

How has Whole Genome Sequencing changed clinical practice? or What are the some other benefits of WGS?

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NW TB Conference
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Outline

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- WGS has created a paradigm shift in TB diagnostics
 - Accurate confirmation of drug susceptibility
 - Huge depth of relatedness of strains, allowing molecular contact tracing
 - Many benefits for laboratory practice
- But also:
 - Offers rapid results
 - May sometimes be more accurate than phenotypic DST
 - Related strains may allow tailored empiric treatment
 - May diagnose mixed strains
 - Direct sputum WGS...



Faster results

SURNAME [REDACTED]		FORENAME [REDACTED]		DOB/AGE [REDACTED]		HEALTHCARE TRUST SEX F	
WARD/LOCATION BARNES 5		CONSULTANT/G.P. UNKNOWN		HOSPITAL NUMBER [REDACTED]		LABORATORY NUMBER T01125Y	

(R10) Page 1 of 1



Microscopy

Auramine film +++ Acid fast bacilli seen

Culture result may take up to 6 weeks

Culture for Acid Fast Bacilli

POSITIVE

Mycobacterium tuberculosis	Pyrazinamide S Isoniazid S Rifampicin S Ethambutol S
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SPECIMEN/SITE SPUTUM - TB EXAMINATION	RECEIVED 16/10/95	REPORTED 16/01/96	AUTHORISED BY Dr J Timms
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Grid



List



Chart

Collected : 24-Dec-2014 17:42

Result : 13-Aug-2015 15:35



Test

Result

UoM

Ref. Range

187240 (187240)

-

FINAL REPORT

Collected 24.12.14 M,14.1505011.X

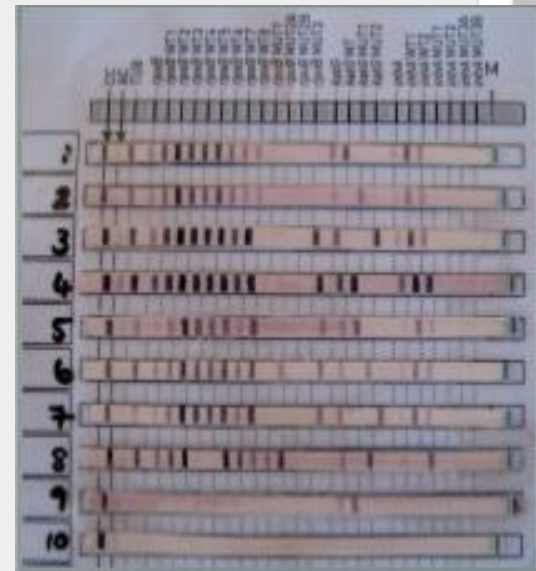
AAFB stain: ***** AAFB +++ SEEN *****

Mycobacterium tuberculosis

Ethambutol	S
Rifampicin	R
Isoniazid	R
Pyrazinamide	S
Azithromycin	S
Clarithromycin	S
Streptomycin	S
Amikacin	S
Capreomycin	S
Kanamycin	S
Moxifloxacin	S
Ofloxacin	S
Prothionamide	S

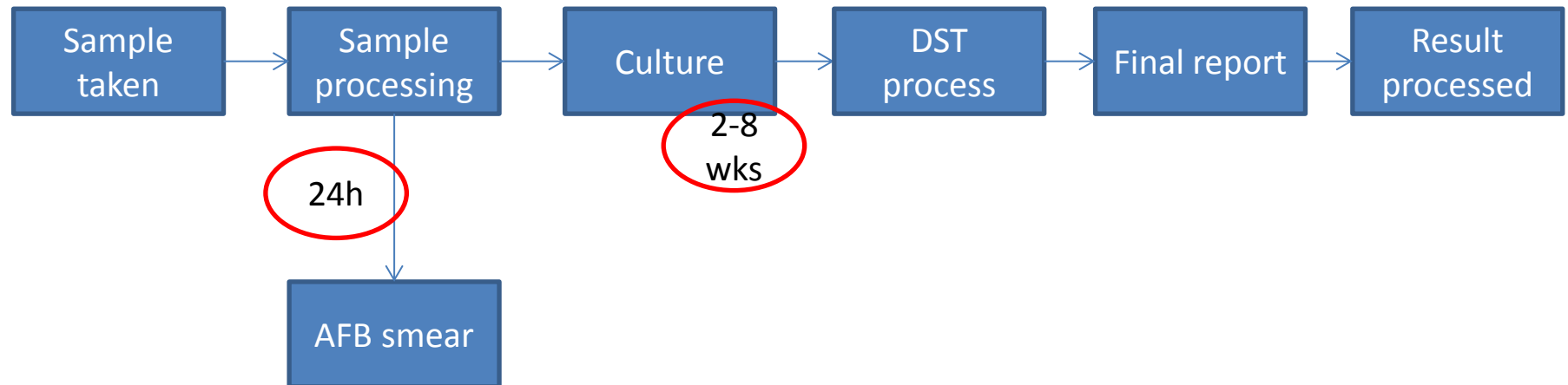
For advice on patient management contact TB unit
Reported to CCDC

Specimen - Sample for AAFBs
Sputum

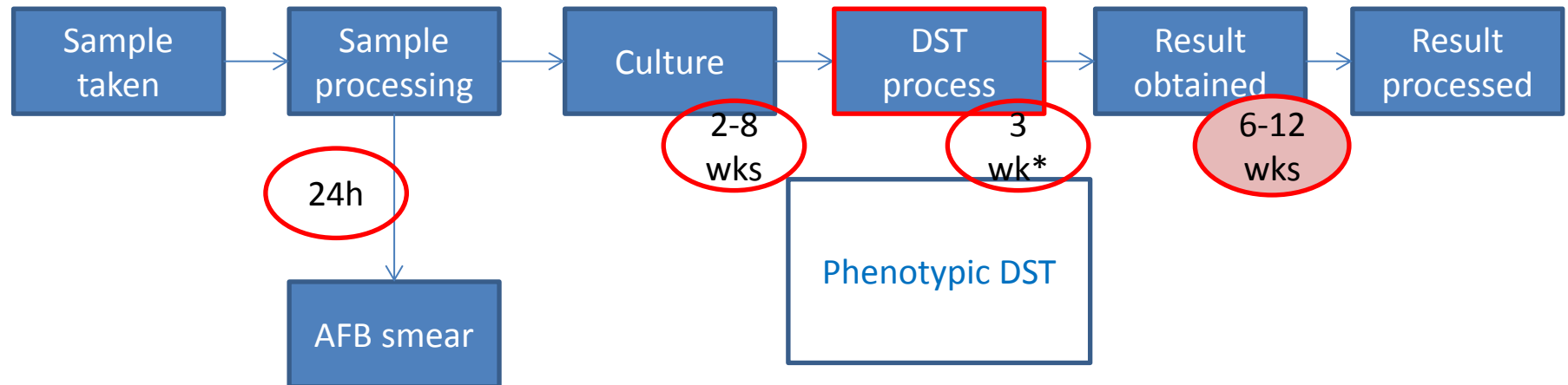


*GenoType MTDRplus strips
(Hain Lifescience)

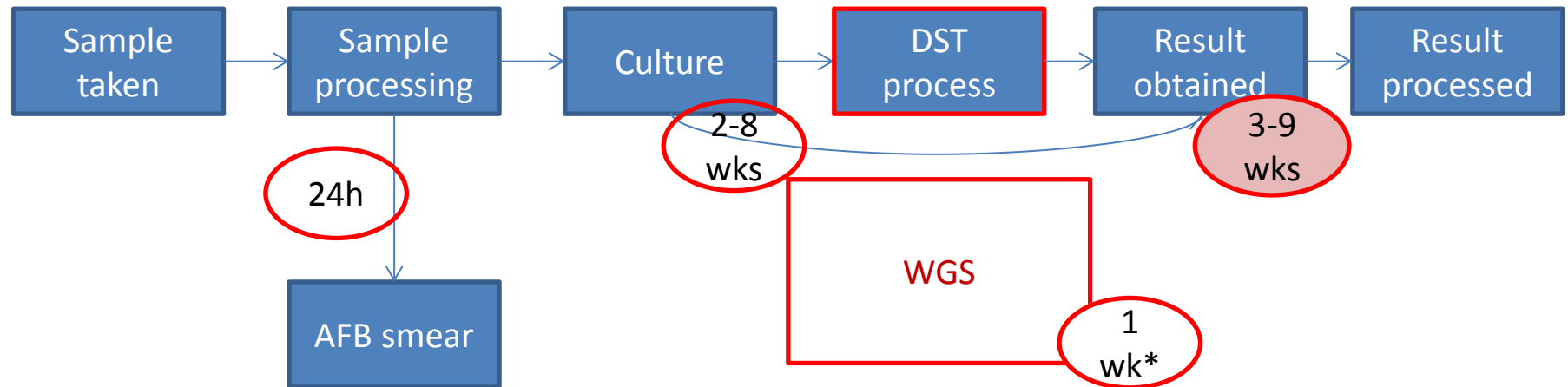
What happens to get from sampling to DST result?



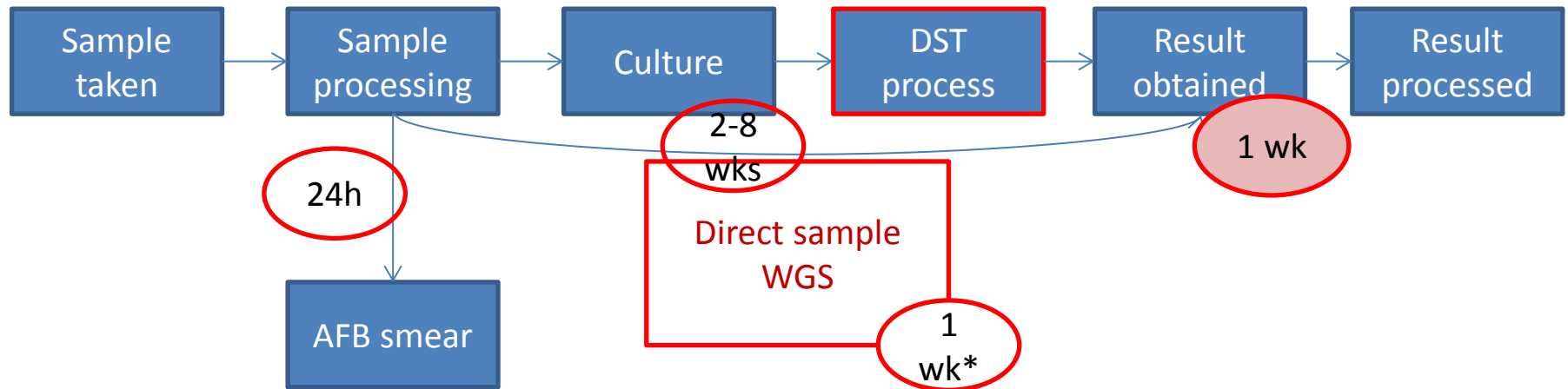
What happens to get from sampling to DST result?



What happens to get from sampling to DST result?



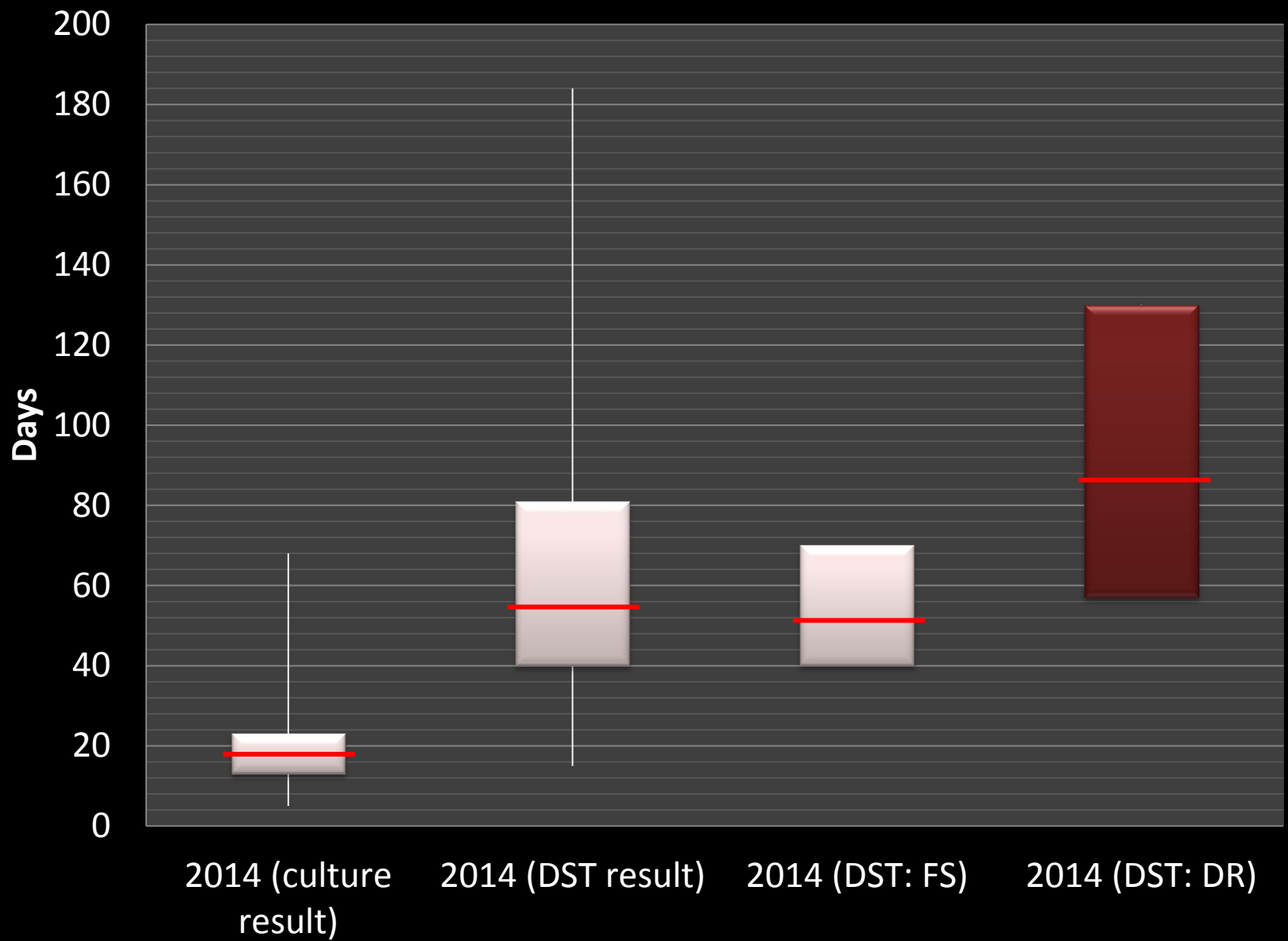
The holy grail of WGS testing...

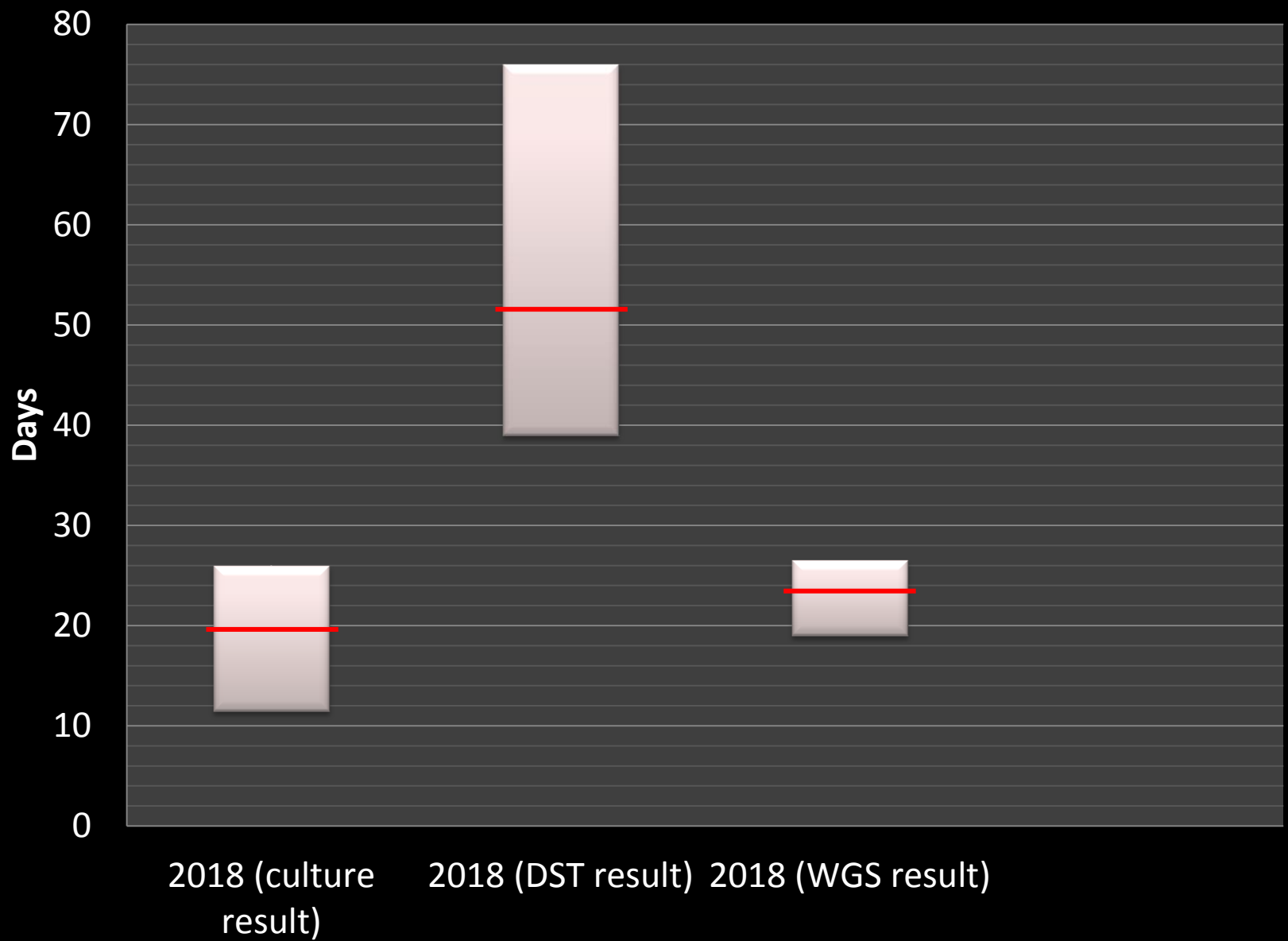


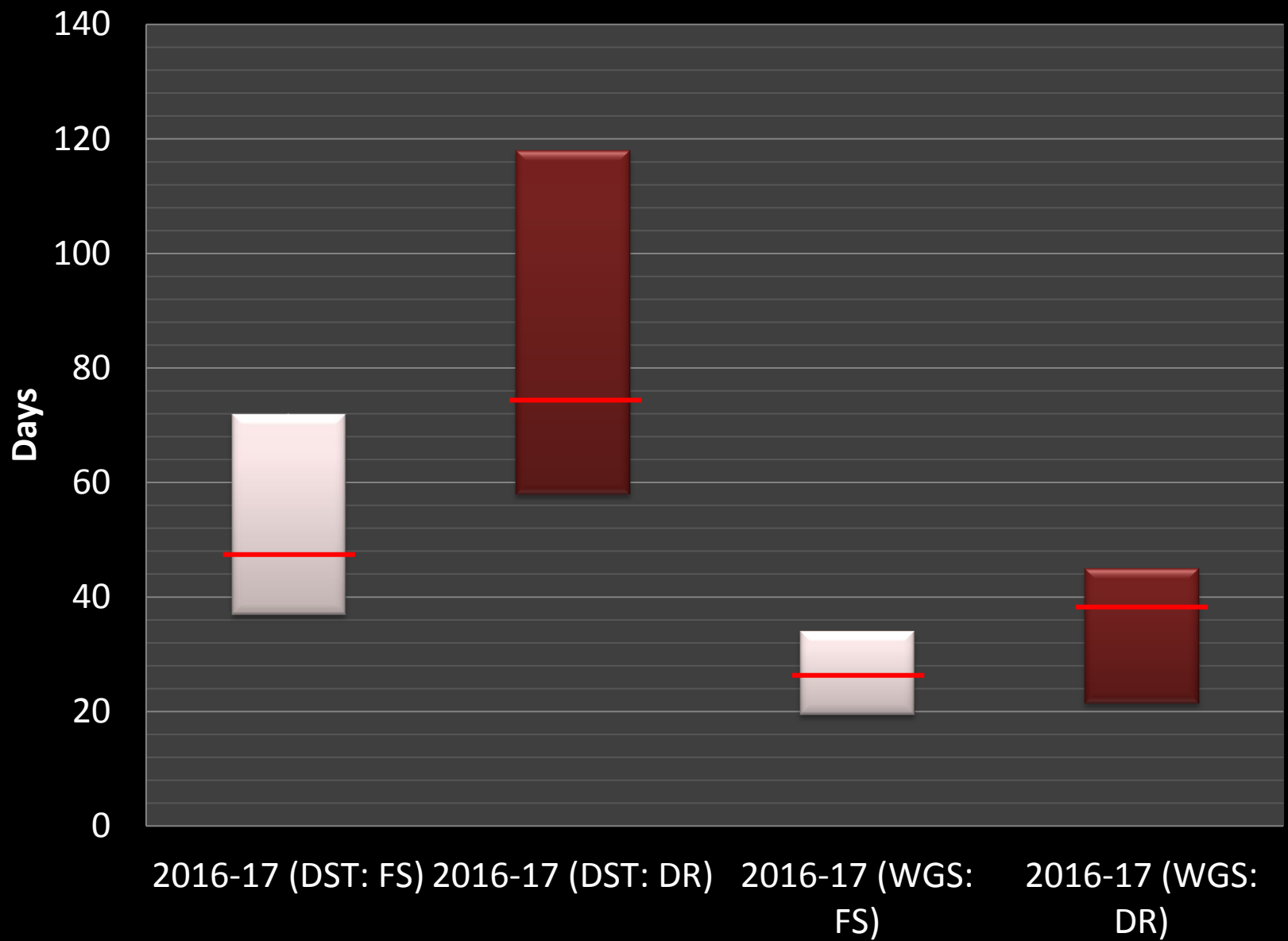
How long to results?

- West Midlands
 - 2014 – 100 consecutive patients
 - 2016-17 – 82 consecutive patients









Summary of time savings

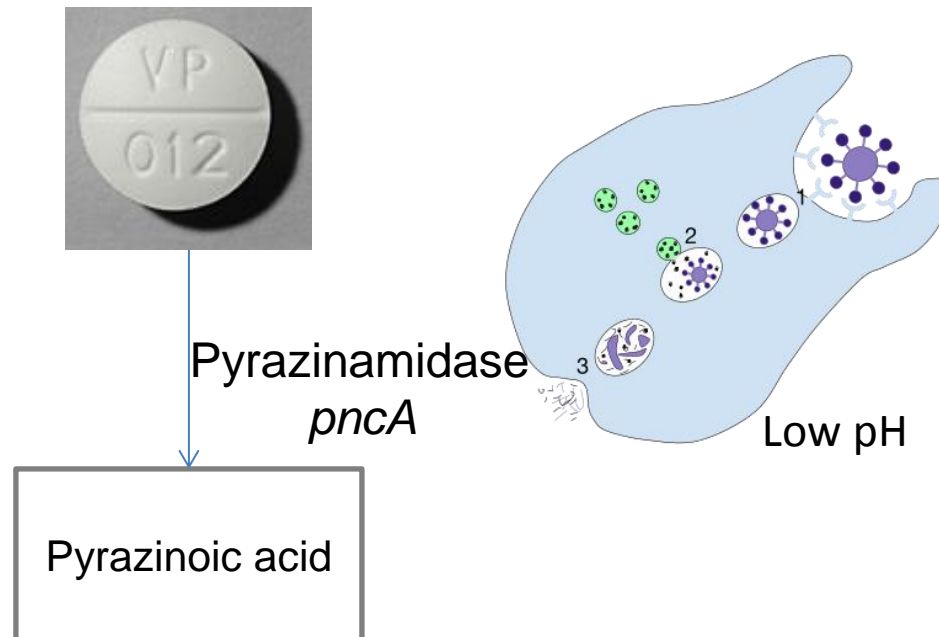
- Time to result around 20 days faster for FS strains
 - over 30 days for DR
- Respiratory samples around 20 days faster
- LN samples around 35 days faster
- Much higher proportion of results obtained within 60 days of sample collection

Accuracy of results

Accuracy of results (pyrazinamide)

Pyrazinamide resistance and testing

- Pyrazinamide (Z) DST is difficult, unreliable and associated with high rates of failure



Is Z WGS better than DST?

- 41 Z-phenotypically sens strains
- 41 Z-phenotypically resistant strains
- How many of each had pyrazinamidase (PZase) activity?
- And what was pncA sequence on WGS?

Is Z WGS better than DST?

- All Z-sens strains had PZase activity
- 17/41 Z-res strains had PZase activity
 - When Z-monoresistant strains were retested for DST, 16 were sensitive to Z
- 23/24 of those with no PZase activity had *pncA* mutations
- DST (vs. PZase activity)
 - 100% sensitive, 59% specific
- WGS (vs. PZase activity)
 - 100% sensitive, 96% specific

Related strains

Related strains

- We often infer resistance patterns from contacts
- Most often, for children of parent with pulmonary TB with known resistance pattern
- Also adult close household contacts where sampling culture-negative or impossible
- Resolution of WGS allows inference of resistance patterns from strains
 - Not necessarily with close epidemiological links

Related strains

- 53y Indian-born man
- Cold abscess and psoas abscess
- Pus aspirated, AFB negative
- Started empirically on (RHZE)
- Culture positive (2 weeks) for M. tuberculosis



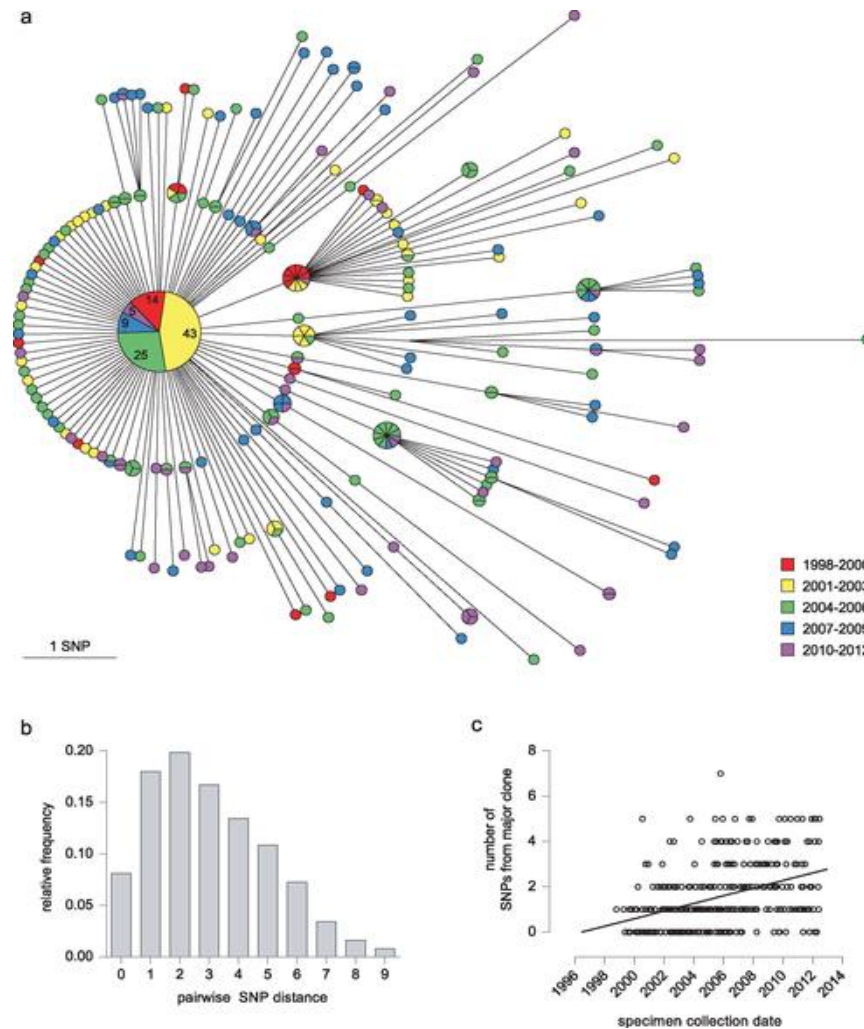
- Hain indicated H resistant but R sensitive
- WGS R failed (H & E resistant)
- Treatment changed to MDR-TB treatment
- Part of a cluster
- Closest isolate was R sensitive on WGS
- R added
- DST subsequently confirmed R sensitive

Mixed strains

Mixed strains

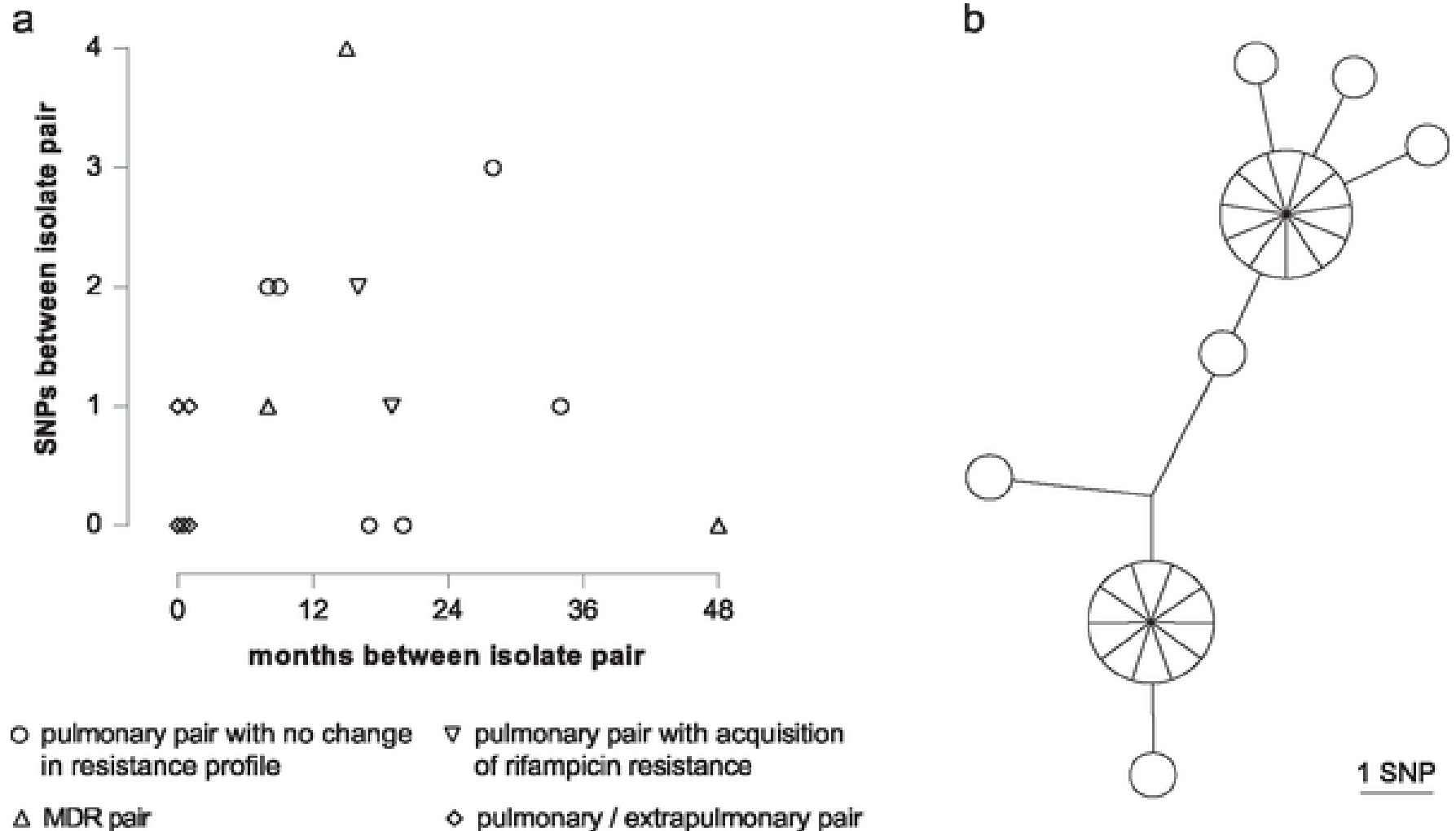
- Large outbreak of isoniazid-resistant TB in London
- WGS carried out on all available isolates retrospectively

Fig 2. Outbreak diversity.



Casali N, Broda A, Harris SR, Parkhill J, Brown T, et al. (2016) Whole Genome Sequence Analysis of a Large Isoniazid-Resistant Tuberculosis Outbreak in London: A Retrospective Observational Study. *PLOS Medicine* 13(10): e1002137. <https://doi.org/10.1371/journal.pmed.1002137>
<https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002137>

Fig 5. Intra-host diversity.



Casali N, Broda A, Harris SR, Parkhill J, Brown T, et al. (2016) Whole Genome Sequence Analysis of a Large Isoniazid-Resistant Tuberculosis Outbreak in London: A Retrospective Observational Study. *PLOS Medicine* 13(10): e1002137. <https://doi.org/10.1371/journal.pmed.1002137>
<https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002137>

Mixed strains

- Difficult to interpret results
- Likely to be much more of an issue in high incidence settings
- Significance on daily clinical practice unclear

Mixed strains, and ID confirmation

< PREV MRI TB 1/2 SPUT... - 16-MAR-2016 13:35 NEXT >

Grid List

Collected : 16-Mar-2016 13:35

Test R

187435 (187435)

FINAL REPORT Collecte
AAFB stain: ***** AAFB +++ S

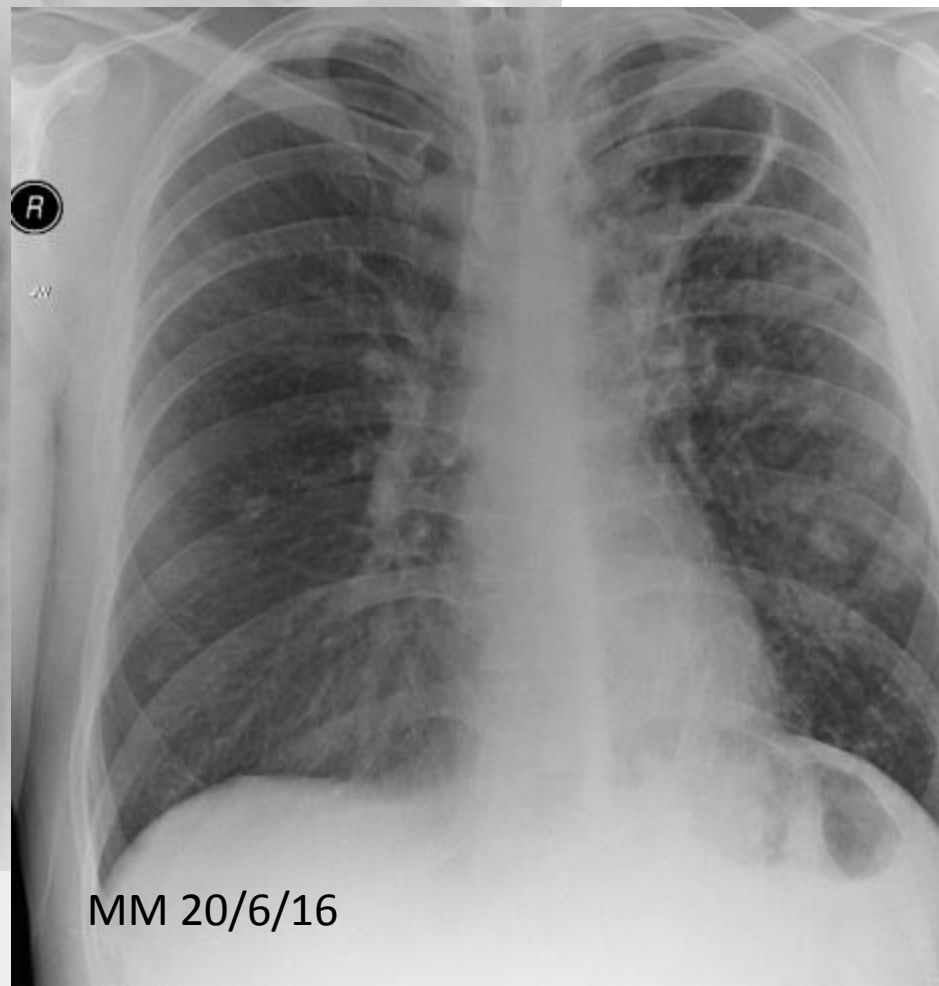
Mycobacterium tuberculosis

Ethambutol
Rifampicin
Isoniazid
Pyrazinamide
Rifabutin
Ciprofloxacin
Azithromycin
Clarithromycin
Linezolid
Prothionamide
Amikacin
Capreomycin
Kanamycin
Moxifloxacin
Ofloxacin

Report from Mycobacterium Reference laboratory Newcastle

For advice on patient management contact TB unit on 64387
Reported to CCDC

Specimen - Sample for AAFBs
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Summary

Summary

- WGS has had a number of impacts on clinical practice
 - Particularly time to get full susceptibility information
 - Assumptions about treatment based on relatedness
 - Mixed strains
- Direct sample WGS will make even more of a difference



With thanks to...

- Grace Smith
- Martin Dediccoat
- Christine Bell



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