

Diagnosis of TB and drug resistance in
15 minutes with a piece of paper in 15
mins for 5 euros



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Swiss Lung Association

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Better diagnosis



Answer four main questions...

Why do we want or need this?

Can we diagnose TB and drug resistant TB accurately?

Can we do this in under 15 minutes....

Can we do this for less than 5 euros...

At this moment right now the answer is.....

Better diagnosis



....No

..(well maybe...)



Actions and recommendations Laboratories



Ensure swift access to rapid tests for rifampicin resistance

Laboratories should aim to identify TB and rifampicin resistance in over 90% of cases from smear-positive sputum directly where resources are available for this..... Rapidly within 1-2 days

Eur Respir J 2006; 28: 1–7
DOI: 10.1183/09031936.06.00084906
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Recommended standards for modern tuberculosis laboratory services in Europe

**F.A. Drobniowski*, S. Hoffner[#], S. Rusch-Gerdes[†], G. Skenders⁺,
V. Thomsen⁵ and the WHO European Laboratory Strengthening Task Force**



Why do we want or need this?



...

MDRTB Mx in general

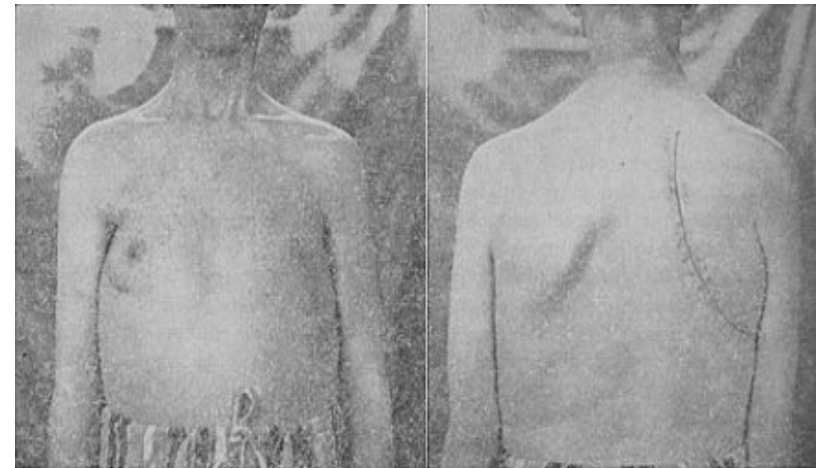


In general, standardised treatment worse outcomes than individualised treatment

Pre-chemotherapy: about 1/3 self-cure, 1/3 die quickly, 1/3 remain ill and die slowly ('consumption')

Start with 5 or more drugs to which the organism is, or is likely to be sensitive.

Once sensitivities are available and there has been clinical response a minimum of 3 or 4 drugs are needed for consolidation Rx.



TB Extensive Resistance to Second-Line Drugs --- Worldwide, 2000--2004



Emergence of *Mycobacterium tuberculosis* with Extensive Resistance to Second-Line Drugs --- Worldwide, 2000--2004

A Wright, G Bai, L Barrera, F Boulahbal,, N Martín-Casabon, C Gilpin, , F Drobniowski, M Havelková, R Lepe, R Lumb, B Metchock, F Portaels, M Rodrigues, S Rüsck-Gerdes A Van Deun, V Vincent, V Leimane, V Riekstina, G Skenders, T Holtz, R Pratt, K Laserson, , C Wells P Cegielski, NS Shah. MMWR March 24, 2006 / 55(11);301-305

XDR: Defined as cases in persons with TB whose isolates were resistant to isoniazid and rifampin and at least three of the six main classes of SLDs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicylic acid).



Weekly

March 24, 2006 / Vol. 55 / No. 11

World TB Day — March 24, 2006

World TB Day is March 24. This annual event commemorates the date in 1882 when Robert Koch announced his discovery of *Mycobacterium tuberculosis*,

Emergence of *Mycobacterium tuberculosis* with Extensive Resistance to Second-Line Drugs — Worldwide, 2000–2004



South Africa Tugela Ferry XDR 2006

52 of 53 (98%) XDR TB patients died

100% of those HIV tested with XDR were HIV positive

Majority never previously treated or had previous cure or treatment completion

Suggests newly infected with drug-resistant TB strains

26 of 30 (87%) XDR TB isolates found to be genetically similar

Nosocomial transmission in hospitals likely

Transmission in community also possible since 36% XDR TB patients with no prior hospitalizations

Lancet 2006

**KwaZulu Natal 18% sensitive TB cases completed therapy,
13% cured (JTLD 2008)**



XDR-TB in the UK, 1995-2009



Between 1995 and 2009 there were 9 confirmed XDRTB cases in the UK

Most were pulmonary

Most were male

Most aged 20-50

Most had had previous TB

Most born abroad.

Distributed across country

Extensively drug-resistant tuberculosis in the UK:
1995 to 2007

I Abubakar,¹ J Moore,¹ F Drobniewski,¹ M Kruijshaar,¹ T Brown,¹ M Yates,¹
C Anderson,¹ E G Smith,² J Magee,³ M Lipman,⁴ J McMenemy,⁵ M Ruddy,⁶
J M Watson¹

Thorax Jun 2009

Second line drug resistance in MDRTB



Table 1 Second-line antituberculosis drug resistance among multidrug-resistant isolates of *M tuberculosis* complex, UK, 1995–2007

Class	Drug	Resistant/tested* (%)
Fluoroquinolones	Ciprofloxacin	34/610 (5.6)
	Moxifloxacin	1/24 (4.2)
	Ofloxacin	1/26 (3.9)
	Any fluoroquinolone	36/647 (5.6)
Serine analogues	Cycloserine	28/551 (5.1)
Aminoglycosides	Amikacin	32/583 (5.5)
	Kanamycin	1/24 (4.2)
Polypeptides	Capreomycin	20/592 (3.4)
Thionamides	Ethionamide	84/601 (14.0)
	Prothionamide	2/5 (40.0)
	Any thionamide	86/606 (14.2)
PAS	PAS	77/462 (16.7)
Others	Clarithromycin	70/587 (11.9)
	Azithromycin	8/92 (8.7)
	Streptomycin	327/636 (51.4)
	Rifabutin	138/160 (86.3)
	Clofazimine	33/94 (35.1)

*Where information on testing was available among a total of 678 isolates.
PAS, *p*-aminosalicylic acid.

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Aggressive treatment -better results

Mitnick et al (NEJM 2008; 359:563-574) showed that in Lima, Peru, cure rates of XDR-TB in HIV negative reaches 60%

29/48(60.4%) completed treatment or were cured, compared with 400 patients (66.3%) with MDRTB (P=0.36).

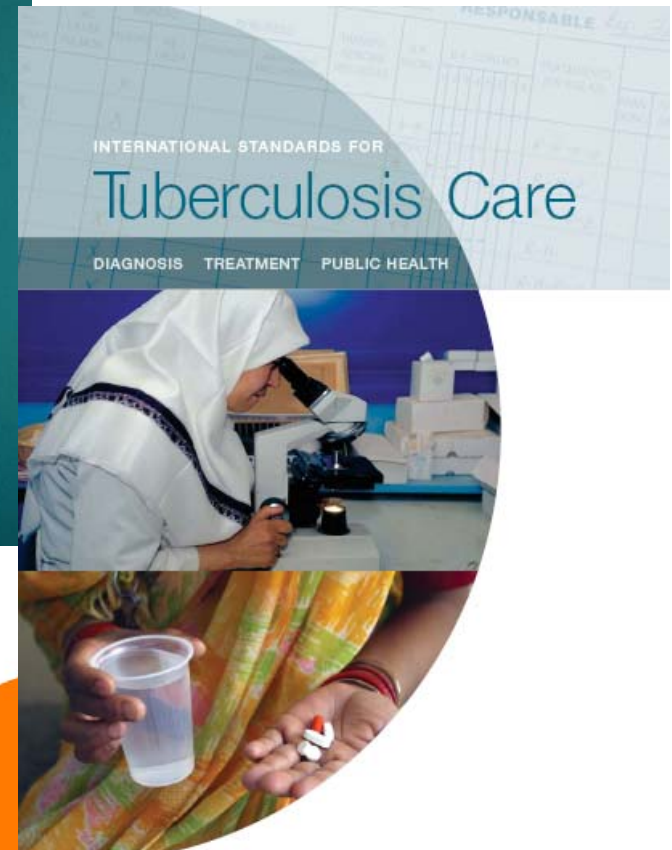
HOW?: early DST, early and rational use of at least 5 drugs including SLD, regimen re-adjustment, strict supervision and patient support, constant bacteriological monitoring

"A hope and a challenge" (Editorial, NEJM, 2008; 359:636-7)

Similar XDRTB and MDRTB success shown in Korea (**Kwon et al CID 2008 15;47(4):496-502**)

Diagnose early, cure early

Actions and recommendations

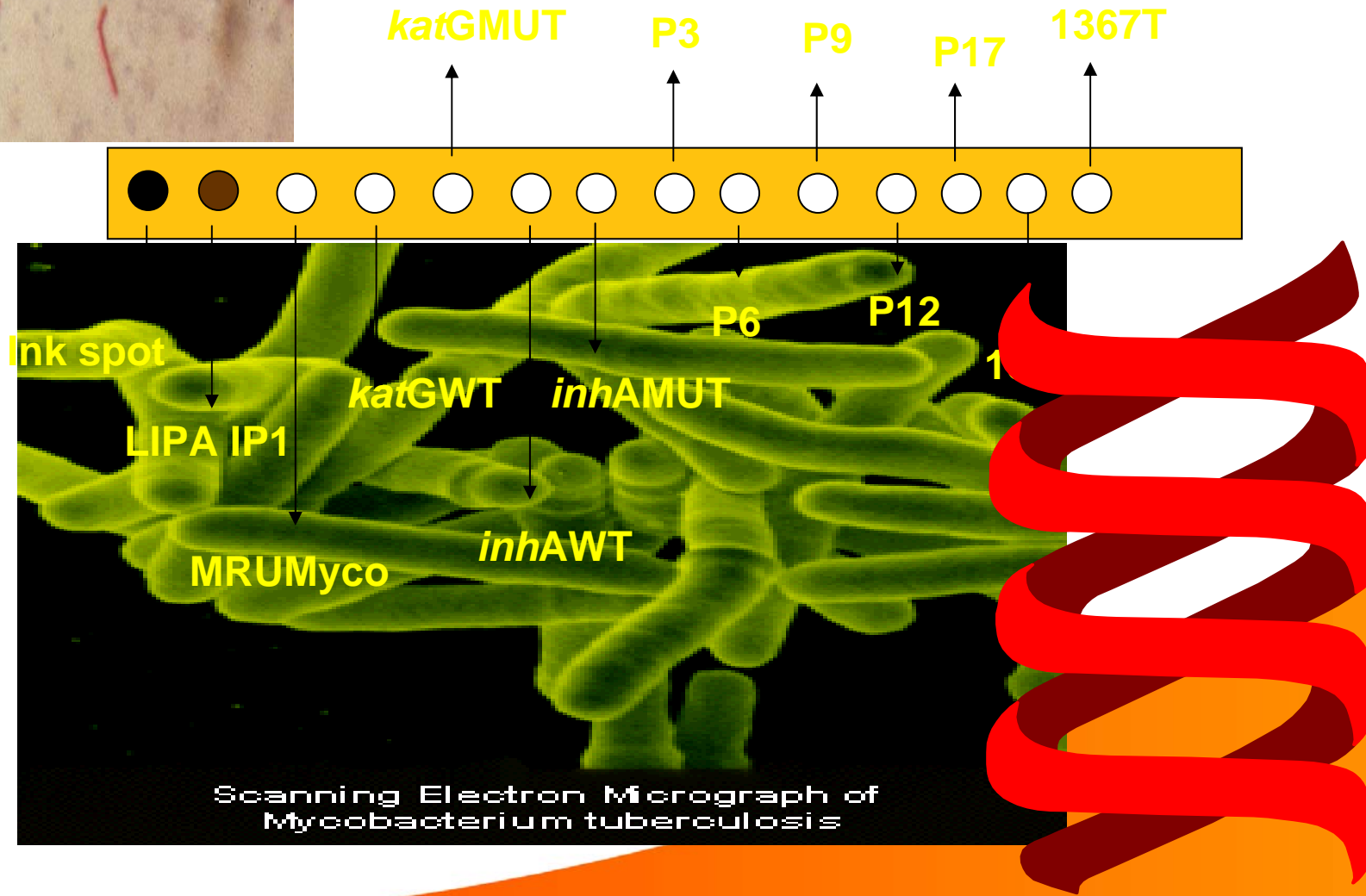


Implement

WHO Guidelines for the programmatic management of drug-resistant tuberculosis

International Standards for TB Care

Can we diagnose TB and drug resistance accurately?



Diagnosis latent and active disease



Latent infection: Skin testing, Gamma Interferon release assays

Active TB: Microscopy-light vs fluorescent (17% more sensitive)

Culture-solid and liquid media, manual vs automated

Serology-fast poor sensitivity and specificity

NAAT: commercial vs in-house PCR,

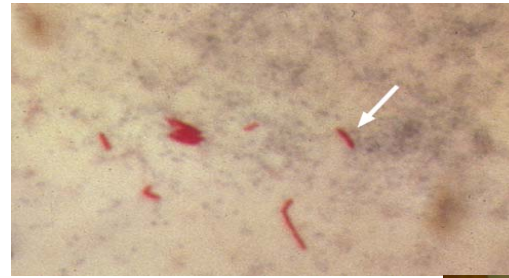
Automated liquid culture most sensitive, relatively slow compared to NAAT

NAAT too insensitive also for childhood TB....



of TR TR meningitic

Diagnosis: Microscopy



Proof of principle to demonstration projects,

Eg Microscopy: fluorescent microscopy more sensitive than light microscopy and higher throughput (right)

But higher cost, mains power, work in dark, bulbs very expensive, 100 hours, mercury vapour if broken

LED-red and green LEDs many years but correct high intensity blue range last 3 years; cheap, long lasting, batteries

Multicenter demonstration projects, analysis May 2009, WHO

Improve smear microscopy by better instruction eg tell women how to cough Sameer Khan et al. Lancet 2007; 369 1955-60



Culture

Solid + rapid liquid systems

Sensitive for diagnosis

Slow but faster for liquid eg approx 14-21 days for smear positive sputum specimens

Also for drug resistance

Commercial=standardised, QA, more expensive, supply chain critical,



Evaluation of MGIT 960-Based Antimicrobial Testing and Determination of Critical Concentrations of First- and Second-Line Antimicrobial Drugs with Drug-Resistant Clinical Strains of *Mycobacterium tuberculosis*

Annika Krüüner,^{1,2} Malcolm D. Yates,¹ and Francis A. Drobniowski^{1*}

Health Protection Agency, Mycobacterium Reference Unit, Clinical Research Centre, Barts and the London School of Medicine, Queen Mary College, University of London, 2 Newark Street, London, United Kingdom E1 2AT,¹ and Tartu University Clinics, United Laboratory, Department of Mycobacteriology, Tartu, Estonia²

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2006, p. 688–692
0095-1137/06/\$08.00+0 doi:10.1128/JCM.44.3.688–692.2006
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Vol. 44, No. 3

Multicenter Laboratory Validation of the BACTEC MGIT 960 Technique for Testing Susceptibilities of *Mycobacterium tuberculosis* to Classic Second-Line Drugs and Newer Antimicrobials

Sabine Rüsç-Gerdes,^{1*} Gaby E. Pfyffer,² Manuel Casal,³ Maureen Chadwick,⁴ and Salman Siddic

National Reference Center for Mycobacteria, Forschungszentrum Borstel, Borstel, Germany¹; Department of Medical Microbiology, Lucerne General Hospital, Lucerne, Switzerland²; Mycobacteria Reference Center, Faculty of Medicine, University of Cordoba, Cordoba, Spain³; Royal Brompton Hospital, London, United Kingdom⁴; and Becton Dickinson Diagnostic Systems, Sparks, Maryland⁵



Health Technology Assessment 2007, Vol. 11, No. 3

A systematic review of rapid diagnostic tests for the detection of tuberculosis infection

J Dinnes, J Deeks, H Kunst, A Gibson, E Cummins, N Waugh, F Drobniowski and A Lalvani

January 2007

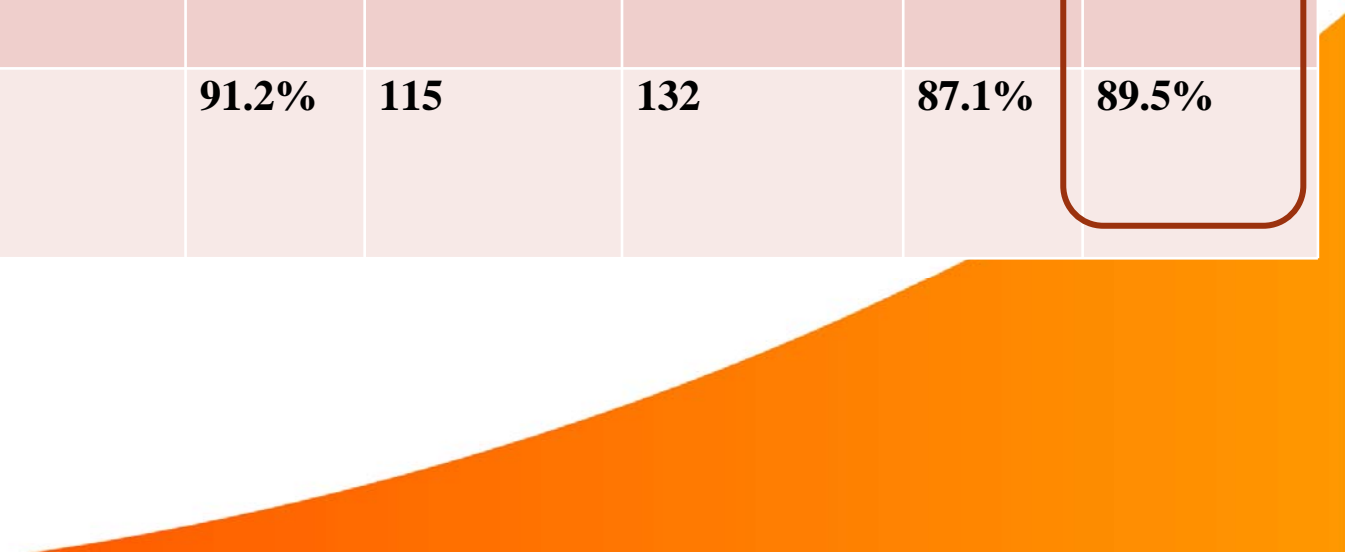
Health Technology Assessment
NHS R&D HTA Programme
www.hta.ac.uk



Comparative agreement of LJ and MGIT-based DST methods-middle income and middle incidence country



<i>DST</i>	<i>No of concordant resistant</i>	<i>Total No resistant any methods</i>	<i>% concordance</i>	<i>No of concordant sensitive</i>	<i>Total No sensitive any methods</i>	<i>% concordance</i>	<i>Total agreement %</i>
Inh (n-315)	190	197	96.4%	111	118	94.1%	95.6%
Rif (n-313)	153	158	96.8%	150	155	96.8%	96.8%
E (n-321)	76	89	85.4%	219	232	94.4%	91.9%
S (325)	176	193	91.2%	115	132	87.1%	89.5%



Where improvements are still needed

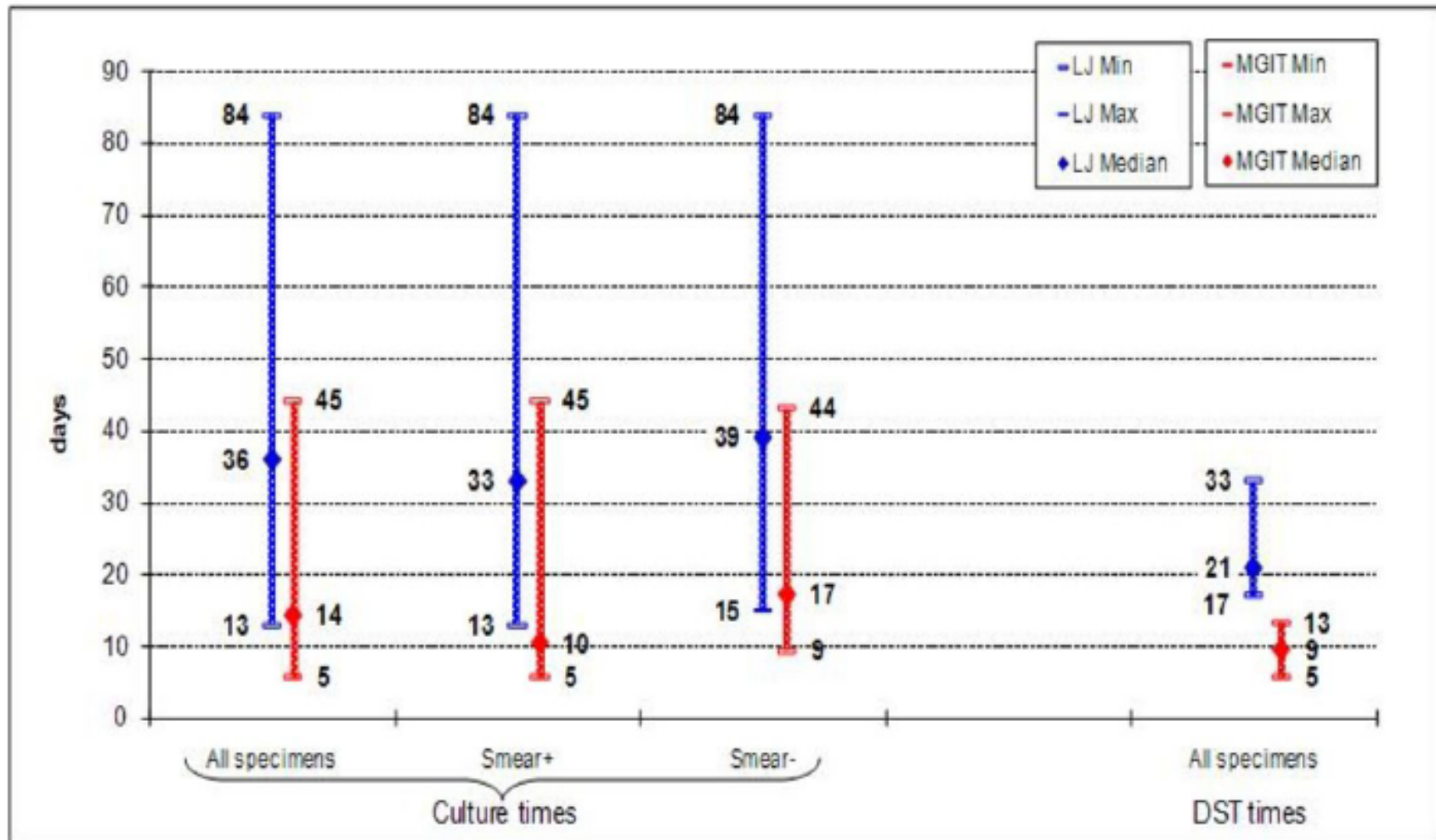


- 1. TB in HIV positive**
- 2. TB in children**
- 3. TB extrapulmonary disease—dilutional fluids such as CSF, pleural fluid but good for fresh tissues**

Can we do this reliably in under 15 minutes...



...



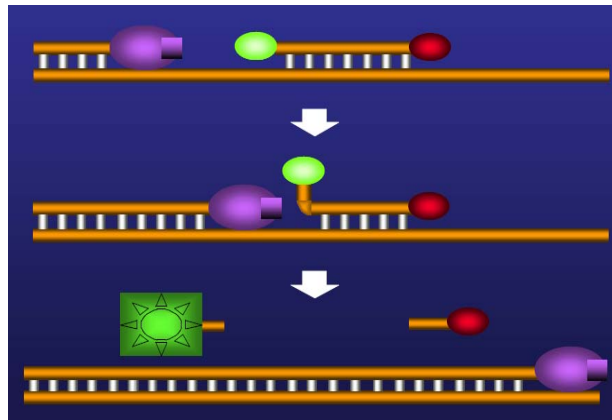
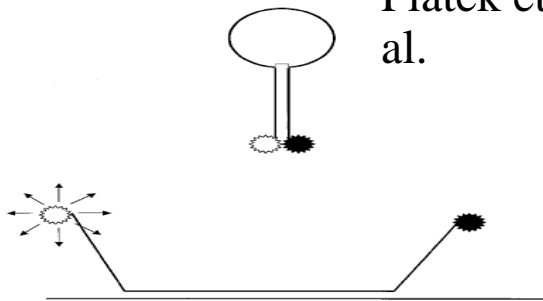
Samara: Median time to detection was 14 days for MGIT; 36 days for LJ ; 10 and 33 days for smear positive specimens

Indirect DST in MGIT took 9 days compared to 21 days on LJ.

Molecular methods of diagnosis/ drug susceptibility testing – PCR-based methods



Molecular Beacons
Piatek et al; Alland et
al.



Torres, M.J., et al. 2000.
J Clin Microbiol 38:3194-3199

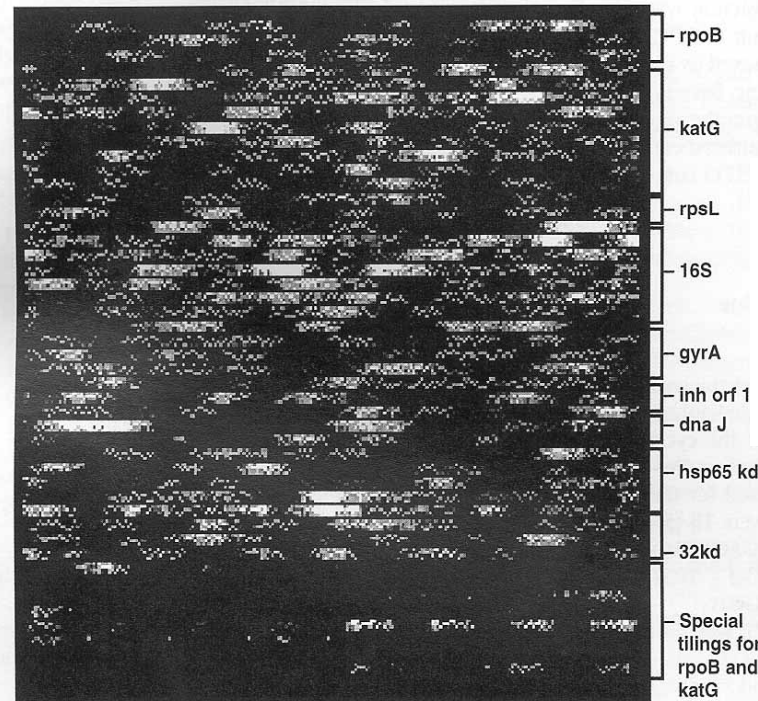


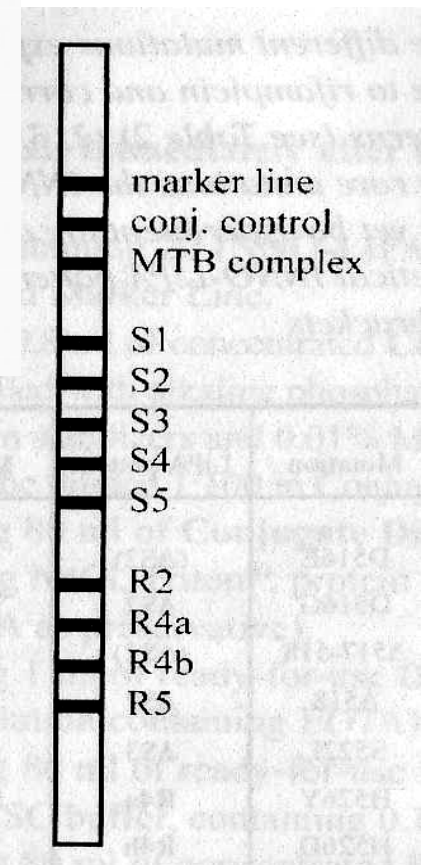
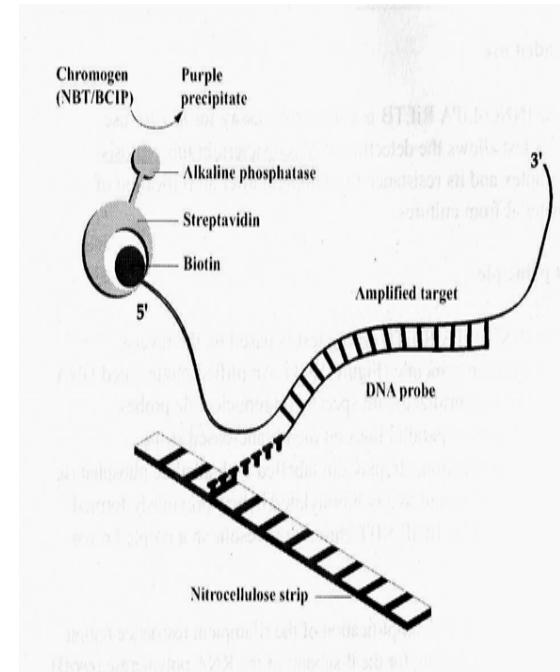
Figure 6 A high-density oligonucleotide array used to genotype 731 bp of *rpoB*, 2286 bp of *katG*, 356 bp of *rpsL*, 1683 bp of 16S, 731 bp of *gyrA*, 281 bp of *inh orf*, 341 bp of *hsp 65 kd*, 1097 bp of *dnaJ*, and 1279 bp of 32 Kd genes. Additionally, specific insertion, deletions, and missense mutations in *rpoB* and *katG* are interrogated by the alternative allele-specific oligonucleotide probes at the bottom of the chip.



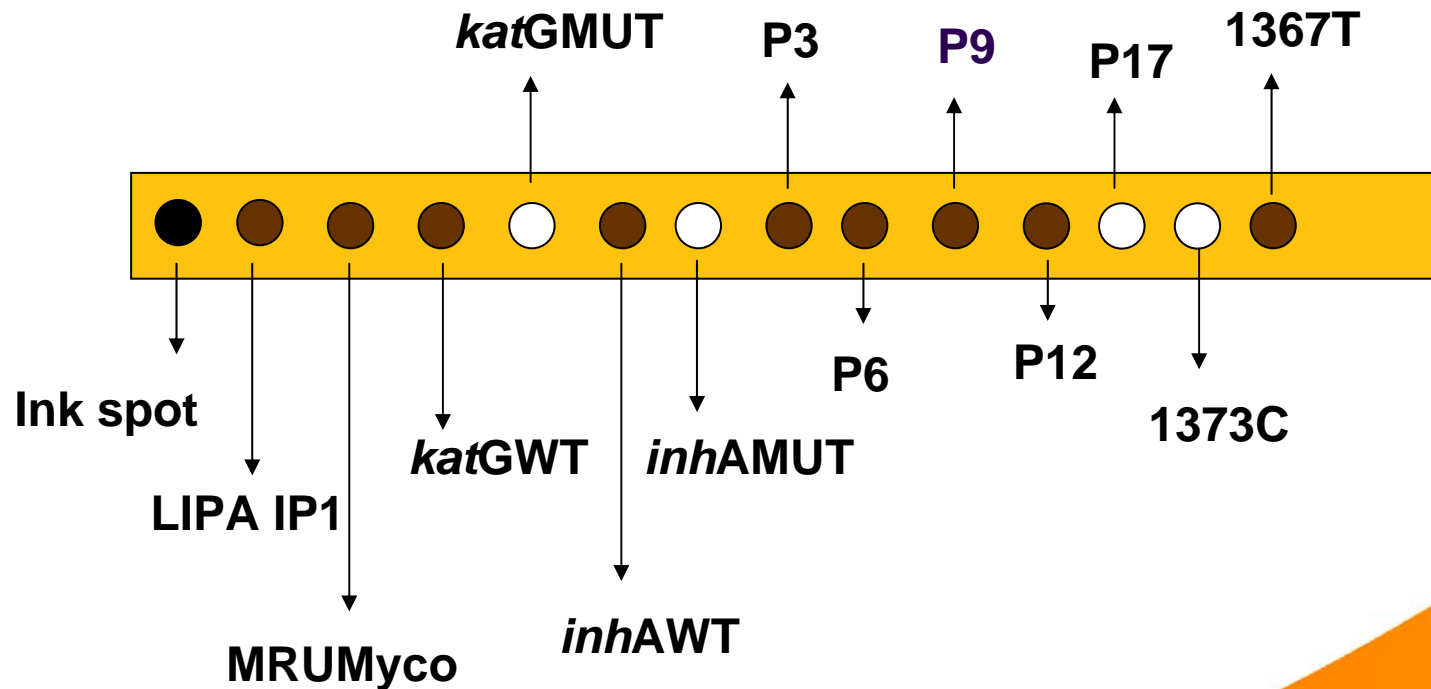
Molecular methods of drug susceptibility testing – PCR+hybridization

Based on amplification of fragments of genes responsible for drug resistance development followed by hybridization with oligonucleotide probes immobilized on membranes;

Commercial kits and in-house macro-arrays have been reported to demonstrate high sensitivity and specificity



MRU: Identification of MDR-TB isolates using low density array technology



Drobniewski et al Emerg Inf Dis 2002; 8:1320-1326;
Nikolayevskyy et al J Clin Micro 2004; 42:4498-4502; Brown et al
J Microbiol Methods 2006;65: 294-300; Nikolayevskyy et al Clin
Microbiol Infect. 2007;13(2):129-38 and Mokrousov et al J
Microbiol Methods. 2004;57(3):323-35



Fastrack: Analysis UK National Fastrack for primary specimens UK and Latvia

UK Jan 1999-Dec 2002: n=1997 patient specimens.

Detection of rifampicin resistance in specimens yielding MTBC on culture:

Concordance, Sensitivity, Specificity, PPV, NPV:

99.1%; 95.0%; 99.6%; 92.7%, 99.7%

Sam *et al.* EID 2006 12(5) 752-759

Latvia: Jan 2003-Mar 2004: n=89 smear positive respiratory specimens

Concordance, Sensitivity, Specificity, PPV, NPV:

91%; 96%; 94% 96%

Skenders *et al* EID 2005 11 1461-3

HAIN GenoType® MTBDR Plus-S Africa



Rapid detection RIF and INH resistance directly on **536 consecutive smear-positive sputum specimens** (patients at increased risk of MDR-TB).
Compared with conventional DST.

97% smear-pos specimens interpretable results in 1-2 days.

Assay performed well on specimens contaminated on culture

Sensitivity, specificity, PPV and NPV were:

98.9%, 99.4%, 97.9% and 99.7% for RIF resistance;

94.2%, 99.7%, 99.1% and 97.9% for INH resistance;

98.8%, 100%, 100% and 99.7% for MDRTB

Barnard et al Am J Respir Crit Care Med. 2008

GenoType MTBDRplus assay



South Africa

Proportion INH resistance, particularly in INH-mono-resistant isolates of lineage X3 in S Africa, due to resistance determinants other than KatG S315T and inhA C215T.

Isolates will be indicated as drug susceptible

Highlights need for determining local patterns of resistance mutations

Evans et al JAC 2009 63; 11-16

Russia

Sensitivity and specificity RIF and INH resistance and MDR was 96.2%, 97.4%, 97.1% and 90.7%, 83.3%, 88.9% respectively.

Mutations codon 531 of rpoB gene and codon 315 of katG gene dominated in RIF and INH resistant strains respectively.

Rare mutations explained disagreements between phenotypical and molecular results (12 samples)

Ability to read/interpret results varied; better results (96.7%–100.0% readable results) achieved with sputum with higher AFB counts (2+...3+),

Nikolayevskyy et al BMC Clin Path 2009 9:2



WHO Expert Committee 2008 (1) Recommendations

- “The expert committee considers that there is sufficient generalisable evidence to justify a recommendation of the use of line-probe assays for rapid screening for MDR TB within country-specific settings”
- Written summary and recommendations to WHO STAG
- WHO STAG endorsed---WHO policy
- ..UNITAID, FIND...Lesotho and Ethiopia will implement above for smear positives as a central process 2008-9.....

GeneXpert



Specific for TB

Specific for rifampicin resistant

Good sensitivity equivalent to culture ..98-99% sm+ culture+...

..less sensitive for sm -ve...

Relatively safe

Relatively fast ..but not 15 minutes



The 15 min test we have...



Ironically the only test that is completed within 15 minutes...is the oldest!



TB bacterial metabolite detection



Giant African pouch rats.

**Used to detect land mines, now
TB**

**Sniff 120 sputum samples per
day (compared to 20 vis
microscopy)**

Waits for reward if sniffs TB

Rapid!

Need to consider total time



Not biology but logistics!!!!!!!!!!!!

1. Pre-diagnosis:

POC: this time becomes zero.....**NOT THERE YET!**

...but any lab-based test..taking specimens and sending to lab takes time and minimising this is the key...eg courier etc

2. Diagnosis”---many advances discussed today eg PCR, HAIN, Genexpert, IGRA

3. Post-diagnosis: POC this time becomes zero

Getting results to clinician is the key eg telephone, fax, IT..

IT..always argued as the defintiteve route..but IT cosnultants are like snake-oil salesmen....promise everything, deliver *****

What was needed to achieve satisfactory performance of MGIT or any assay?

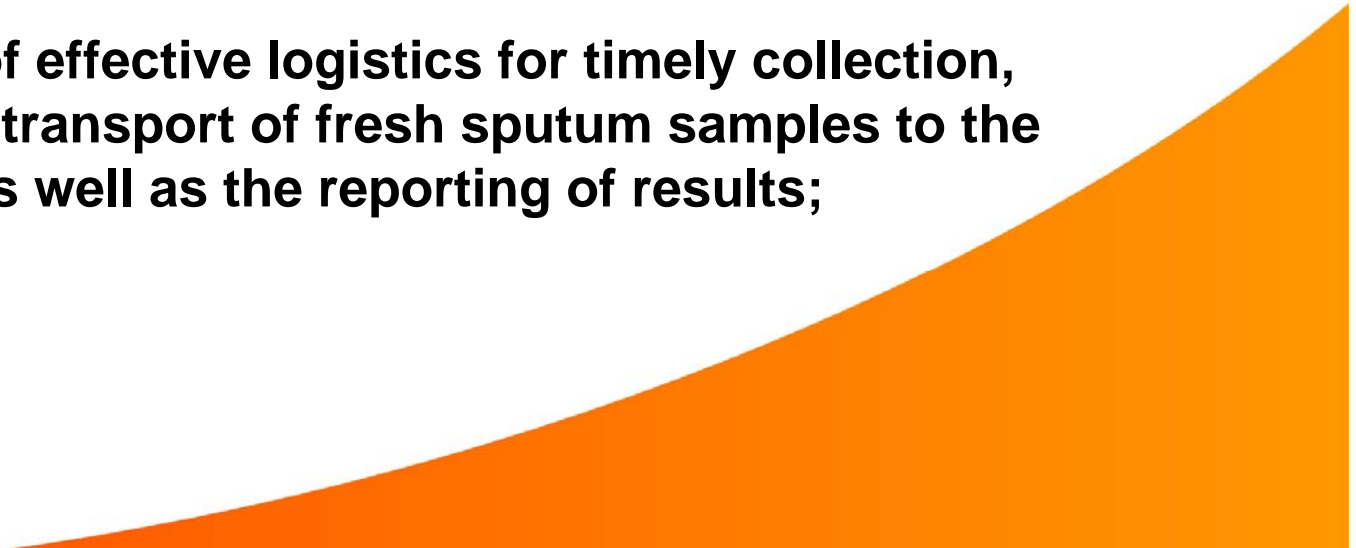


availability of appropriate category III level laboratory infrastructure

repetitive on-site training of laboratory personnel in methodology (using detailed SOPs and system manual) and molecular methods to create a multi-skilled cadre of staff;

initial expert observation of the performance and implementation of internal and external quality control of laboratory work;

development of effective logistics for timely collection, storage and transport of fresh sputum samples to the laboratory as well as the reporting of results;



What was needed to achieve satisfactory performance of MGIT?



the creation of algorithms for laboratory work flow and computerized laboratory record keeping;

an agreed maintenance plan for the system; timely maintenance of equipment

introduction of a robust stock control system....

and ensuring a safe continuous supply of reagents by establishing a commercial contract with a manufacturer;



Do a test correctly



Reliable SOP- no variation! Positive and negative controls

Correct materials: eg Zhao et al Int J TLD 2009; 13;126-9 variation in fuchsin content in 10 samples from routine field checks China 2005-6)

Correct workload/time: Significant extra % positive with smear examination of 10 mins compared to 2 min (Cambanis et al Int J TLD 2007; 11;40-5)

Can we do this for under 5 euros.....



...

Costs comparisons: LJ vs MGIT (per specimen, USD)



Test	MGIT INT'L	MGIT FIND- BD/Local	LJ INT'L	LJ Local
Primary culture	13.74	11.66	11.49	10.50
FLD DST (SIRE)	55.68	32.42	19.83	17.00
FLD DST (IR)	27.73	16.71	14.84	13.47
SLD DST	-----	33.39	-----	-----

Economic cost



Market price vs “special public health price” (FIND)

Volume discount

Make your own vs commercial supplier

Commercial-consistency but potential supply problems

Manufacturer may agree

Agreements eg FIND.....?How enforceable are they?

Middleman distributors profiteering

In USA..

Depending on testing strategy

Cost per pulmonary TB suspect diagnosis

\$68.3 to 90.9 for probability of correct diagnosis of 0.91 to 0.96

JCM 2008 46; 3811-3812



Economic cost-conclusion



Reality:

global agencies will not enforce discount deals

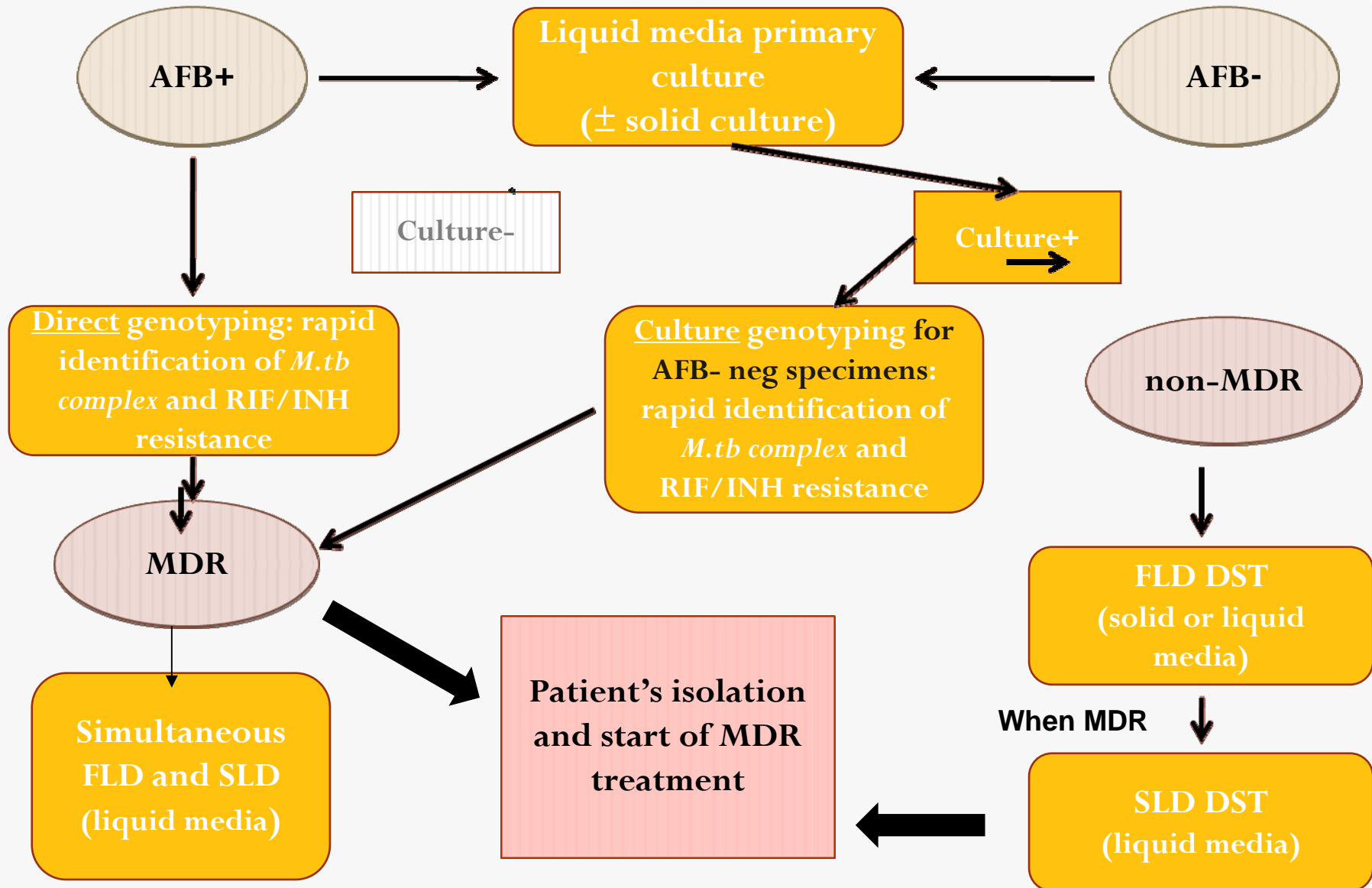
Two good competitors for tests essential...

Manufacturers need to police their distributors....and honour any institutional agreements ahead of national distributor agreements

Cheaper patient materials eg blood, urine eg trans-renal on urine supernatants

Good lab/POC tests...high volume..lower cost

Proposed algorithm for rapid diagnosis of resistance



Diagnose TB early...use best bacteriological and cultural techniques