



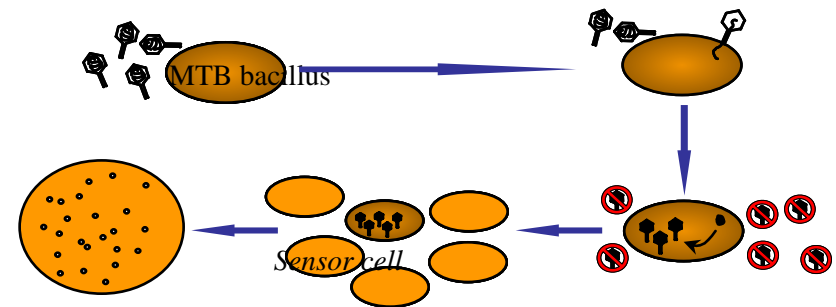
Phage-based assays for detecting rifampicin-resistant TB

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Phage-based techniques

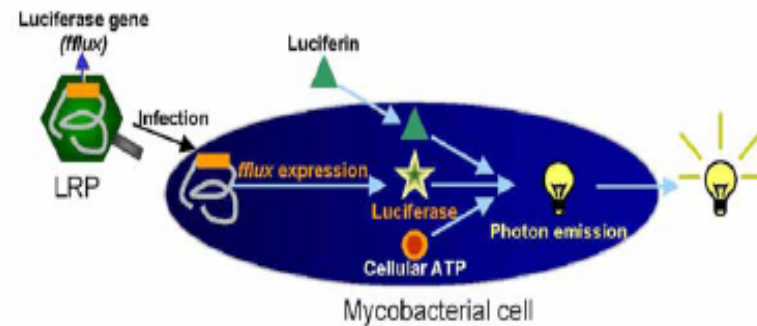
- **Phage amplification**

Amplification of phage in rapid-growing helper cells following phage replication and lysis of viable MTB



- **Lux phage**

Detection of light produced by luciferase reporter phage (LRP) after infection of viable MTB



Summary of lux phage data

- From TB culture

Study site	Sensitivity	Specificity	Reference
USA	100% (10/10)	94% (16/17)	Riska, 1999
China	92% (12/13)	89% (8/9)	Lu, 2000
Mexico	100% (3/3)	100% (47/47)	Banaiee, 2001
Mexico	100% (7/7)	100% (65/65)	Banaiee, 2003
Colombia	100% (11/11)	89% (33/37)	Hazbon, 2003
South Africa	100% (9/9)	100% (182/182)	Banaiee, 2004

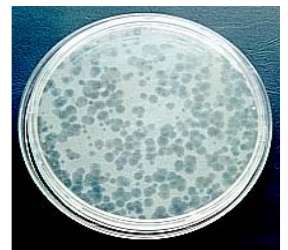
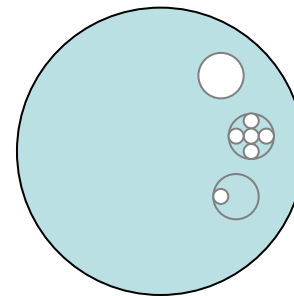
From Pai *et al*, 2005

Phage amplification

- **Rapid (2 days)**
- **Manual**
- **No specialised equipment needed**
 - **facilities for manual TB culture required**
- **Commercial and in-house methods**
- **Technology applied to other TB drugs, most data on rifampicin**

Phage amplification

- Test format (tube or microwell plate)
- Rifampicin concentration
 - (2 -) 4 - 10 μ g/ml in culture (single or multiple concentrations)
 - 10 μ g/ml in sputum
- End-point detection and interpretation of results
 - Single cut-off number of plaques
 - Proportion of plaques cf. drug-free sample
 - Lysis / no lysis on indicator plates
 - MTT colorimetric end-point



Summary of phage amplification data from cultures

Study site	Method	Sensitivity	Specificity	Reference
South Africa	FASTPlaque	100% (81/81)	95.5% (105/110)	Albert, 2001
South Africa	FASTPlaque	100% (43/43)	98.8% (89/90)	Albert, 2002
Turkey	FASTPlaque	100% (9/9)	74.2% (23/31)	Aktepe, 2001
Turkey	FASTPlaque	81.0% (17/21)	81.8% (9/11)	Oguz, 2002
Turkey	FASTPlaque	100% (21/21)	92.5% (62/67)	Kisa, 2003
UK	In-house	100% (9/9)	94.6% (35/37)	Wilson, 1997
UK	In-house	100% (31/31)	100% (46/46)	Eltringham, 1999
South Africa	In-house	100% (17/17)	100% (20/20)	McNerney, 2000
Spain	In-house	100% (18/18)	100% (71/71)	Gali, 2003
India	In-house	97% (98/101)	84.0% (84/100)	Mani, 2003
Argentina	In-house	100% (42/42)	98.9% (86/87)	Simboli, 2005
Cuba	In-house	97.8% (44/45)	100% (57/57)	Yzquierdo, 2006
Peru	In-house	100% (43/43)	98.0% (49/50)	Chauca, 2007
Uganda	In-house	100% (39/39)	96.5% (110/114)	Traore, 2007

Meta-analysis of phage-based tests for rifampicin resistance

- Pai *et al* (2005)
- *“Current evidence is largely restricted to the use of phage assays for the detection of rifampicin resistance in culture isolates. When used on culture isolates these assays appear to have high sensitivity, but variable and slightly lower specificity.”*

Phage amplification directly from sputum



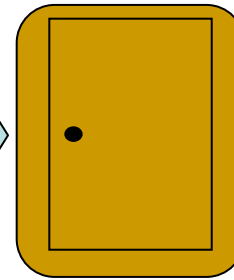
FASTPlaque-Response



Sputum collection
>1+ smear-positive



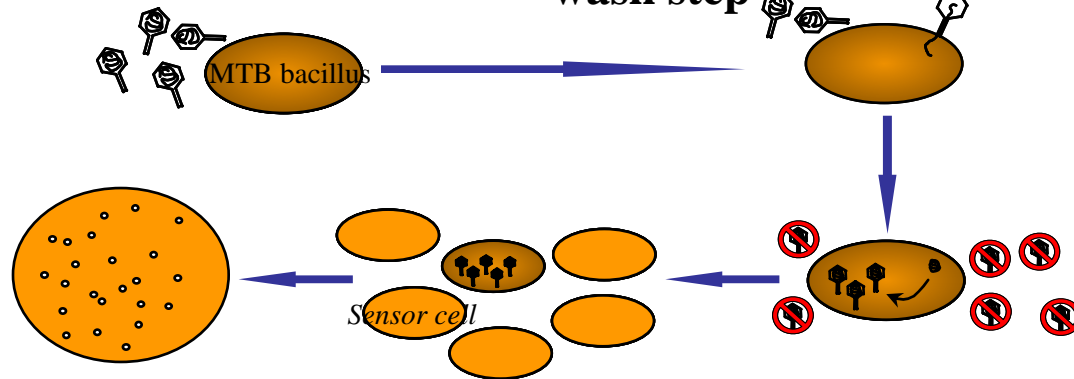
Specimen processing
NALC-NaOH +
wash step



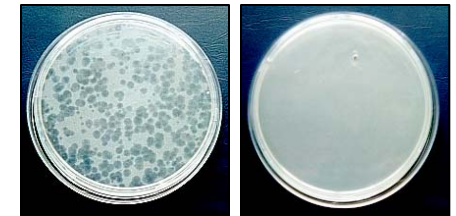
o/n incubation
± rifampicin



FASTPlaque-
Response
test procedure



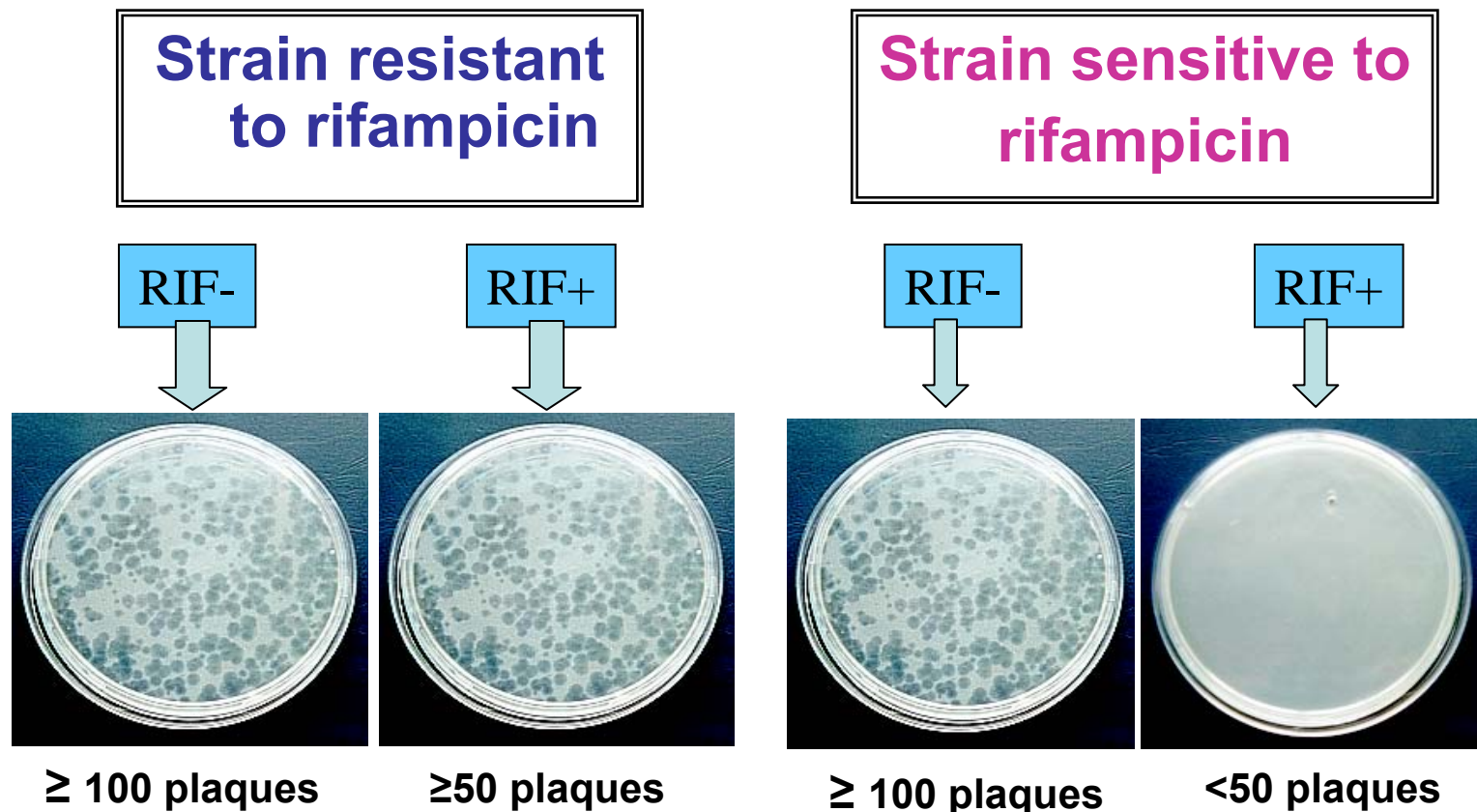
2 days



Interpretation of results



FAST *Plaque-Response* results interpretation



Summary of phage amplification data from sputum

Study site	Sens	Spec	Overall accuracy	% interpret. results	Reference
Pakistan	86% (24/28)	75.0% (9/12)	82.5% (33/40)	100%	Butt, 2004 †
South Africa	100% (11/11)	100% (134/134)	100% (145/145)	83%	Albert, 2004
South Africa	95.6% (86/90)	99.3% (138/139)	97.8% (224/229)	71%	Albert, 2007
South Africa	92.9% (39/42)	95.7% (287/300)	95.3% (326/342)	69%	Mole, 2007
Peru (TDR/FIND)*	94.7% (54/57)	94.6% (420/444)	94.6% (474/501)	75%	FIND, 2007

† 5µg/ml rifampicin, and different interpretation criteria used.

* Comparison to MDR status

Level of interpretable results

- The level of interpretable results is an area of concern.
- Loss of results due to two causes:
 - Fewer than 100 plaques in the drug free (RIF-) control
 - phage inhibitor present in sputum
 - metabolic state of MTB post-decontamination may not support efficient phage infection and/or replication.
 - Contamination
 - NOA antimicrobial supplement

Contamination control

- Cape Town and Peru data
 - High level of contamination (14%; 19%)
- Development of NOA supplement¹
 - Nystatin, oxacillin, aztreonam
- Evaluation of NOA
 - No adverse effect on test performance
 - Contamination reduced from 14% to 4% in Cape Town² and 19% to 1% in Peru
 - Level of interpretable results increased to 88% (CT) and 90% (Peru)

¹ Albert *et al*, J Appl Microbiol, 2007

² Mole *et al*, J Med Microbiol, 2007.

South Africa demonstration (FIND-MRC-NHLS)

- Objective: to assess feasibility and impact of rapid MDR assays under operational conditions
- Phage assay did not meet target specifications during initial phase of project in 2 laboratories in South Africa
- FIND is discontinuing demonstration of phage assay in South Africa and other sites
- Manufacturer is investigating possible causes of poor performance and remedial action

Conclusions

- Phage-based testing for rifampicin resistance
 - Potential for low cost and relatively low tech method
 - Most data, esp. in-house methods, are from cultures not sputum
 - Issues remain to be addressed regarding test performance