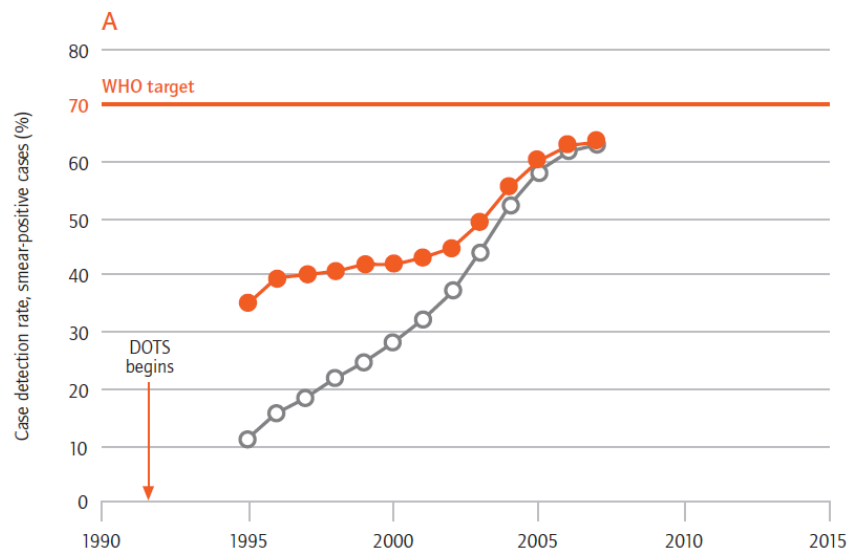# Rapid culture and drug susceptibility testing using non-commercial methods

Jessica Minion, MD
McGill University, Montreal, Canada

GLI Meeting
Veyrier-du-Lac, France
October 15th, 2009

Global TB Case Detection

Global
Tuberculosis
Control
2009

EPIDEMIOLOGY
STRATEGY
FINANCING

World Health
Organization

- 2.6 million new smear + cases notified in 2007

- 64% of the estimated 4.1 million cases

- 5.3 million new cases overall notified in 2007

- 57% of the estimated 9.3 million cases

TUBERCULOSIS
MDR-TB & XDR-TB
THE 2008 REPORT
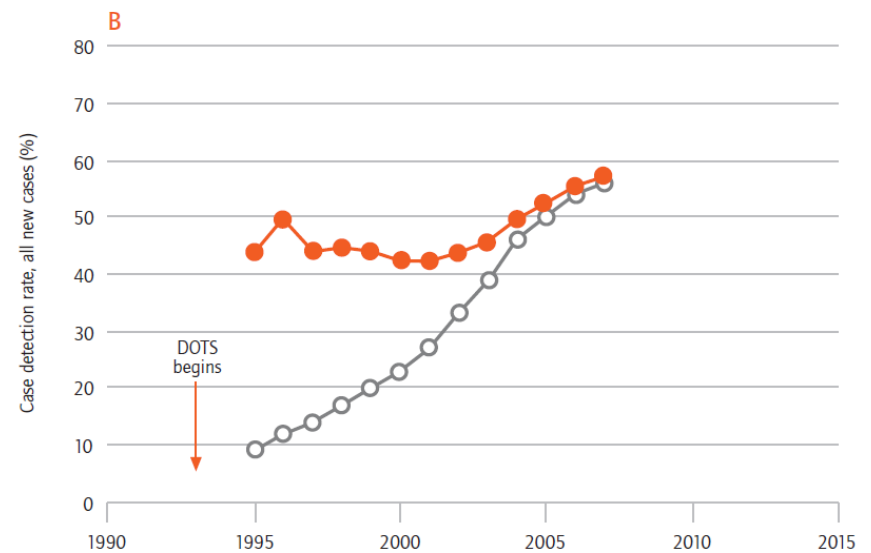
World Health Organization

THE **STOP TB** DEPARTMENT

Conclusions:

• **Highest rates ever recorded** of MDR-TB

• **Highest rates** are in countries of the former Soviet Union and China

• **Severely limited laboratory capacity** has meant limited data availability in Africa

• **Insufficient efforts** in many areas of the world to treat and control MDR-TB

• Equipment to **rapidly diagnose** MDR-TB in 1 week instead of 3 months exists but most patients cannot access such services

• **XDR-TB in 45 countries** threatens to derail 10 years of progress in TB control and HIV management

• **Extraordinary measures** are needed in Eastern Europe: rapid detection, effective care, access to drugs

> 6 %
3-6 %
< 3%
No data

% of MDR-TB among new TB cases 1994-2007

Countries reporting XDR-TB as of June 2008
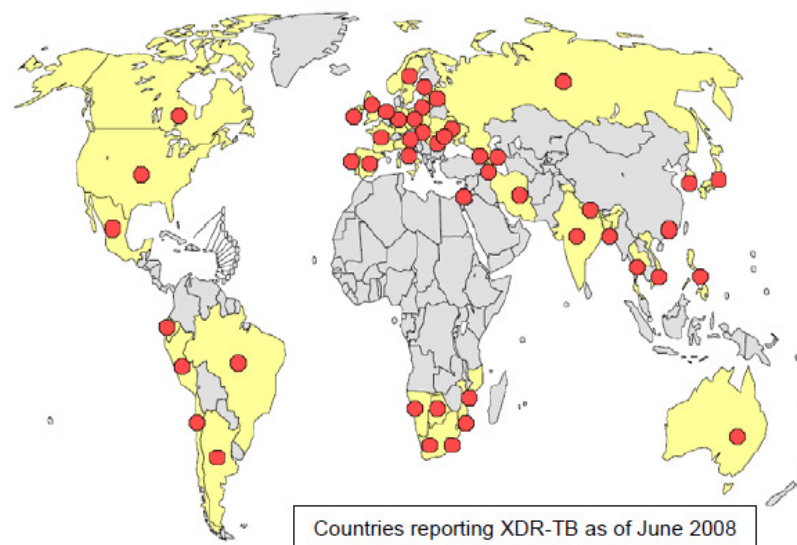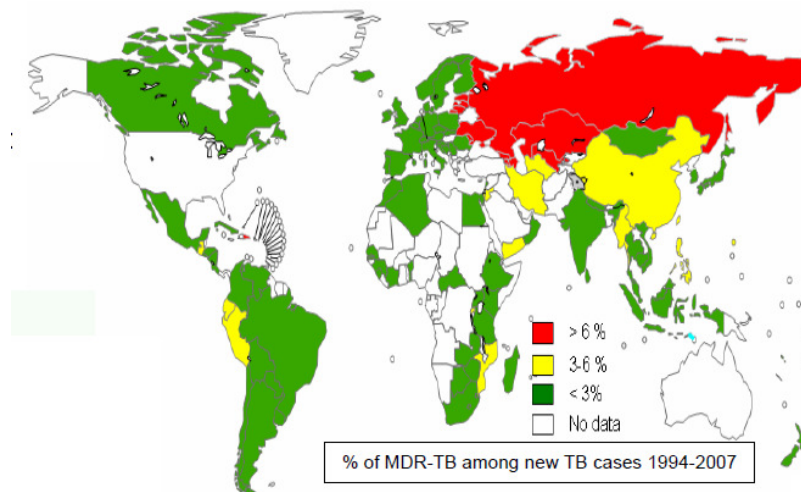
Current Policy Recommendations

World Health Organization

20, AVENUE APPIA – CH-1211 GENEVA 27 – SWITZERLAND – TEL CENTRAL +41 22 791 2111 – FAX CENTRAL +41 22 791 3111 – WWW.WHO.INT

Use of Liquid TB Culture and Drug Susceptibility Testing (DST) in Low and Medium Income Settings

World Health Organization

WHO policy statement: molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis

- 2007
  - Liquid medium for culture and DST

- 2008
  - Line probe assays for rapid MDR-TB screening

Initiatives to Expand Access

FIND
foundation
or innovative new diagnostics

Partnering for better diagnosis for all

| ABOUT US | OUR PROGRAMS | OUR PARTNERS | RESOURCE CENTER | NEWS ROOM |

SEARCH FOR: [   ] GO

LOSIS

SICKNESS

ORY PREPAREDNESS

FEEDBACK | DONATE TO FIND

FIND prices for BACTEC and MGIT and Country List

Enhancement of diagnostic capacity for TB and MDR-TB is urgently needed to scale-up access to care and treatment of MDR-TB. To help meet this challenge, FIND has collaborated in the development and evaluation of new TB diagnostic tools, including TB liquid culture and DST, rapid species identification, and line probe assay. WHO has officially endorsed the use of these technologies based on the thorough evaluation of the evidence of their effectiveness under actual program conditions. As part of its role in the development and evaluation process of these tools, FIND has successfully negotiated with three of the manufacturing partners to obtain significant price reductions in order to facilitate access to these diagnostic technologies. These discounts average 50% on diagnostic instruments, and 75% on reagents, and are available to high TB burden countries that wish to procure TB diagnostics for use in the public and non-profit health care sectors, and who procure these tools with funding from the government, UNITAID, or the Global Fund. Furthermore, the FIND-negotiated agreements contain provisions for further discounts as procurement volumes of reagents increase. As these become available FIND shall communicate the new prices to the TB community. In the case of the BBL MGIT Tubes List Nr. 245122 below, it is expected that price shall be reduced in the second quarter of 2009.

Current FIND-negotiated prices, along with the list of countries eligible for the discounts, as with BD are listed below:

BD BACTEC™ MGIT™ 960 System	US$ 38,950.00
BBL MGIT Tubes List Nr. 245122 Reagents	US$ 205.00/100

UNITAID TOGETHER TO HEAL

| ABOUT | PROJECTS | RESOURCES | GOVERNANCE |

Expanding and accelerating access to diagnostics for patients at risk of MDR-TB

Print
E-mail

Description of the project

A. Project title: Expanding and accelerating access to diagnostics for patients at risk of multi-drug resistant tuberculosis (MDR-TB)

B. Timeframe: Project duration: 2009-2011, starting on the date of the final signature of the Memorandum of Agreement.

C. Amount committed by UNITAID: US$ 26 129 897

D. Lead partner: Global Laboratory Initiative (GLI), Stop TB Department, World Health Organization

E. Other partner(s): - Global Drug Facility (GDF), Stop TB Partnership, World Health Organization
- Foundation for Innovative New Diagnostics (FIND)

Are there non-commercial options for detection and DST that could serve as temporary solutions during capacity building?

- 2009

  - Microscopically Observed Drug Susceptibility (MODS)

  - Thin Layer Agar (TLA)

  - Nitrate Reductase Assay (NRA)

  - Colorimetric Redox Indicators (CRI)

 - Phage-based Assays (including FAST*Plaque*^{TM})

Review Questions

- "To perform systematic reviews of the literature and meta-analysis (where appropriate) of data examining the diagnostic accuracy and performance characteristics [of the assay] for the detection of drug resistance in MTB"
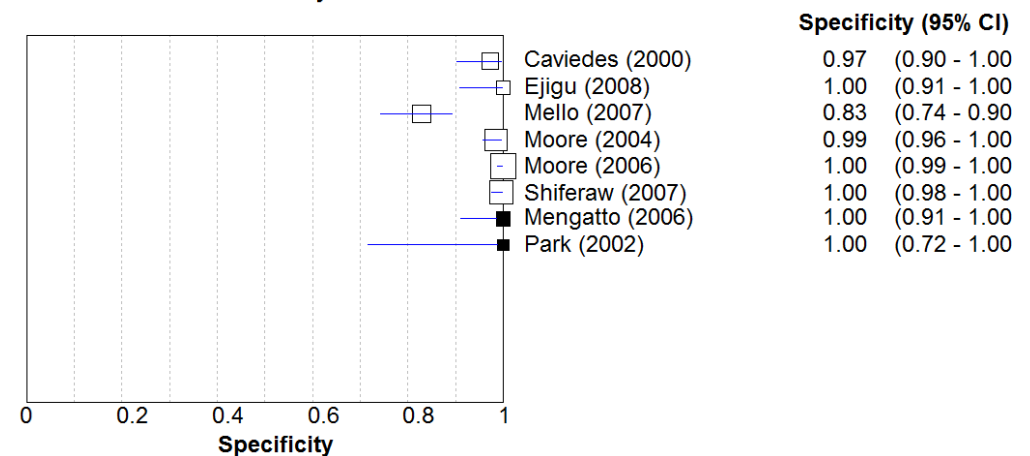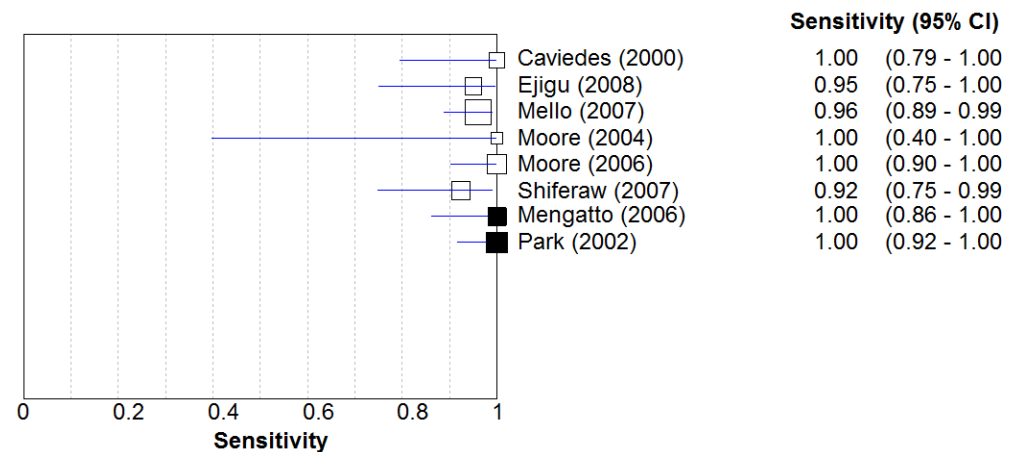
Microscopically Observed Drug Susceptibility (MODS)

- Direct or indirect inoculation of patient specimens for detection & DST

- Liquid media – increased sensitivity and faster growth

- Microcolony detection – faster turnaround time
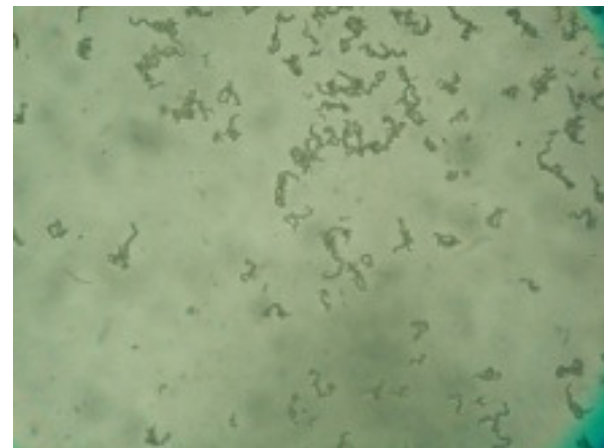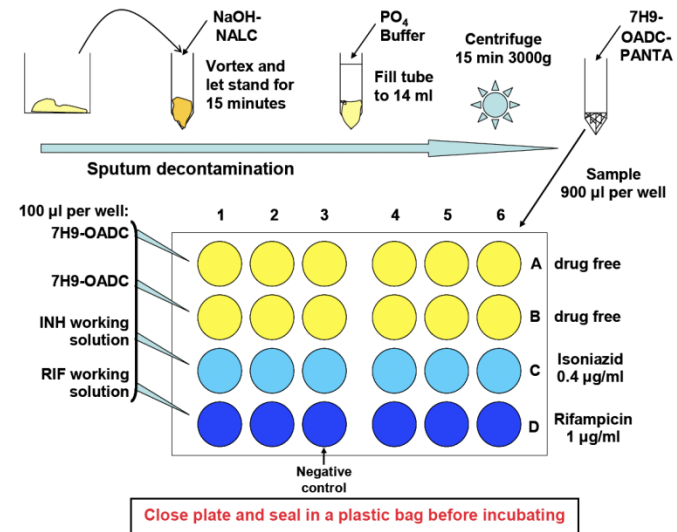
Results of Systematic Review (MODS)

- 9 studies identified
  - 6 direct inoculation
  - 3 indirect inoculation

- Overall (Rifampin, n=8)
  - Sensitivity = 98.0% (94.5, 99.3)
  - Specificity = 99.4% (95.7, 99.9)

- If more stringent exclusion criteria (n=5)
  - Sensitivity = 98.7% (89.4, 100)
  - Specificity = 100% (95.8, 100)

Sensitivity (95% CI)

Study	Sensitivity	95% CI
Caviedes (2000)	1.00	(0.79 - 1.00)
Ejigu (2008)	0.95	(0.75 - 1.00)
Mello (2007)	0.96	(0.89 - 0.99)
Moore (2004)	1.00	(0.40 - 1.00)
Moore (2006)	1.00	(0.90 - 1.00)
Shiferaw (2007)	0.92	(0.75 - 0.99)
Mengatto (2006)	1.00	(0.86 - 1.00)
Park (2002)	1.00	(0.92 - 1.00)

Specificity (95% CI)

| Study	Specificity	95% CI
Caviedes (2000)	0.97	(0.90 - 1.00)
Ejigu (2008)	1.00	(0.91 - 1.00)
Mello (2007)	0.83	(0.74 - 0.90)
Moore (2004)	0.99	(0.96 - 1.00)
Moore (2006)	1.00	(0.99 - 1.00)
Shiferaw (2007)	1.00	(0.98 - 1.00)
Mengatto (2006)	1.00	(0.91 - 1.00)
Park (2002)	1.00	(0.72 - 1.00)

Results of Systematic Review (MODS)

- Direct only (n=6)
  - Sensitivity = 96.8% (92.4, 98.7)
  - Specificity = 99.0% (94.3, 99.8)

- Contamination Rates (n=7)
  - MODS: 6.3%
  - vs. solid media comparisons: 10.4%
  - vs. liquid media comparisons: 4.1%

- Turnaround Time (n=6)
  - Direct Inoculation: 11.6 days (range 6 – 21)
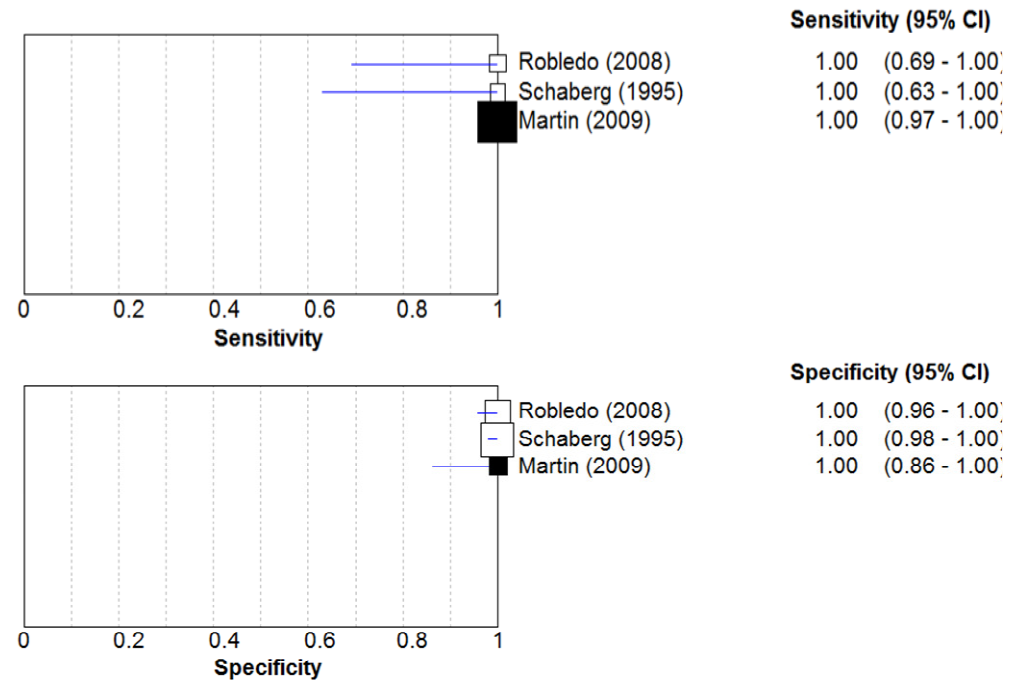 - Indirect Inoculation: 6.5 days (range 6 – 7)

Thin Layer Agar (TLA)

- Direct or indirect inoculation of patient specimens for detection & DST

- Solid media – easier to manipulate

- Microcolony detection – faster turnaround time
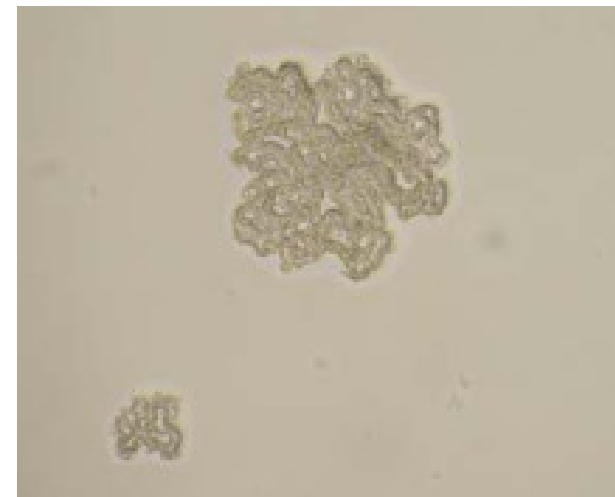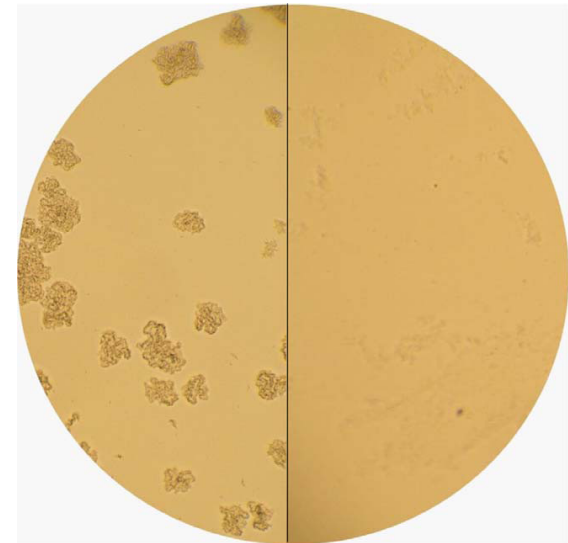
Results of Systematic Review (TLA)

- 3 studies identified
  - 2 direct inoculation
  - 1 indirect inoculation

- All reporting 100% accuracy

	Sensitivity (95% CI)
Robledo (2008)	1.00 (0.69 - 1.00)
Schaberg (1995)	1.00 (0.63 - 1.00)
Martin (2009)	1.00 (0.97 - 1.00)

	Specificity (95% CI)
Robledo (2008)	1.00 (0.96 - 1.00)
Schaberg (1995)	1.00 (0.98 - 1.00)
Martin (2009)	1.00 (0.86 - 1.00)

Results of Systematic Review (TLA)

- Contamination Rates (n=9)
  - TLA: 11.8%
  - vs. solid media comparisons: 5.5%
  - vs. liquid media comparisons: 9.7%

- Turnaround Time (n=2)
  - 11.1 days (range 11 – 11.2)

Nitrate Reductase Assay (NRA)

- Based on MTB's ability to reduce nitrate to nitrite

- Simple, direct or indirect inoculation of patient specimens for detection & DST

- Sensitive detection of small amounts of metabolic biproduct improves turnaround time

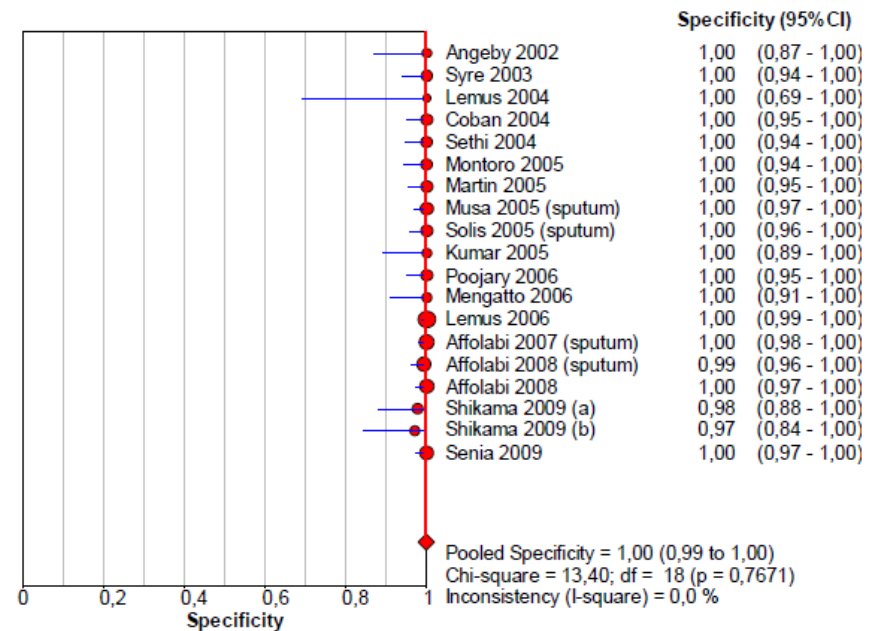
KNO_3 - containing media

↓

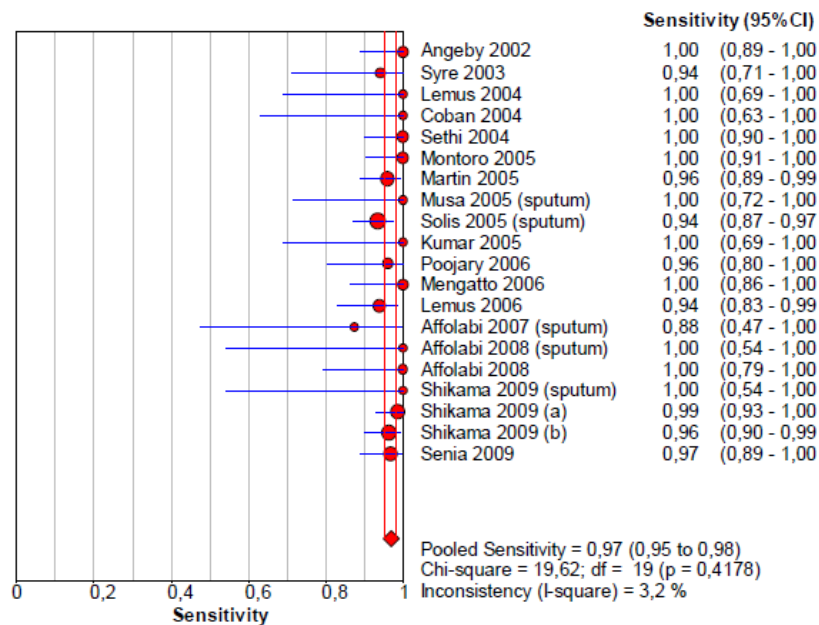Add reagent to drug-free slant day 7 (repeat day 10, 14)

↓

Color development = growth

Results of Systematic Review (NRA)

- Overall (n=20)
  - Sensitivity = 97.0% (95.0, 98.0)
  - Specificity = 100% (99.0, 100)

Results of Systematic Review (NRA)

- Direct only (n=5)
  - Sensitivity = 96.0% (92.0, 98.0)
  - Specificity = 99.6% (98.7, 100)

- Contamination Rate
  - 4.8%

- Turnaround Time
  - 7 – 14 days

Colorimetric Redox Indicators (CRI)

- Based on reduction of indicator by metabolically active MTB

- MIC determination using microdilution

- Sensitive detection metabolic activity improves turnaround time

Incubate microdilution plate 7 days

⬇

Add indicator to all wells, incubate overnight

⬇

Color change = growth

Results of Systematic Review (CRI)

- Overall (n=31)
  - Sensitivity = 98.0% (96.0, 99.0)
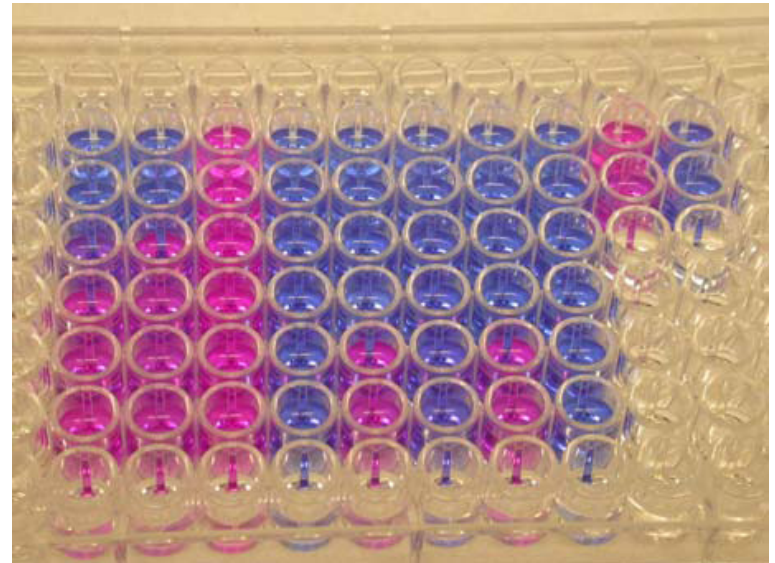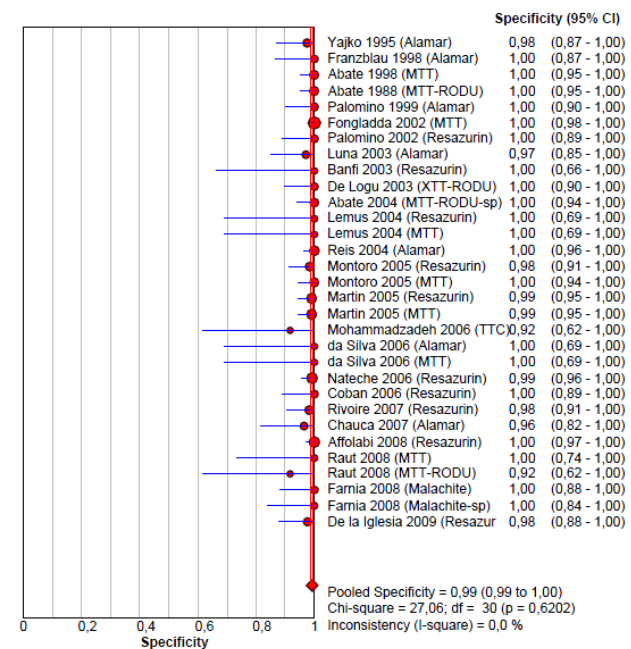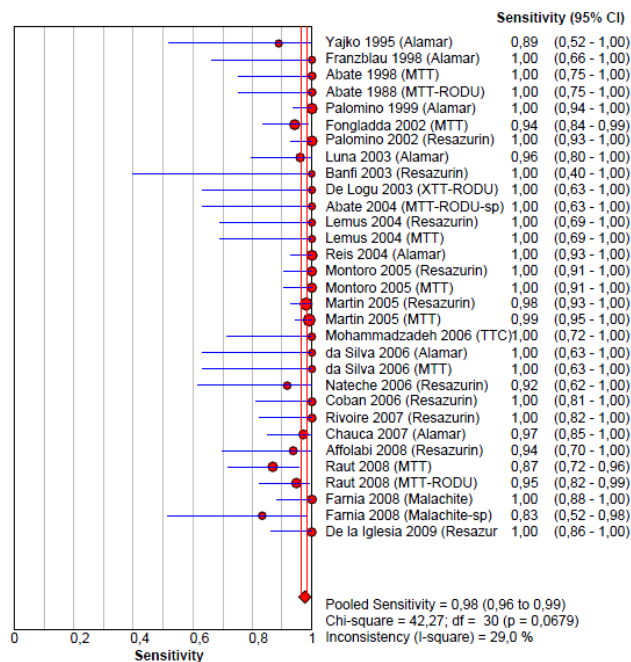 - Specificity = 99.0% (99.0, 100)

Results of Systematic Review (CRI)

- Direct only (n=2)
  - Sensitivity = 90.0% (68.3, 98.8)
  - Specificity = 100% (98.7, 100)

- Contamination Rate
  - 5%

- Turnaround Time
  - 7 days

Mycobacteriophage Assays:
FAST*Plaque*™, in-house amplification, in-house luciferase reporter phage (LRP)

- Uses bacteriophage viruses to infect and detect viable MTB

- Amplification approach or luciferase light production

- 2 day turnaround time, direct or indirect detection & DST

Actiphage

Actiphage infection

TB bacillus

Virusol addition

Sensor cell

Actiphage detection as plaques on lawns of Sensor cells grown in a Petri dish

Neutralisation then amplification of Actiphage in Sensor cells
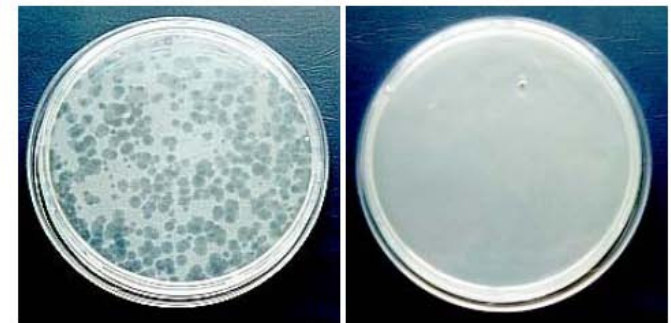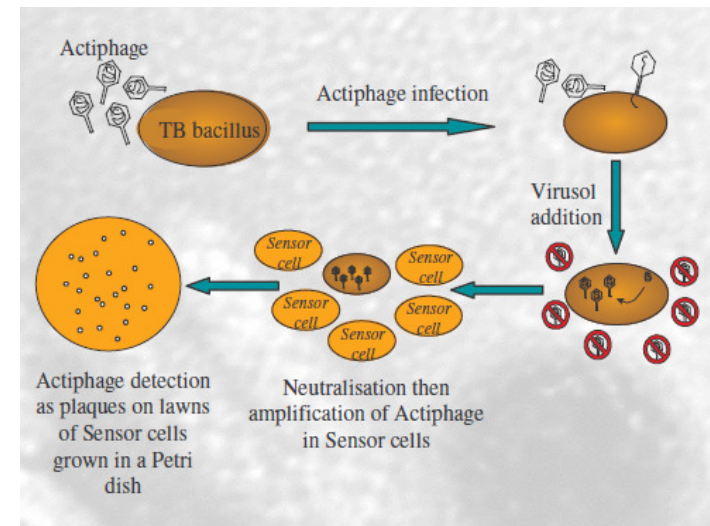
Plaques – viable MTB cells present

No plaques – no viable MTB cells present

Results of Systematic Review (Phage)

- Overall (FAST*Plaque*™, n=15)
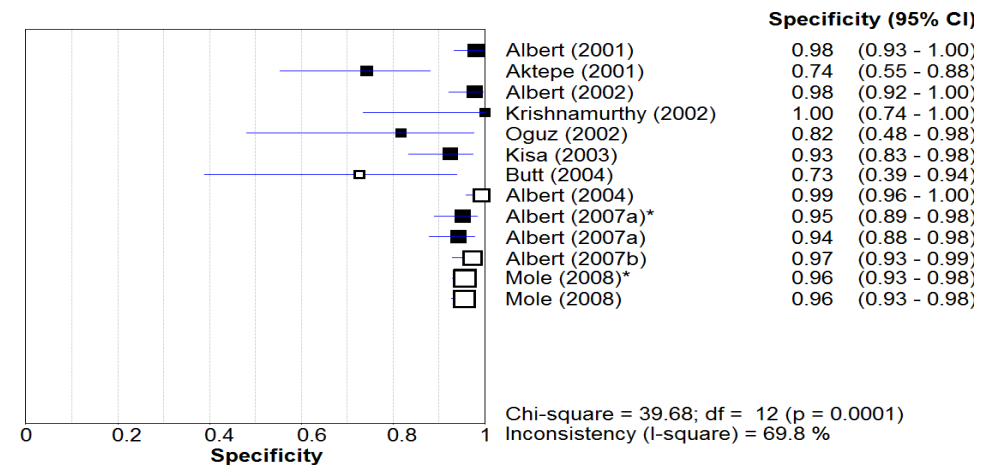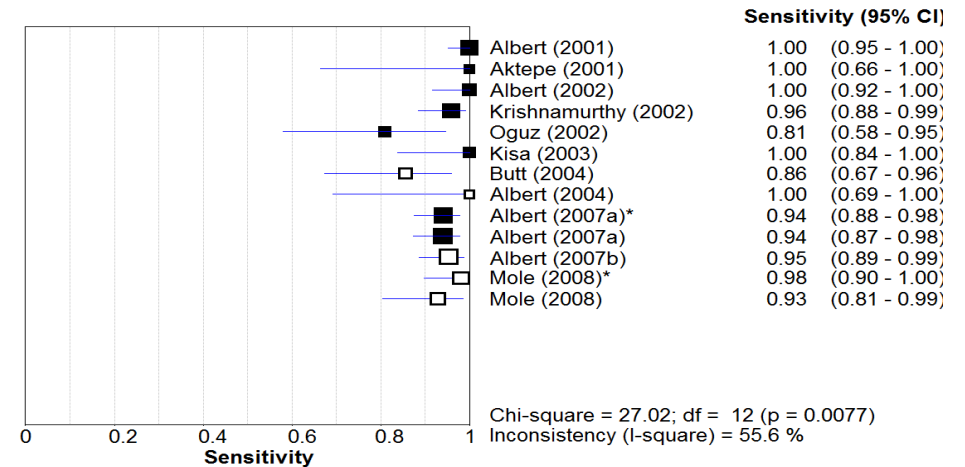 - Sensitivity = 95.0% (91.5, 97.1)
  - Specificity = 95.3% (91.1, 97.6)

- Overall (in-house amplification, n=11)
  - Sensitivity = 98.7% (96.3, 99.6)
  - Specificity = 98.2% (94.9, 99.4)

- Overall (LRP, n=8)
  - Sensitivity = 99.6% (35.6, 100)
  - Specificity = 99.4% (93.4, 99.9)

Sensitivity (95% CI)

Albert (2001)	1.00	(0.95 - 1.00)
Aktepe (2001)	1.00	(0.66 - 1.00)
Albert (2002)	1.00	(0.92 - 1.00)
Krishnamurthy (2002)	0.96	(0.88 - 0.99)
Oguz (2002)	0.81	(0.58 - 0.95)
Kisa (2003)	1.00	(0.84 - 1.00)
Butt (2004)	0.86	(0.67 - 0.96)
Albert (2004)	1.00	(0.69 - 1.00)
Albert (2007a)*	0.94	(0.88 - 0.98)
Albert (2007a)	0.94	(0.87 - 0.98)
Albert (2007b)	0.95	(0.89 - 0.99)
Mole (2008)*	0.98	(0.90 - 1.00)
Mole (2008)	0.93	(0.81 - 0.99)

Chi-square = 27.02; df = 12 (p = 0.0077)
Inconsistency (I-square) = 55.6 %

Sensitivity

Specificity (95% CI)

Albert (2001)	0.98	(0.93 - 1.00)
Aktepe (2001)	0.74	(0.55 - 0.88)
Albert (2002)	0.98	(0.92 - 1.00)
Krishnamurthy (2002)	1.00	(0.74 - 1.00)
Oguz (2002)	0.82	(0.48 - 0.98)
Kisa (2003)	0.93	(0.83 - 0.98)
Butt (2004)	0.73	(0.39 - 0.94)
Albert (2004)	0.99	(0.96 - 1.00)
Albert (2007a)*	0.95	(0.89 - 0.98)
Albert (2007a)	0.94	(0.88 - 0.98)
Albert (2007b)	0.97	(0.93 - 0.99)
Mole (2008)*	0.96	(0.93 - 0.98)
Mole (2008)	0.96	(0.93 - 0.98)

Chi-square = 39.68; df = 12 (p = 0.0001)
Inconsistency (I-square) = 69.8 %

Specificity

Results of Systematic Review (Phage)

- Direct only (n=5, FAST*Plaque*™ only)
  - Sensitivity = 93.0% (88.0, 96.7)
  - Specificity = 96.3% (91.6, 98.4)

Results of Systematic Review (Phage)

- Large range of contaminated or indeterminate results: 0 – 36% (mean = 5.8%)

- Primarily a problem for studies using direct specimens: 3 – 36% (mean = 20.4%)
  - 18 out of 28 arms using indirect specimens did not report any contaminated/indeterminate results

- 3 studies with arms using antibiotic supplement (NOA) showed decreased contamination by 36 – 94%
  - No statistically significant difference in accuracy

Summary Findings

Diagnostic (Reference)	# Studies (Participants)	Pooled Accuracy Estimates from Meta-Analyses		Turnaround Time (direct)	Contamination Rates (direct)	Quality of Evidence	Costs (as per NDWG)	Resources (as per NDWG)
		Sens	Spec					
MODS	9 studies (n=1474)	0.980	0.994	11.6 days	6.3%	Moderate	Equipment: ++ Consumables: ++	Training: extensive Infrastructure: ++/+++
TLA	3 studies (n=439)	1.00	1.00	11.1 days	11.8%	Low	Equipment: + Consumables: ++	Training: extensive Infrastructure: ++/+++
Phage – FASTPlaque	12 studies (n=2945)	0.950	0.953	1 – 2 days	20.4%	Very Low	Equipment: ++ Consumables: +++	Training: moderate Infrastructure: ++/+++
CRI	31 studies (n=2498)	0.980	0.990	7 days	5%	Moderate	Equipment: + Consumables: ++	Training: extensive Infrastructure: +++
NRA	19 studies (n=2304)	0.970	1.00	7 – 14 days	4.8%	Moderate	Equipment: + Consumables: ++	Training: moderate Infrastructure: ++/+++
WHO-endorsed rapid test for DST (for comparison)								
LPA	12 studies (n=4937)	0.981	0.987	1 – 2 days		Moderate	Equipment: +++ Consumables: +++	Training: moderate Infrastructure: ++/+++

Concerns and Issues

- Lack of data on outcomes other than accuracy

- Quality of primary studies
  - Case control vs. Cross-sectional designs
  - Convenience sampling vs. Consecutive/Random
  - Retrospective vs. Prospective data collection
  - Reporting of blinding

Concerns and Issues

- Non-commercial methods generally suffer from lack of standardization

- Large scale demonstration studies have not been performed, and are not likely to be performed

- Limited data using direct patient specimens, even though this would be the most important application

- Setting of implementation? Peripheral vs. central laboratories

- Biosafety concerns

- Specificity of species identification

STAG Meeting – November 2009

- Final policy recommendations to be determined

Acknowledgements

- Anandi Martin, Madhukar Pai, Karin Weyer

- Juan Palomino, Dick Menzies, Erika Leung

- David Moore, Jaime Robledo, Susan Dorman, Rick O'Brien, Heidi Albert, Andre Trollip, Ruth McNerney, Sonya Shin, Peter Cegielski, Martin Yagui, Freddie Bwanga, Sven Hoffner, Senia Rosales, Parissa Farina, P. Narang