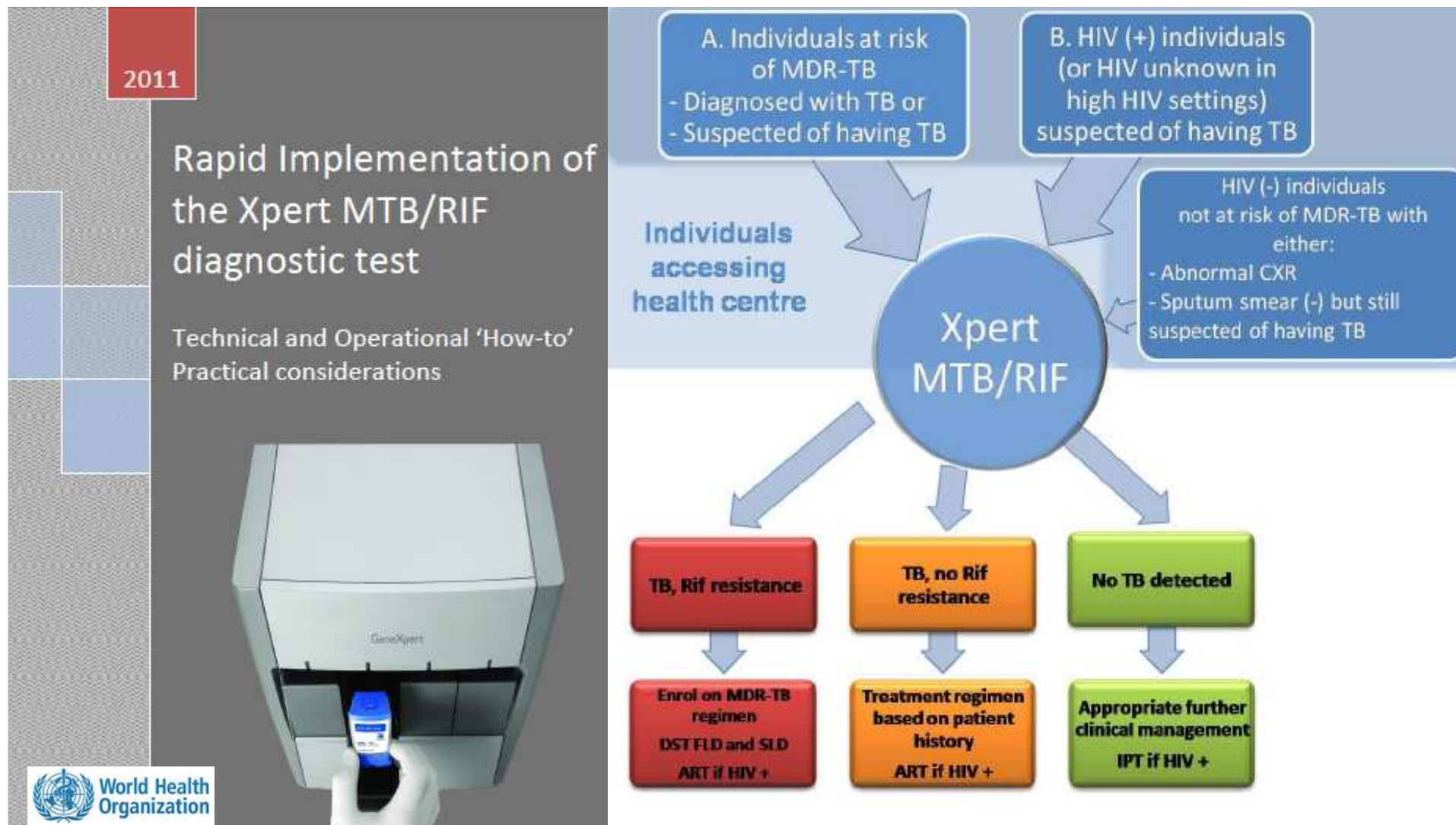
Data
presented
public forum

WHO
recommendations

Pricing public

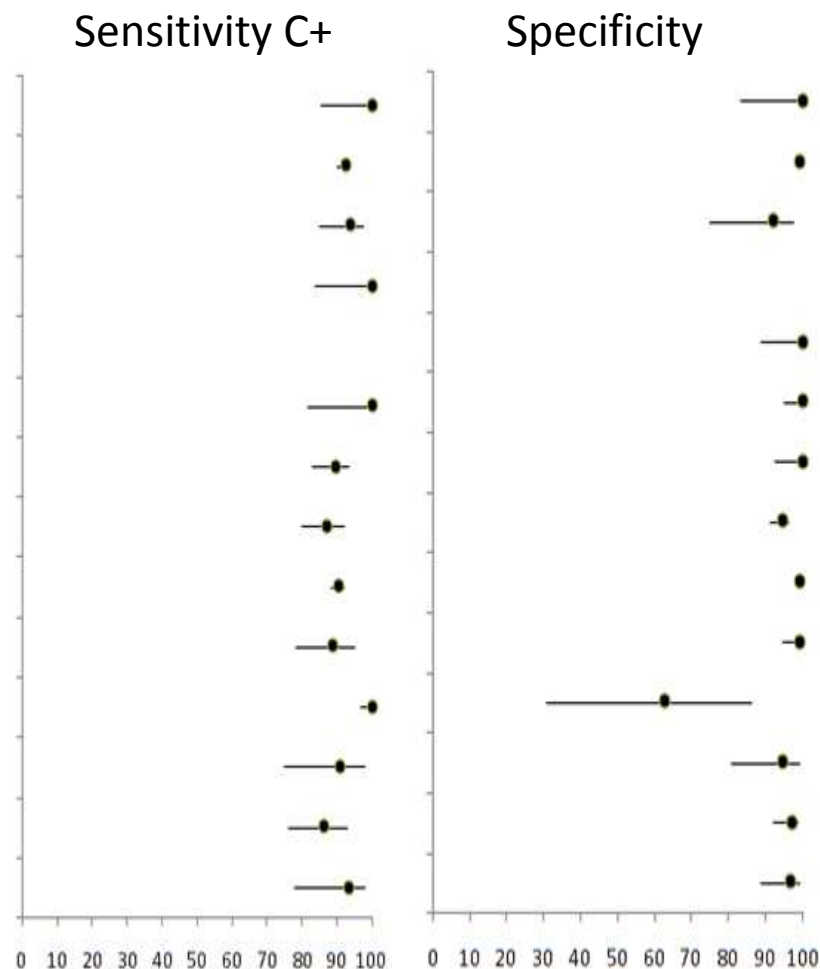


Dec 2010: WHO recommendation on use of Xpert MTB/RIF



Xpert accuracy for pulmonary TB

Reference	Sensitivity C+	95% CI	Specificity	95% CI
Helb et al, JCM, Jan 2010	100.0%	85.4-100.0	100.0%	83.4-100.0
Boehme et al, NEJM, Sep 2010	92.2%	90.0-93.9	99.2%	98.1-99.6
Bowles et al, IJTLD, Jan 2011	93.8%	85.0-97.5	92.0%	75.0-97.8
Armand et al, JCM, Mar 2011	100.0%	83.9-100.0	NA	NA
Moure et al, JCM, Mar 2011	NA	NA	100.0%	88.8-100.0
Malbruny et al, IJTLD, Mar 2011	100.0%	81.6-100.0	100.0%	95.0-100.0
Marlowe et al, JCM, Apr 2011	89.2%	82.7-93.5	100.0%	92.4-100.0
Theron et al, AJRCCM, Apr 2011	87.1%	79.8-92.0	94.4%	91.4-96.4
Boehme et al, Lancet, Apr 2011	90.3%	88.4-92.0	99.0%	98.5-99.3
Rachow et al, PLOS One, Jun 2011	88.4%	78.4-94.9	99.0%	94.7-100.0
Friedrich et al, JCM, Jun 2011	100.0%	96.7-100.0	62.5%	30.6-86.3
Ioannidis et al, JCM, Jun 2011	90.6%	74.9-97.9	94.3%	80.8-99.1
Scott et al, PLoS Med, Jul 2011	86.0%	76.0-93.0	97.0%	92.0-99.0
Miller et al, JCM, Aug 2011	93.1%	78.0-98.1	96.7%	88.6-99.1



TB in children: Fumbling in the dark...

Based on clinical diagnosis, we think that

- ❖ **Culture is a poor reference standard (20-50%)**
- ❖ **Microscopy is infrequently helpful (<10%)**

However, clinical diagnosis is probably even worse

- ❖ **Chest radiography interpretation is variable**
- ❖ **Clinical scoring systems seldom concur**



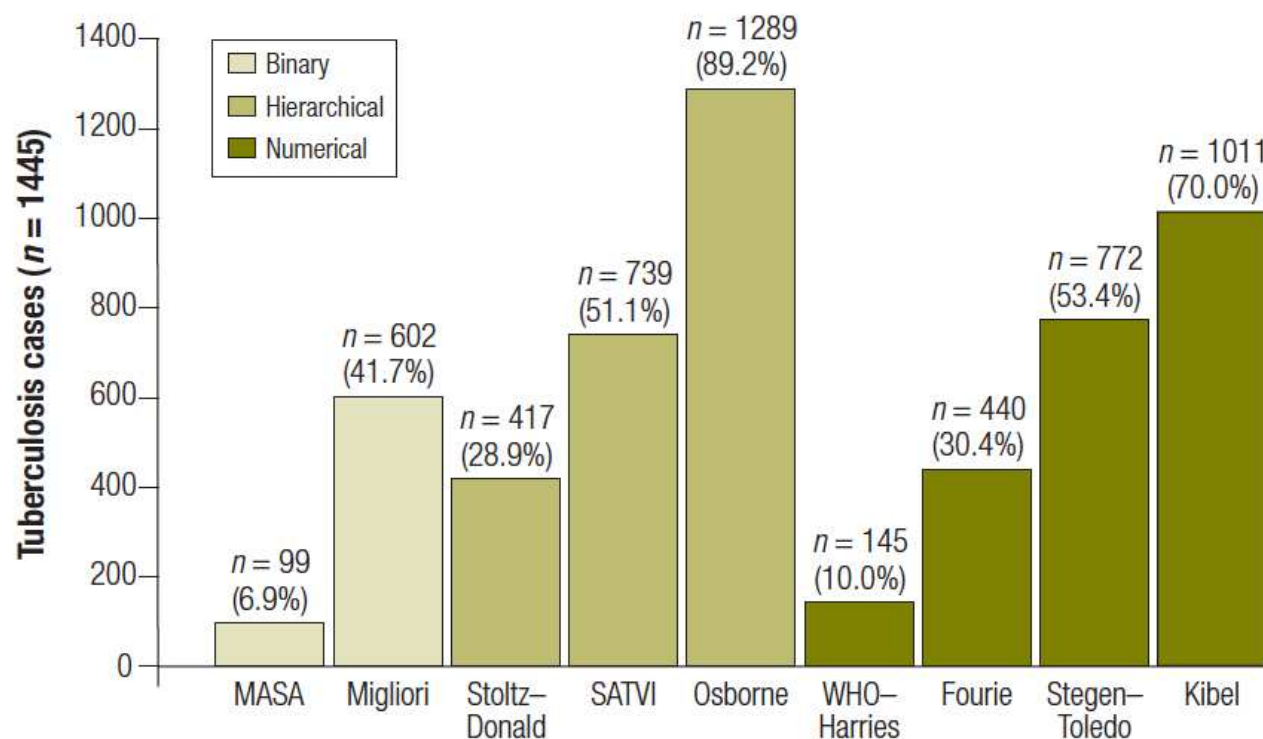
Interpretation of CXR is highly inconsistent

Table 1. Results of chest radiograph assessment by three independent paediatric reviewers, grouped by certainty of tuberculosis diagnosis, South Africa, 2001–2006

Diagnostic certainty ^a	Reviewer 1		Reviewer 2		Reviewer 3		Final classification	
	No.	%	No.	%	No.	%	No.	%
Highly likely to have tuberculosis	16	1.1	29	2.0	171	11.8		
Likely to have tuberculosis	20	1.4	38	2.6	323	22.4		
Suspected of having tuberculosis	124	8.6	145	10.0	242	16.7		
Positive	160	11.1	212	14.6	736	50.9	271	18.8
Inconclusive	45	3.1	35	2.4	82	5.7		
Abnormal but not tuberculosis	102	7.1	139	9.6	312	21.6		
Normal	1038	71.8	778	53.9	59	4.1		
Negative	1185	82.0	952	65.9	453	31.4	1174	81.2
Not read	100	6.9	281	19.5	256	17.7		
Total	1445	100	1445	100	1445	100	1445	100

Standardized scoring systems?

Fig. 1. Frequency of cases classified as tuberculosis with various scoring systems, with hierarchical and numerical outcomes condensed to a binary “tuberculosis/not tuberculosis” output, South Africa, 2001–2006



MASA, Medical Association of South Africa; SATVI, South African Tuberculosis Vaccine Initiative; WHO, World Health Organization.

Xpert performance in pediatric TB



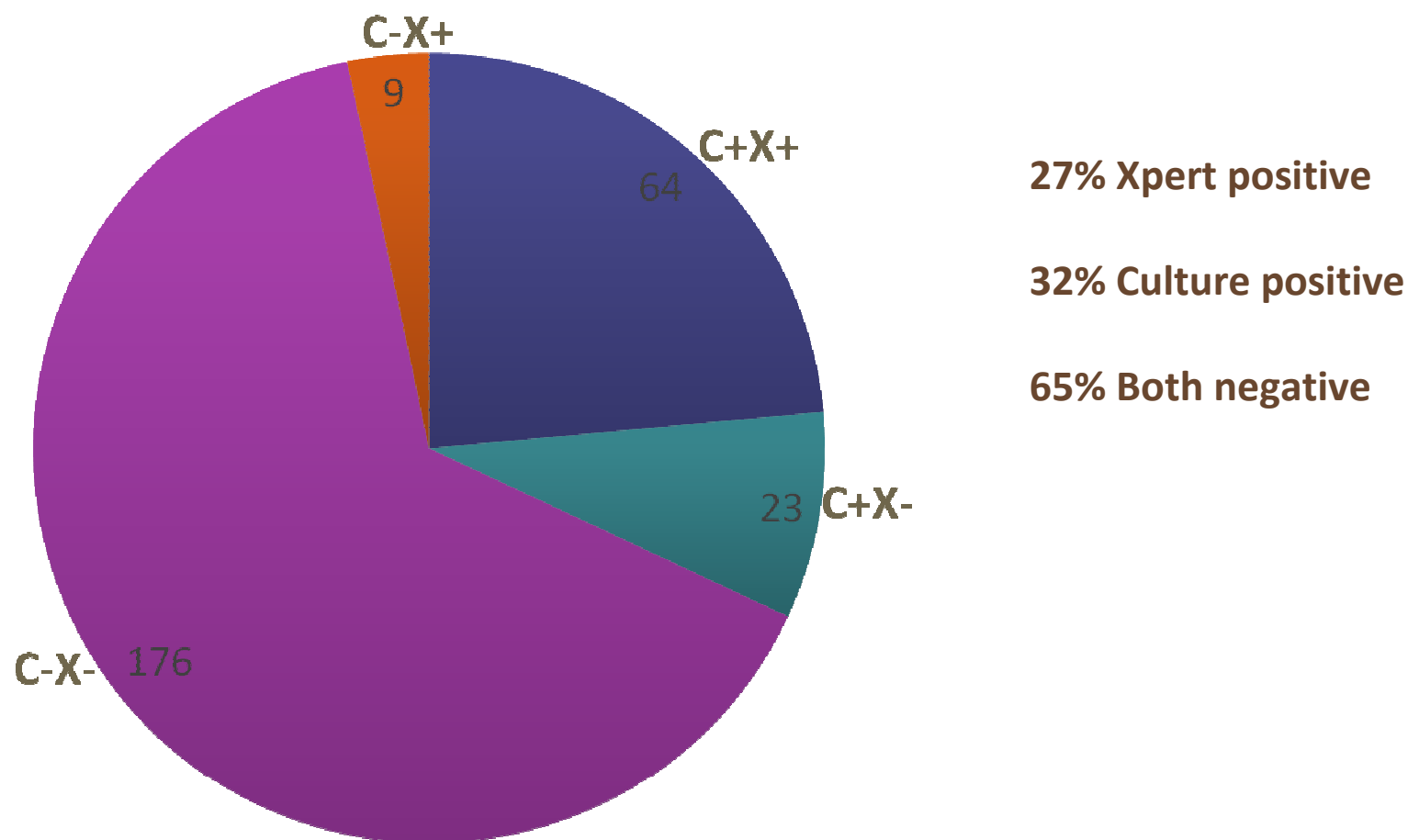
UCT, South Africa

- ❖ 535 children (median age 19 m; 23% HIV-infected)
- ❖ Induced sputum

	Sensitivity	Specificity	PPV	NPV	Sensitivity in smear-positive	Sensitivity in smear-negative
<i>All children with complete results from at least one specimen (n=535)</i>						
Xpert						
All	64/87 73.6 (64.1-83.0)	443/448 98.9 (97.9-99.9)	92.8	95.1	29/30 96.7 (89.9-100)	35/57 61.4 (48.4-74.4)
HIV infected	14/15 93.3 (79.0-100)	102/102 100 (96.4-100)	100	99.0	9/9 100 (66.4-100)	5/6 83.3 (40.5-100)
HIV uninfected	50/72 69.4 (58.5-80.3)	340/345 98.6 (97.3-99.8)	90.9	93.9	20/21 95.2 (85.3-100)	30/51 58.8 (44.8-72.8)
Smear	28/87 32.2 (22.2-42.2)	448/448 100 (99.2-100)	100	88.4		

Sputum tests alone are not the solution

Culture and Xpert results in children started on TB treatment
n=272



Non-respiratory specimens - Xpert Sensitivity compared to culture

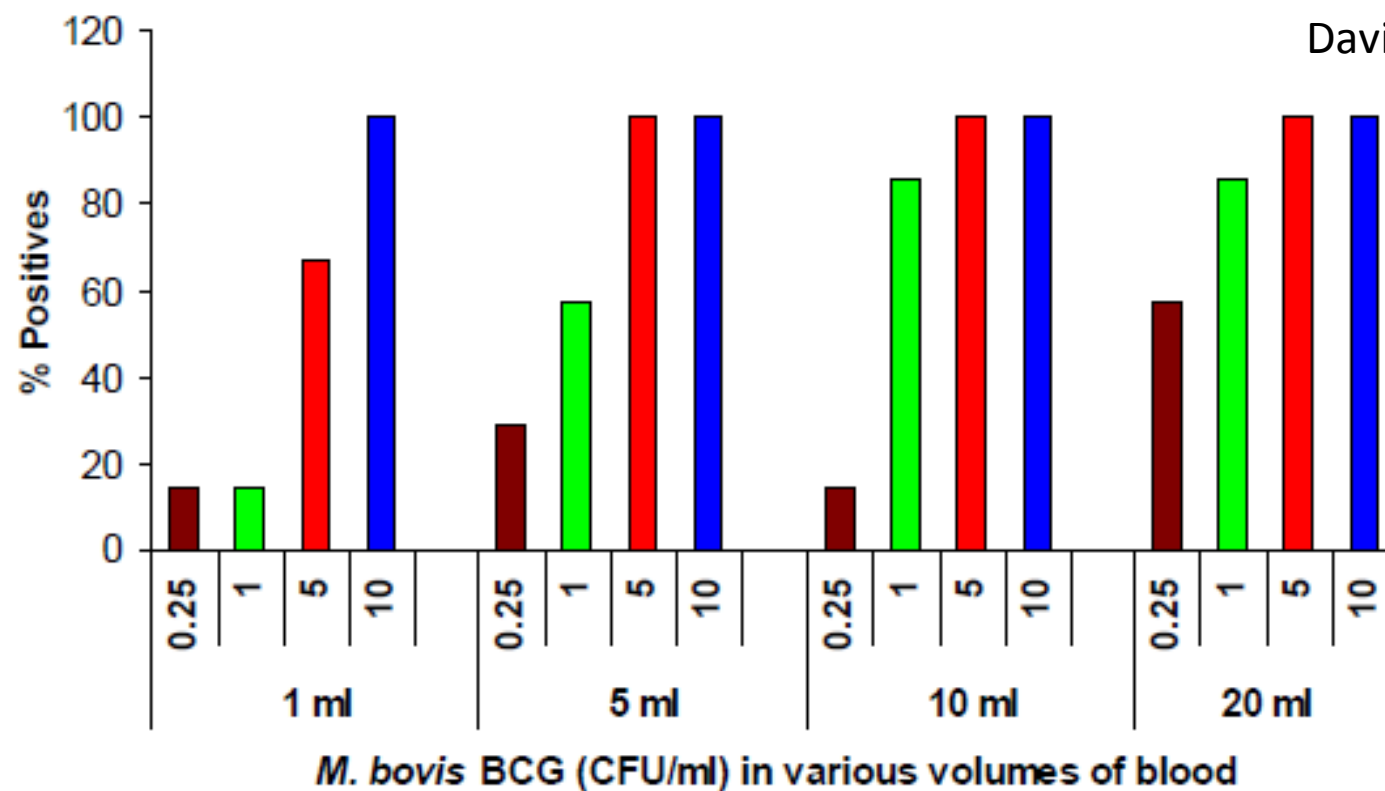


Reference	Tissue	Node	CSF	Gastric	Pleural	Urine	Stool	Comment
Ligthelm et al., JCM 2011		28/29 (96.6%)						FNAB material added to PBS then treated SR:sample = 2:1
Hillemann et al., JCM 2011	20/29 (69%)			7/8 (87.5%)		5 /5 (100%)	2/2 (100%)	SR:sample = 3:1
Teo et al., JCM 2011			2/3 (66%)	4/4 (100%)				SR:sample = 2:1
Vadwai et al., JCM 2011	Biopsy 54/70 (77%)		1/3 (33%)	Body fluids 16/21 (76%)				SR:sample = 2:1 Many patients already treated before biopsy
Miller et al., JCM 2011	Smear positive 4/4 (100%)			Smear negative 3/4 (75%)		Total 7/8 (88%)		SR:sample = 3:1
Causse et al., JCM 2011	Total 39/41 (95%)							Cobas TaqMan 78% sensitive and 98% specific

Optimized sample protocols for stool, CSF and blood



David Alland, UMDNJ, US



Detection of *M. bovis* BCG spiked in blood using new large volume blood protocol

TB diagnosis in women

- ❖ **Reduced access to qualified health services** (socio-economic status)
- ❖ **Maternal TB** increases complications in pregnancy and neonatal morbidity/mortality

Boeree M.J. et al.; IUATLD 2000.

Diwan V.K. et al.; Lancet 1999.

Long N.; Journal of Clinical Epi 2002.

Review Article

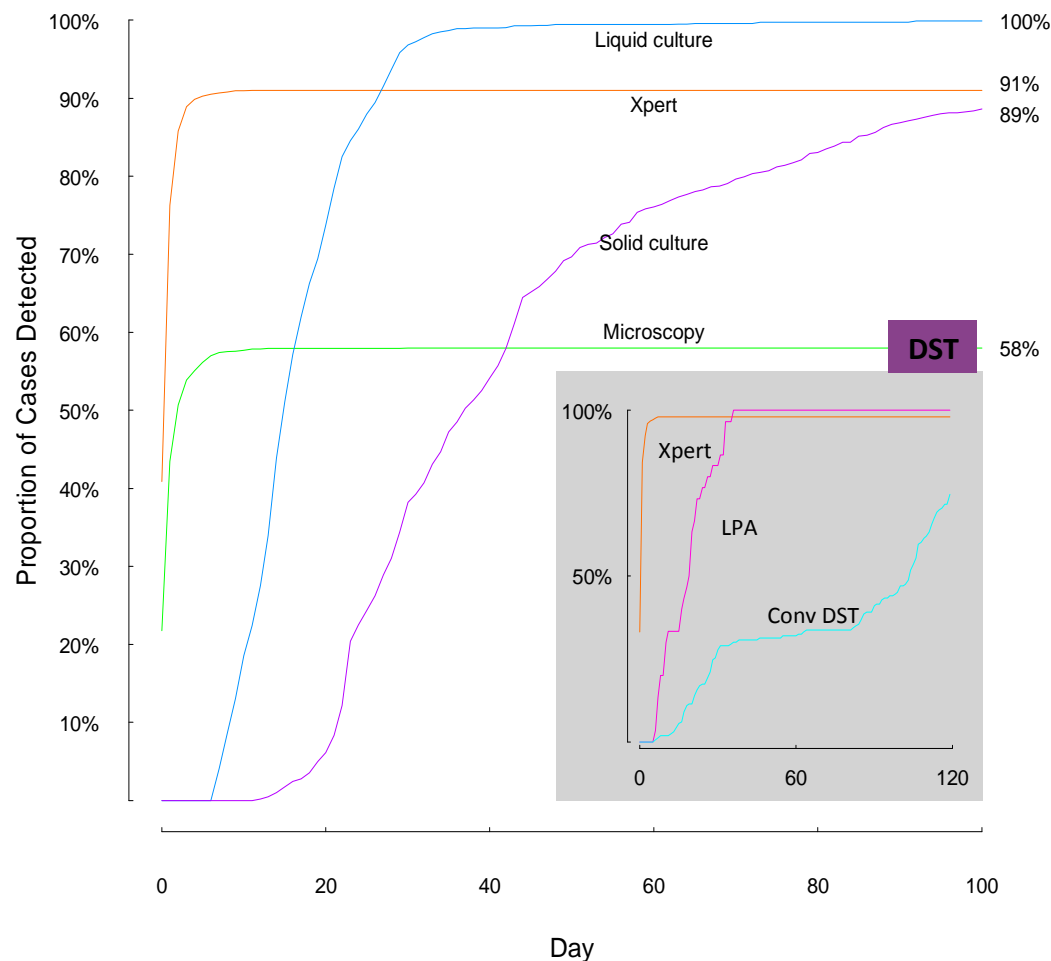
A Model of Tuberculosis Screening for Pregnant Women in Resource-Limited Settings Using Xpert MTB/RIF

Turnbull et al, J Pregnancy

Case detection rate increased by >30%

0.7 d mean time to detection from sputum collection

Proportion of cases detected over time, by test method;
Maximum proportion detected



❖ Significantly reduced time to treatment, notably for smear-negative and MDR cases

❖ Drop out rate: 39.3% before to 14.7% after implementation

Negotiated per test costs in public sector and non-for-profit private sector in 145 countries

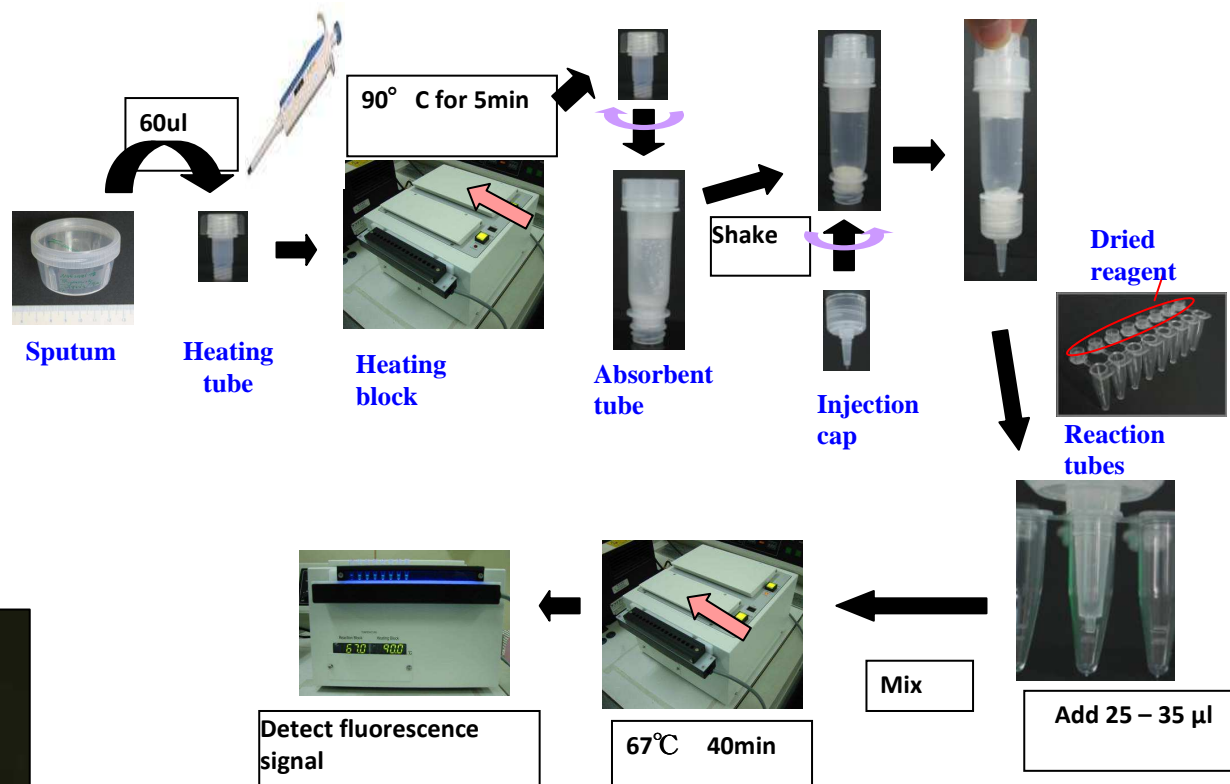
Estimated annual global volumes (cartridges)	> 600,000	>1,700,000	>3,700,000
Estimated year	2011	2012	2014
Price (Ex Works)	US\$ 16.86	US\$ 14.00	US\$ 10.72
Ave % reduction over EU*	75%	79%	84%



Interactive map: <http://www.stoptb.org/wg/gli/assets/documents/map/1/atlas.html>

Molecular testing for TB: Fast followers

- ❖ Idaho
- ❖ Alere
- ❖ Great Basin
- ❖ Hain
- ❖ Seegene
- ❖ Tulip
- ❖ Etc.

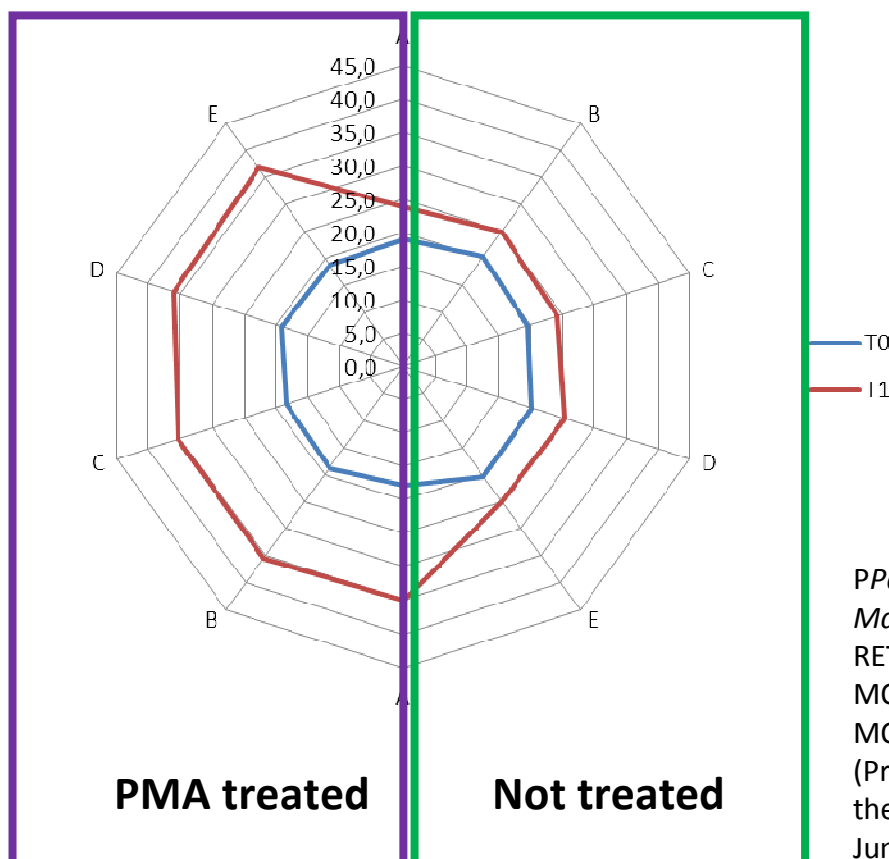


TB BioChip™
Engelhardt Institute, Moscow

TB LAMP
Eiken, Tokyo

Molecular assays for treatment monitoring using propidium monoazide?

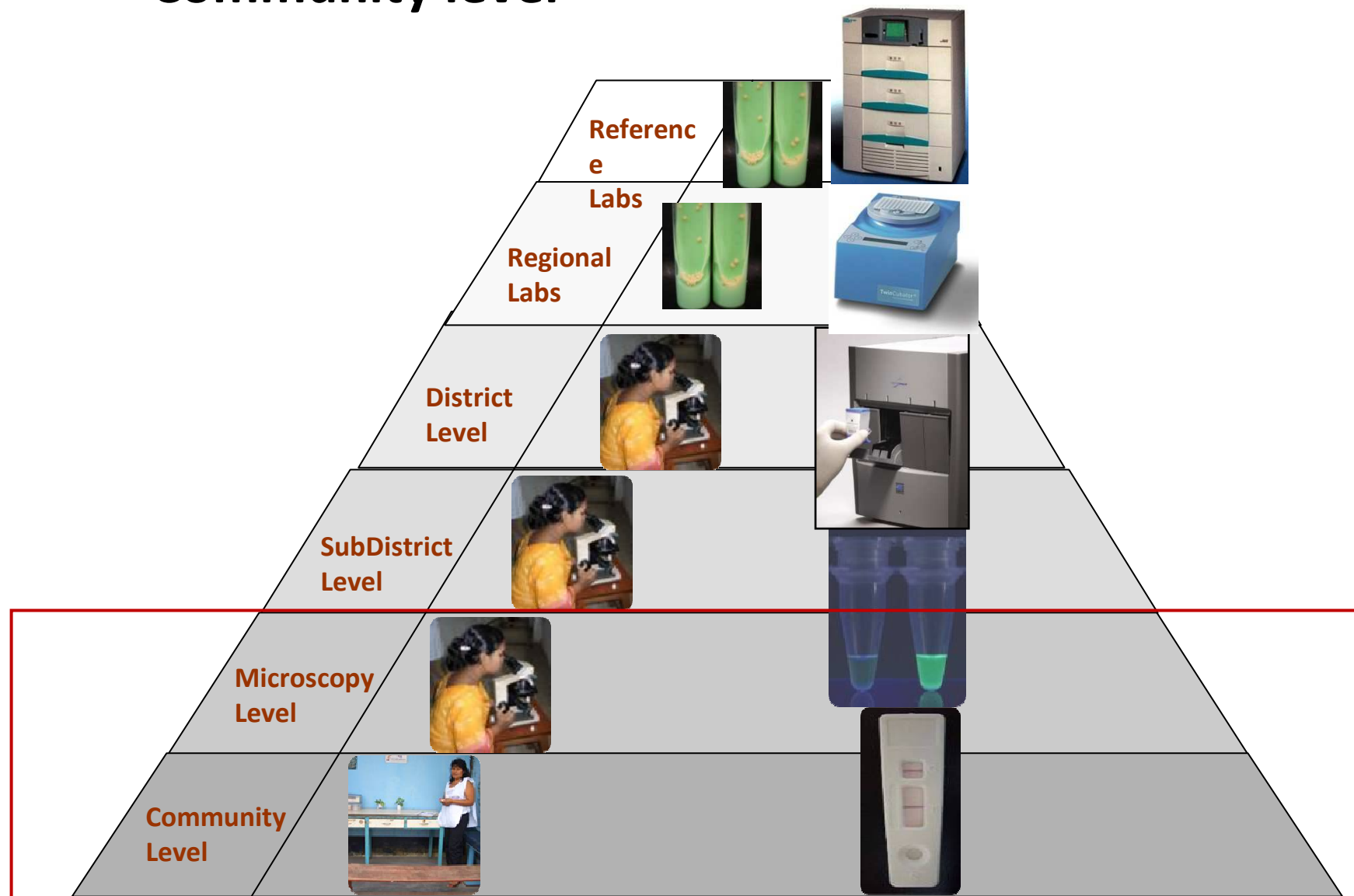
Comparison C_t mean values obtained from sputum samples collected before starting treatment (t_0) and 10-20 days after the beginning of anti-TB therapy (t_1).



PPaolo Miotto, Andrea M. Cabibbe, Sara Bigoni, Alberto Matteelli, Daniela M. Cirillo.

RETREATMENT OF CLINICAL SPECIMENS WITH PROPIDIUM MONOAZIDE ALLOWS THE USE OF MOLECULAR ASSAYS FOR MONITORING THE RESPONSE TO THERAPY IN TB PATIENTS (Presented as oral presentation at the 32nd Annual Congress of the European Society of Mycobacteriology, Lubeck – D, 26-29 June 2011)

Filling the diagnostic gap between District and Community level



Towards a point of care test

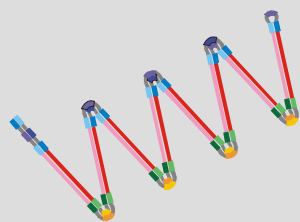


- Access at low health care level
- Rapid
- High sensitivity in S-C+
- Works with blood, urine, stool, saliva / nasopharyngeal swab
- Ability to diagnose extrapulmonary TB

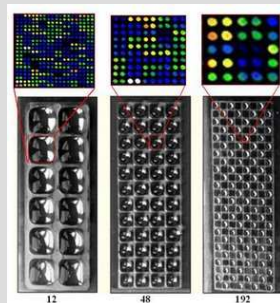
Diagnostic markers



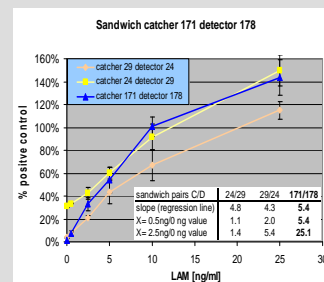
POC enabling technologies



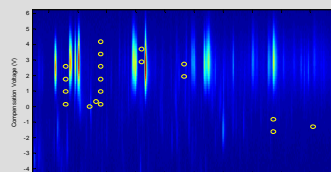
Molecular Detection



AB pattern Detection



AG Detection

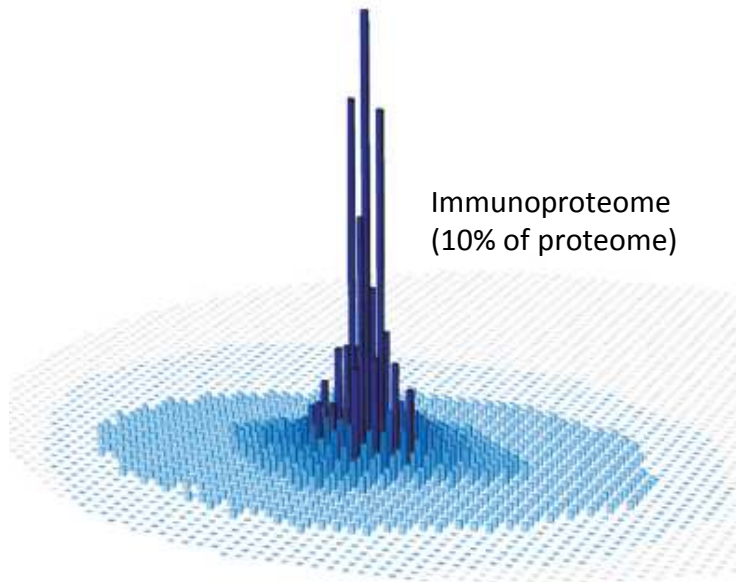


Physical Detection

WHO recommendation against the use of existing serologic tests



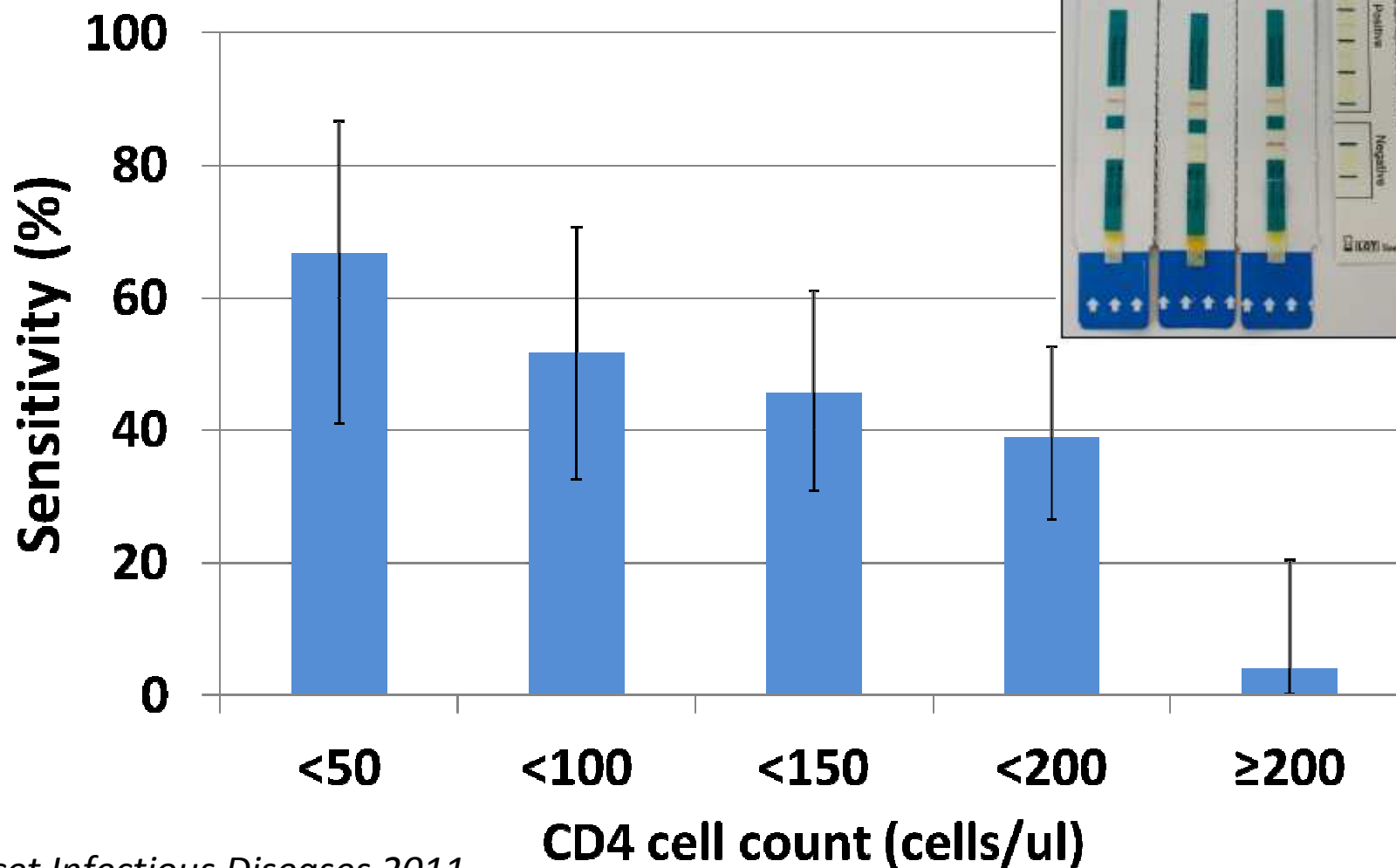
Recent progress towards an antigen array for TB detection in serum



- Whole proteome screening based on high throughput E. coli expression system
- 60 proteins identified as promising markers for active TB (13 in HIV-neg)
- Purified, recombinant proteins in mBio multiplex assay undergo early phase trials
- Expect multi-center trial to start in Apr 2012

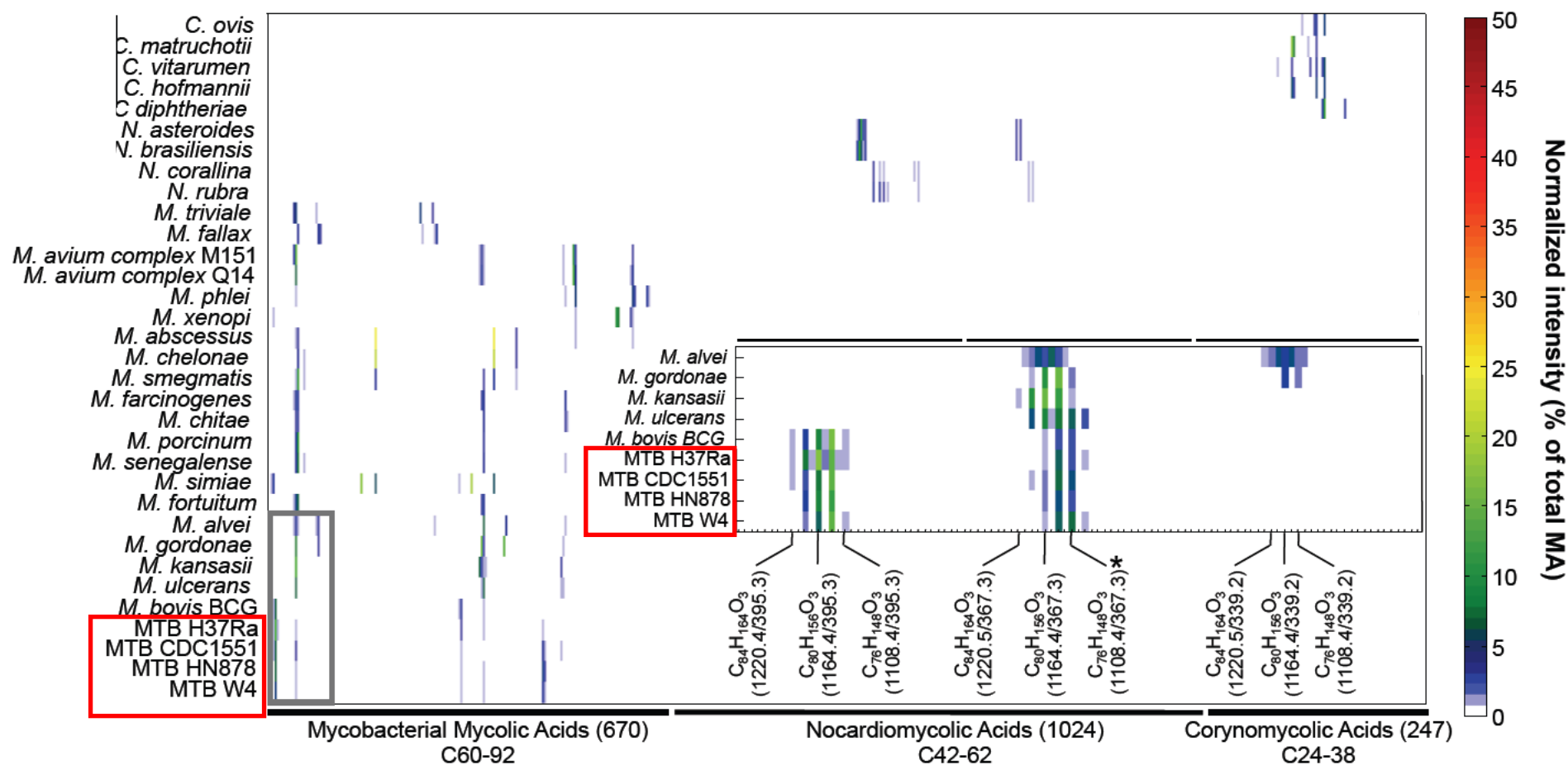
Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study

Stephen D Lawn, Andrew D Kerkhoff, Monica Vogt, Robin Wood



“Library” of Mycolic Acids identifies Novel TB Biomarkers

- Mass Spec Resolution = 670 MA / run
- Differentiation of branched chain MA
- Synthetic MA standards
- Simple sputum extraction

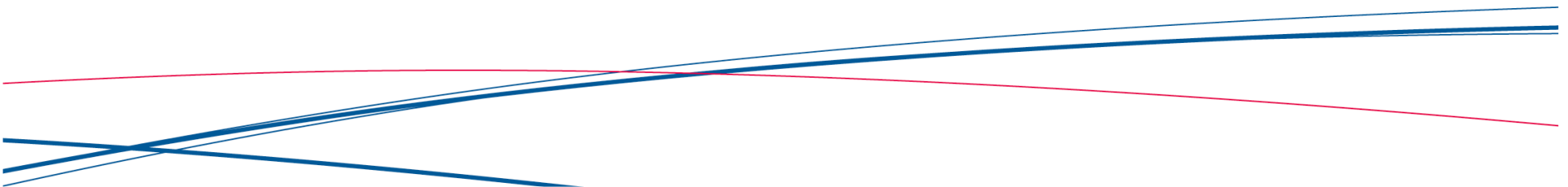


TB biomarker research - *Leaving the shallow end of the biomarker pool*



- Systematic approaches
- Large sample repositories
- Much more resources

Thank you



The New England Journal of Medicine

Методы

Мы оценили характеристики автоматического молекулярного теста Xpert MTB/RIF для определения *Mycobacterium tuberculosis* (MTB) и устойчивости к Рифампицину (RIF) с помощью полностью интегрированной системы обработки анализов, проведённой на 1730 больных с подозрением на лекарственно-чувствительные или лекарственно-устойчивые лёгочные формы туберкулёза. Каждый квалифицирующийся больной из Перу, Азербайджана, Южной Африки и Индии предоставил по три образца мокроты. Два образца были обработаны N-ацетил-L-цистеином и гидроксидом натрия перед микроскопией и перед твёрдым и жидким культуральным анализом, а также перед MTB/RIF тестом, а один образец использовался напрямую как для теста микроскопией, так и для MTB/RIF

Результаты

Среди культурально-положительных больных, одиночный прямой анализ MTB/RIF определил 551 из 561 больных с мазок-положительным туберкулёзом (72,5%) и 124 из 171 с культурально-отрицательным туберкулёзом (72,5%). Анализ являлся специфичным в случае 604 из 609 больных без туберкулёза (99,2%). Среди больных с отрицательными мазками, но культурально положительным туберкулёзом, добавление повторного анализа MTB/RIF увеличило чувствительность на 12,6 процента, а добавление третьего повторного анализа увеличило чувствительность на 5,1 процента до общей чувствительности 90,2%. По сравнению с анализом фенотипа на лекарственную чувствительность, анализ методом MTB/RIF правильно определил 200 из 205 больных (97,6%) с рифампицин-устойчивой микобактерией и 504 из 514 (98,1%) с рифампицин-чувствительной микобактерией. Секвенирование подтвердило все кроме двух случаев в пользу MTB/RIF анализа.

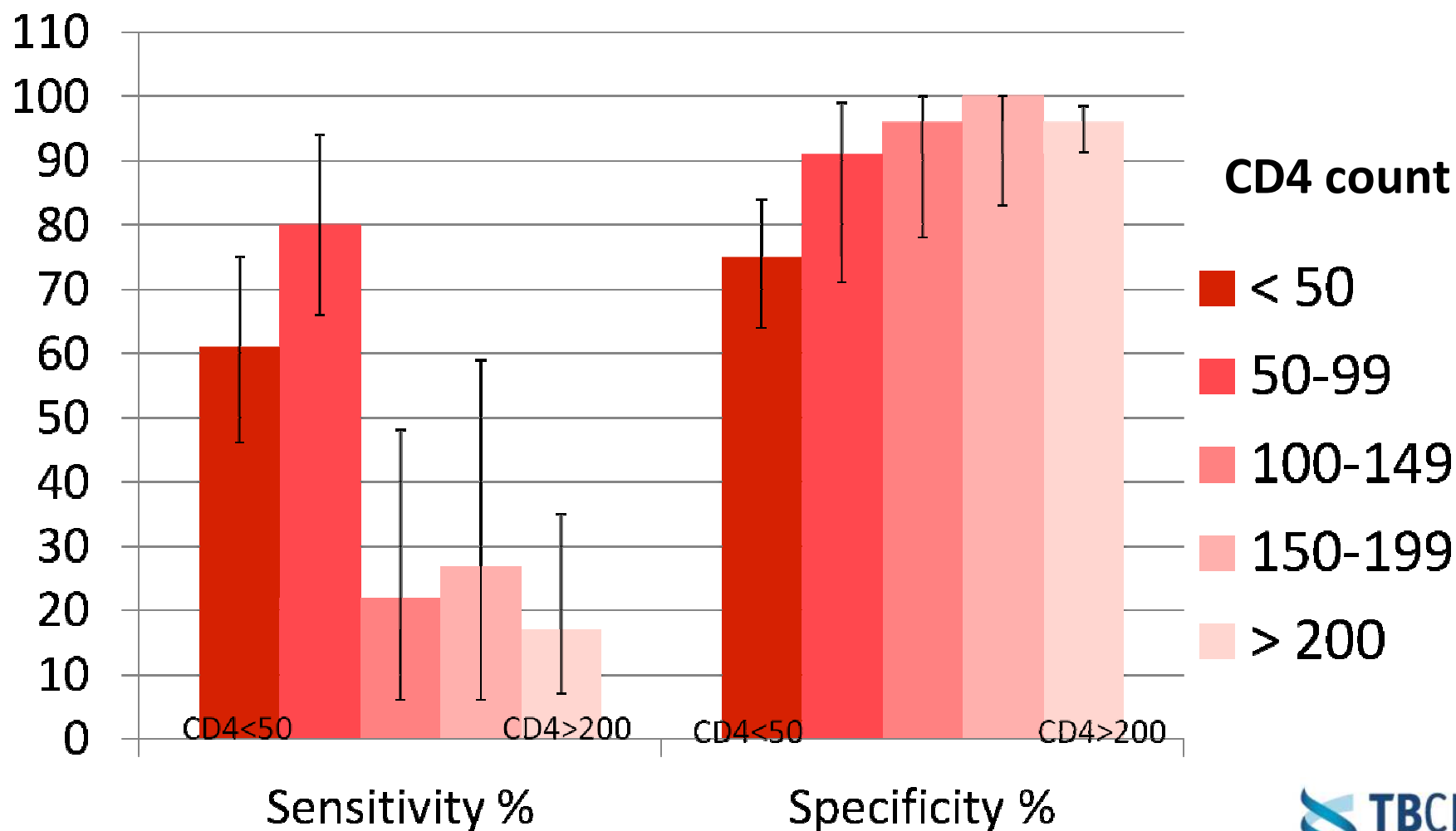
Заключение

Анализ MTB/RIF предоставляет чувствительное определение туберкулёза и устойчивости к рифампицину напрямую из необработанной мокроты менее чем за два часа с минимальной ручной затратой времени. (Спонсировано Организацией Инновационных Новых Диагностик, Foundation for Innovative New Diagnostics).

Alexander Gall
Alexei Korobitsyn

Спасибо!

Alere “Determine LAM” lateral flow assay performance by CD4 count (LF cut-point > or = 2+)



TB LAMP performance

	Lima, Peru	Kampala, Uganda	Sevegram, India	All
Sensitivity in C+	94.3% (100/106) [88.2% - 97.4%]	76.1% (140/184) [69.4% - 81.7%]	87.3% (89/102) [79.4% - 92.4%]	83.9% (329/392) [80.0% - 87.2%]
Sensitivity in S+C+	100.0% (67/67) [94.6% - 100.0%]	95.5% (105/110) [89.8% - 98.0%]	95.9% (70/73) [88.6% - 98.6%]	96.8% (242/250) [93.8% - 98.4%]
Sensitivity in S-C+	84.6% (33/39) [70.3% - 92.8%]	47.3% (35/74) [36.3% - 58.5%]	65.5% (19/29) [47.3% - 80.1%]	61.3% (87/142) [53.1% - 68.9%]
Specificity in S-C-	97.8% (364/372) [95.8% - 98.9%]	97.2% (520/535) [95.4% - 98.3%]	93.7% (448/478) [91.2% - 95.6%]	96.2% (1332/1385) [95.0% - 97.1%]

- Sensitivity target in S+C+ (>95%) met
- Point estimates close to meeting S-C+ target (65%) and S-C- target (97%)

Fluoroquinolones

Sensitivity:

75.6% (31/41)

90.2% (37/41)

87.5% (28/32)

87.5% (21/24)

100% (7/7)

85.5%

Specificity:

100% (21/21)

100% (129/129)

100% (19/19)

96.4% (27/28)

100% (22/22)

99.5%

Kiet et al., (Vietnam)

Hillemann et al., (Germany)

Nikolayevskyy V et al., (Russia)

Brossier V et al., (France)

van Ingen et al., (Russia)

Ethambutol

Sensitivity:

59.0% (46/78)

64.2% (34/53)

57.1% (16/28)

60.4%

Specificity:

100% (92/92)

100% (9/9)

91.7% (22/24)

98.4%

Hillemann et al., (Germany)

Kiet et al., (Vietnam)

Brossier V et al., (France)

Injectable drugs

Amikacin

Sensitivity:

86.8% (46/53)

100% (10/10)

100% (8/8)

90%

Specificity:

100% (117/117)

100% (42/42)

100% (21/21)

100%

Hillemann et al., (Germany)

Brossier V et al., (France)

van Ingen et al., (Russia)

Kanamycin

Sensitivity:

87.5% (10/13)

75.6% (5/5)

83.3%

Specificity:

100% (39/39)

100% (57/57)

100%

Brossier V et al., (France)

Kiet et al., (Vietnam)

Capreomycin

Sensitivity:

86.8% (46/53)

90.9% (10/11)

100% (8/8)

88.9%

Specificity:

99.1% (116/117)

97.6% (40/41)

100% (21/21)

98.9%

Hillemann et al., (Germany)

Brossier V et al., (France)

van Ingen et al., (Russia)