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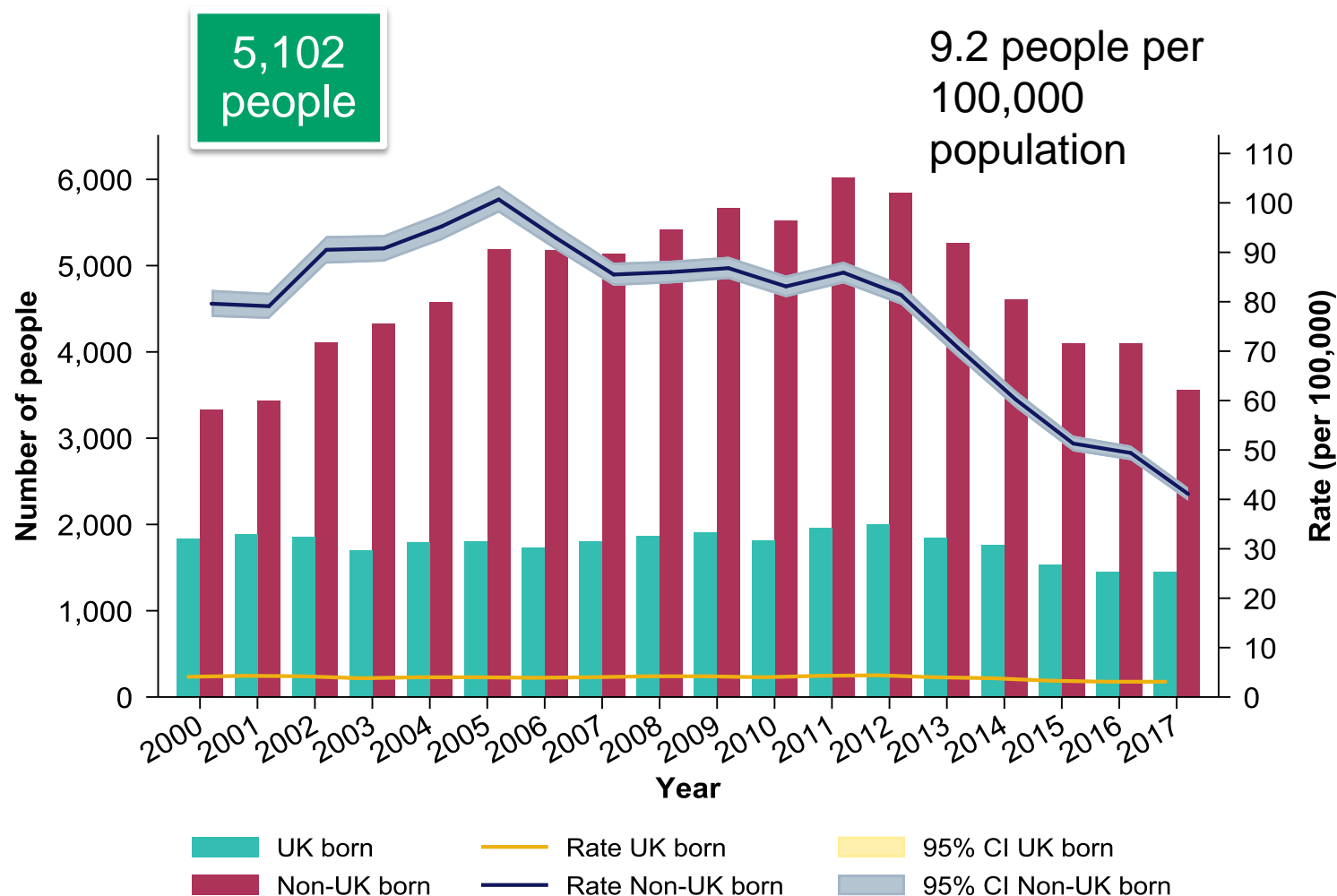
Sequencing to Diagnose Tuberculosis-What we've learned and where we go next

Dr Grace Smith

Lead for National Mycobacterial Reference Service
Interim Head of the TB Unit, PHE National Infection Service



Number of TB notifications and rates by place of birth, England, 2000-2017

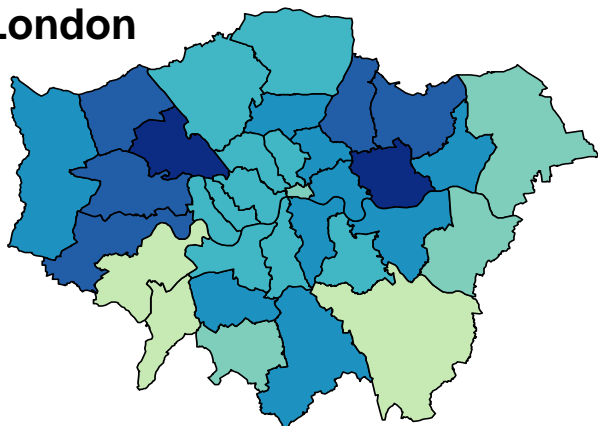




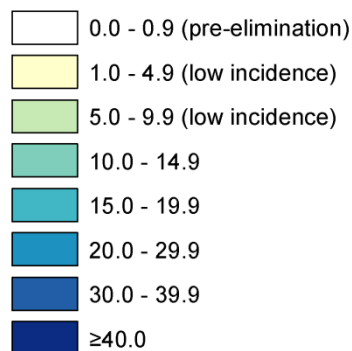
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Three-year average TB rates by clinical commissioning group, England, 2015-2017

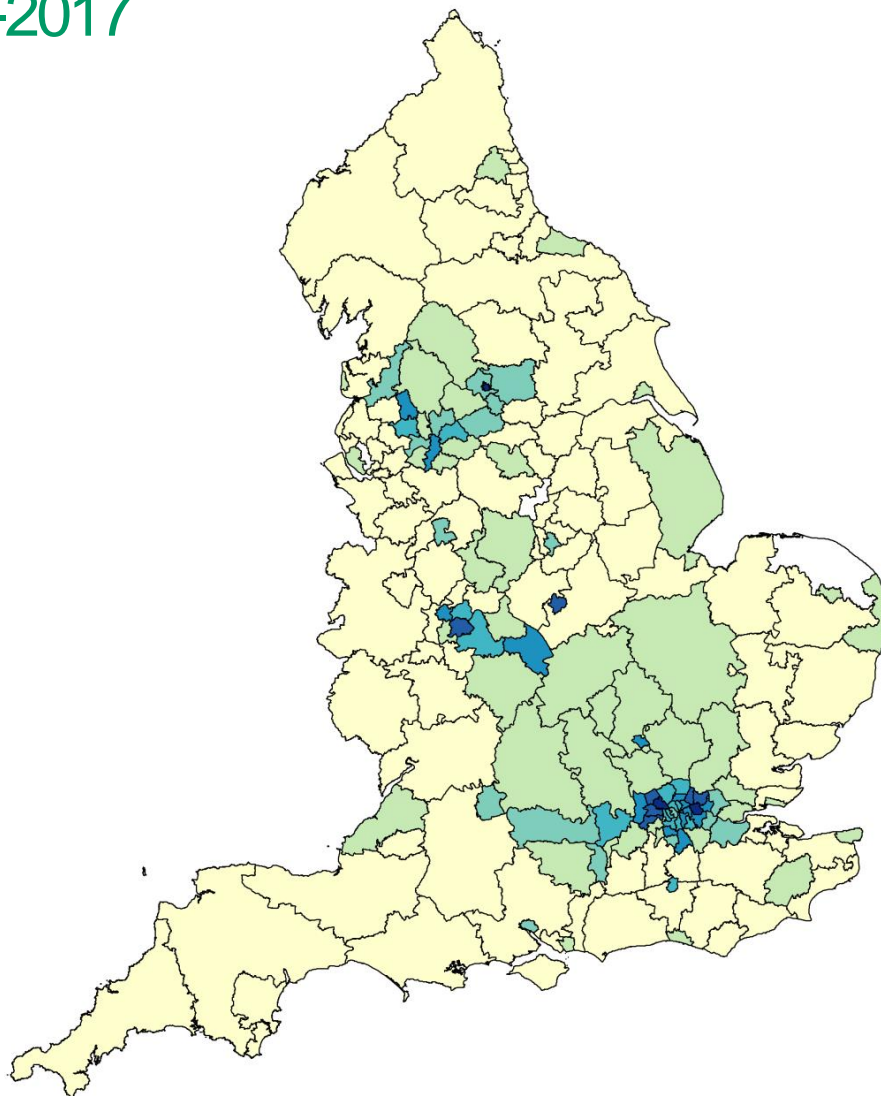
London



Tuberculosis rate (per 100,000)



10 CCGs had a 3-year average rate of 30 per 100,000



Contains Ordnance Survey data © Crown copyright and database right 2018
Contains National Statistics data © Crown copyright and database right 2018



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Modernising diagnostics



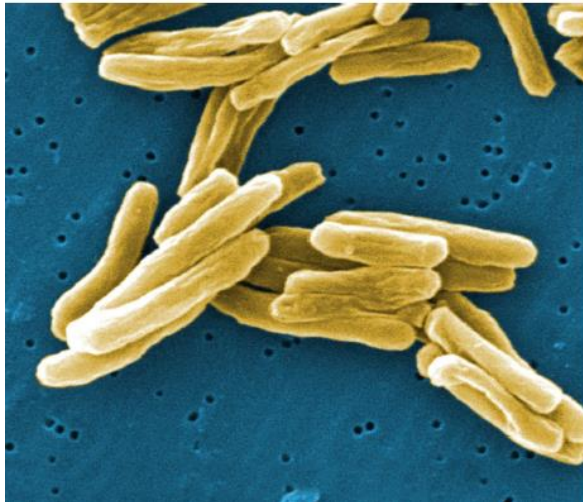
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Protecting and improving
the nation's health



Collaborative Tuberculosis
Strategy for England

2015 to 2020

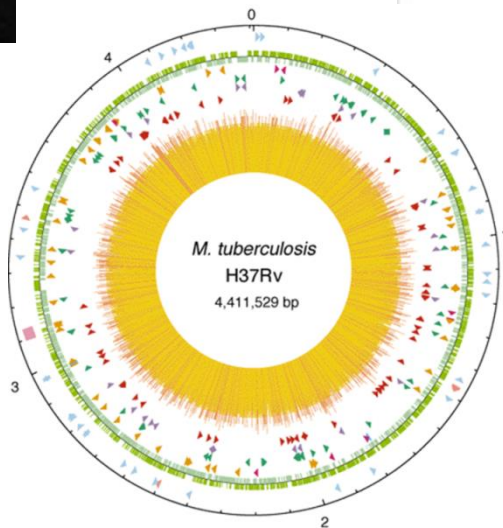


- **A2 Provide universal access to high-quality diagnostics**
- **A6 Reduce drug-resistant TB**
- **A9 Strengthen surveillance**



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WGS



Identification

**Sensitivity
prediction**

**Relatedness
(clustering)- the
SNP type**



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Why WGS?

WGS workflow

Result: Identification,
susceptibility prediction, SNP
type

Result:
phenotypic
susceptibility

Positive culture of
Mycobacterium

Day 1

Day 5-7

Day 14-21

Day 42

Result: Identification by LPA

Result:
phenotypic
susceptibility

Result: TB typing
(MIRU-VNTR)

Conventional workflow

Quality, speed and cost

THE LANCET Respiratory Medicine

Volume 4, Issue 1, January 2016, Pages 49-58



Articles

Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study

Dr Louise J Pankhurst PhD ^{a,*}, Carlos del Ojo Elias MSc ^{a,*}, Antonina A Votintseva PhD ^{a,*}, Timothy M Walker MRCP ^{a,*}, Kevin Cole BSc ^d, Prof Jim Davies PhD ^c, Gilles M Ferment MSc ^b, Deborah M Gascoyne-Binzi PhD ^f, Thomas A Kohl PhD ^j, Clare Kong BSc ⁱ, Nadine Lemaitre PhD ^g, Stefan Niemann DSc ScD ^{j,k}, John Paul MD ^d, Thomas R Rogers FRCPATH ^l, Emma Roycroft MSc ^l, E Grace Smith FRCPATH ^e, Philip Supply PhD ^{g,h}, Patrick Tang PhD ^l... Derrick W Crook FRCPATH ^a

Show more

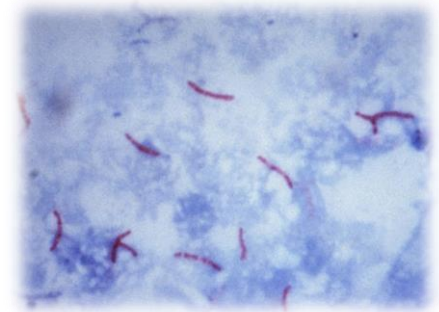
[https://doi.org/10.1016/S2213-2600\(15\)00466-X](https://doi.org/10.1016/S2213-2600(15)00466-X)

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PHE WGS for mycobacteria: the how

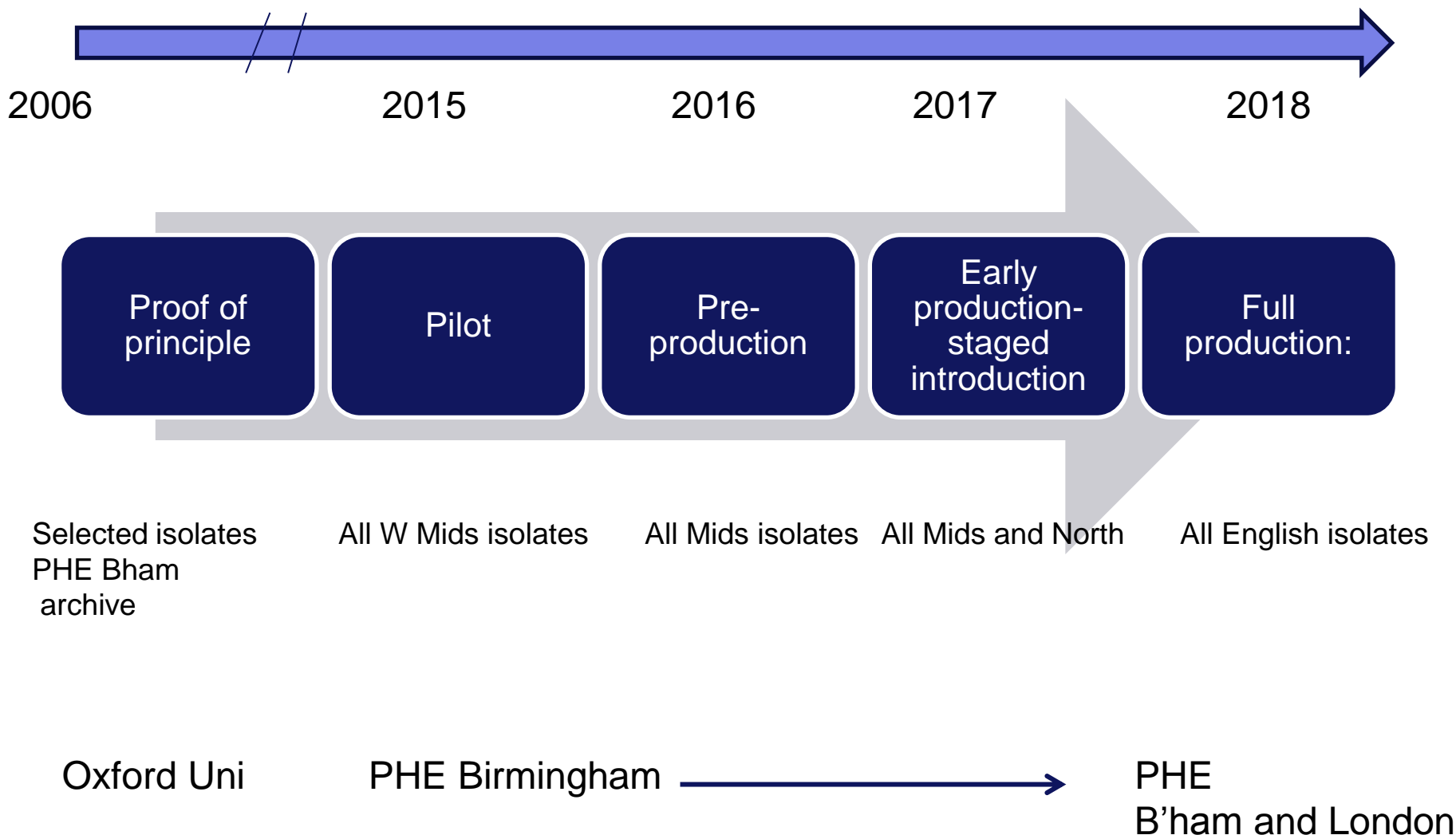
- NMRS: National Mycobacterial Reference Service
- Receive cultures from NHS labs in England
- Distributed hub model: London and Birmingham
 - Resilience
 - Distributed and diffusing expertise
 - Different models for WGS delivery:
 - Birmingham locally run MiSeq
 - London PHE CSU HiSeq & NextSeq
- All first positive mycobacterial cultures
- All positives with previous TB and previous isolate ≥ 2 months previously
- NTM : pathway still being discussed, but likely no repeat if previous isolate 3-6 months





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Getting the results flowing





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Sending Mycobacterial Cultures to the National Mycobacterial Reference Service (Central & North)

Check what the positive MGIT culture looks like:



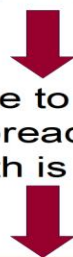
Breadcrumb
like growth



No visible
growth



Non-breadcrumb
growth (i.e. cloudy
or mucoid growth)



Continue to incubate
until breadcrumb
growth is visible

Decontaminate
and re-culture

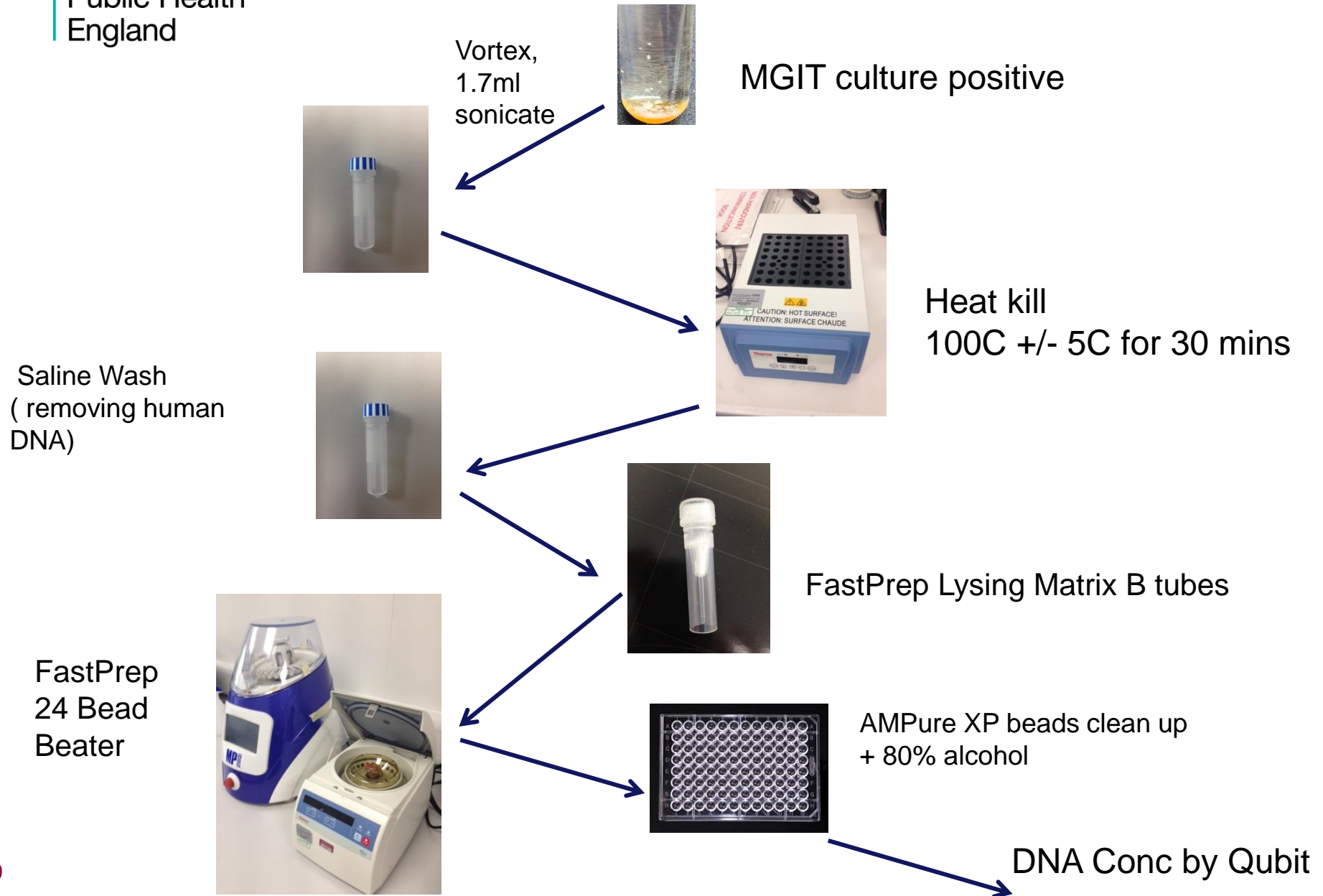
Vortex the MGIT and add 3 ml of MGIT
culture to a plastic bijou. **Make sure that
the breadcrumbs are included.**



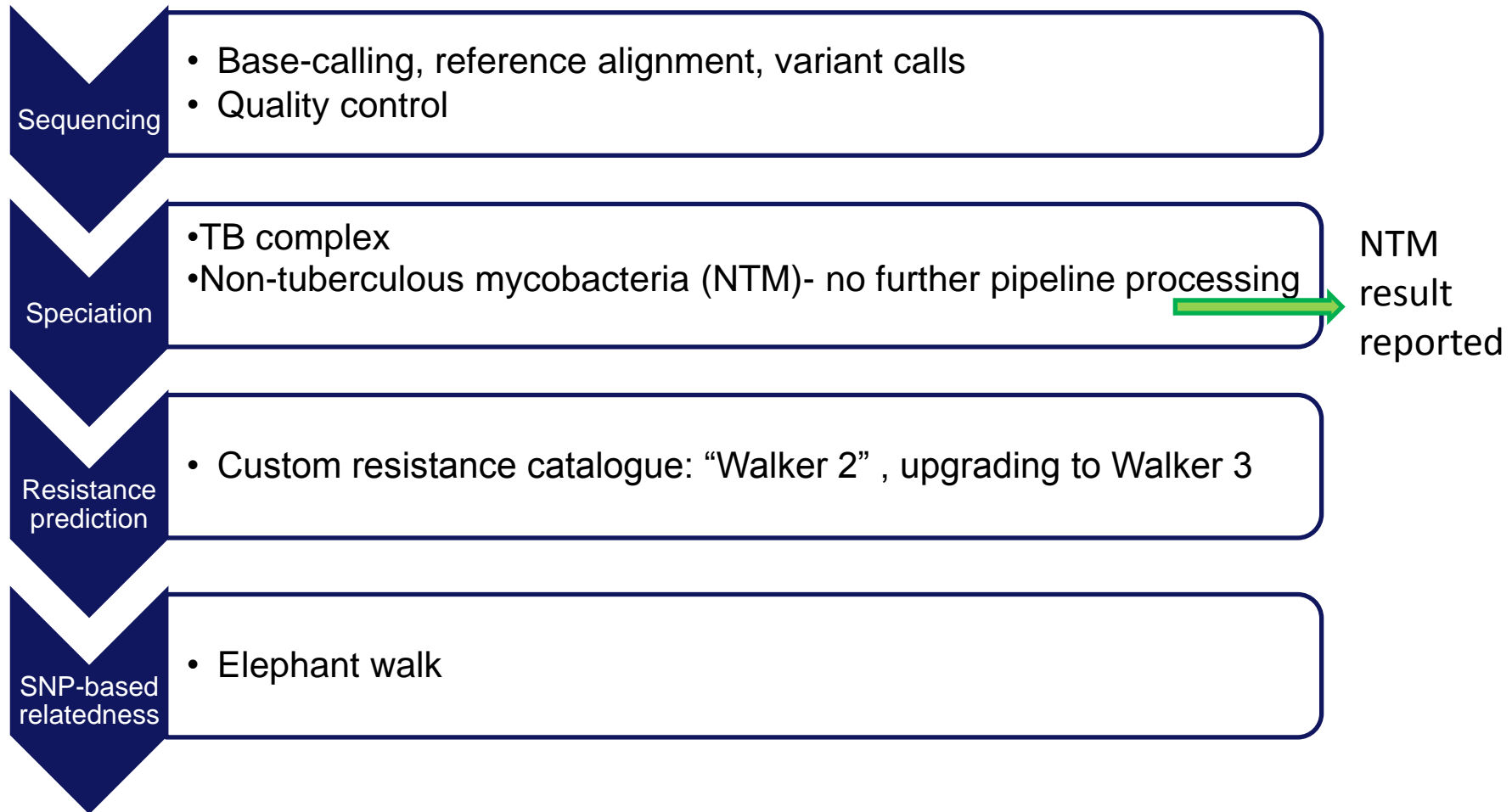
Fill to 3ml line



DNA Extraction



Mycobacterial Computational Pipeline



TB report with resistance prediction- sent to user labs

TB report with resistance prediction and relatedness- imported into PH pilot database system



The story so far...

300-350 isolates/ week

30-40% are MTB complex

TAT 5-7 working days (from
isolate to WGS result) met
(monitored weekly)

2 days in 18m when LPA used
as backup

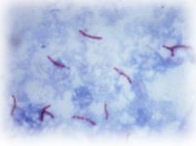
2 instances of use of
contingency pipeline

Continued though loss of CL3
for 6 weeks (Bham)

Conventional vs WGS: real-life “head to head”

Days to result from
specimen collection

	2014	2018
BAL	44.2 (11.8)	23.1 (3.8)
Sputum	66.1 (41.9)	30.7 (21.8)
Lymph node	74.9 (37.5)	40.3 (10.9)



Patient A Sputum 14.12.2016
Result: Mycobacterium tuberculosis identified by WGS

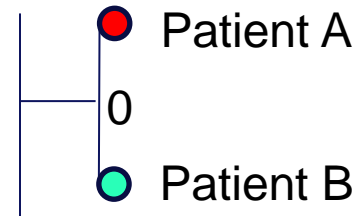
	WGS Resistance
Isoniazid	Resistant
Rifampicin	Resistant
Ethambutol	Resistant
Pyrazinamide	Unknown
Quinolones	Sensitive
Streptomycin	Resistant
Aminoglycosides	Sensitive

49M smear positive,
cavitary MDR TB, no
known risk factors



Where from?
How to treat?
Who to contact
trace?

Raises significant clinical
and public health issues



**Cluster
AA609**
Typing shows Patient A
has same strain as a
known patient B

Month 0: Started
treatment
Month 3: Smear and
culture negative
Month 4: discharged
from inpatient care
Month 9: Half way
through 18 month
therapy

MDR-TB



Amikacin, moxifloxacin,
prothionamide, linezolid,
cycloserine



A knew B previously,
but had **not** identified
him as contact
**No need to hunt for
further source**



WGS reports: identification

Organism Identification

Kraken (percentage)

Human 0.02

Mykrobe

Phylo_group: Mycobacterium_tuberculosis_complex

Species: Mycobacterium_tuberculosis

Lineage: European_American

Percentage	Median
99.63	32
97.75	32
100.00	18

Sequencing Quality

Mapped to: R00000039

Total reads (~millions)	Mapped %	No reads mapped (~millions)	Coverage %
1.39	98.90	1.37	91.58

- Mapping based- works extremely well for MTB
- Very well for well-described species of NTM
- Less well for minority species, heavily dependent on quality of the reference genome (often single)



WGS reports: resistotype

Resistance Summary

INH	RIF	EMB	PZA	QUI	SM	AG
R	R	R	S	S	F	F

- R = predicted resistant: previously described mutation in defined position of known gene associated with resistance to that antibiotic
- S = predicted susceptible: no mutation in defined position of known gene associated with resistance to that antibiotic
- U = mutation, not previously described, detected in known gene associated with resistance to that antibiotic
- F = unable to make a genotypic prediction based on sequence data



Resistotype

Drug	Mutation	Nucleotides	Support (A/C/G/T)	Source	Prediction
INH	katG_S315T	AGC->ACC	(136/0/0/0) (0/135/0/0) (0/134/0/0)	Line-probe/derived-(471/480)	R
RIF	rpoB_*435*	GAC->TAC	(0/0/0/114) (119/0/0/0) (0/124/0/0)	Line-probe	R
SM	gidB_L59R	CTC->CGC	(0/145/0/0) (0/0/148/0) (0/153/0/0)	novel	U



Analysis of 10,000 isolates

WGS and phenotypic DST

16 countries in 6 continents

- Isoniazid, rifampicin, ethambutol and pyrazinamide resistance correctly predicted , meeting the WHO target profiles for new molecular assays of over 90% specificity and 95% sensitivity overall.
- Targets met for individual drugs except ethambutol specificity-93.6%
- Targets met for collections not enriched for drug resistance (consecutively sampled isolates from UK, Italy, the Netherlands and Germany)
- Targets met for predicted pan-susceptibility in all collections
- Targets met in simulated drug profiles with drug resistance rates up to 47%

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

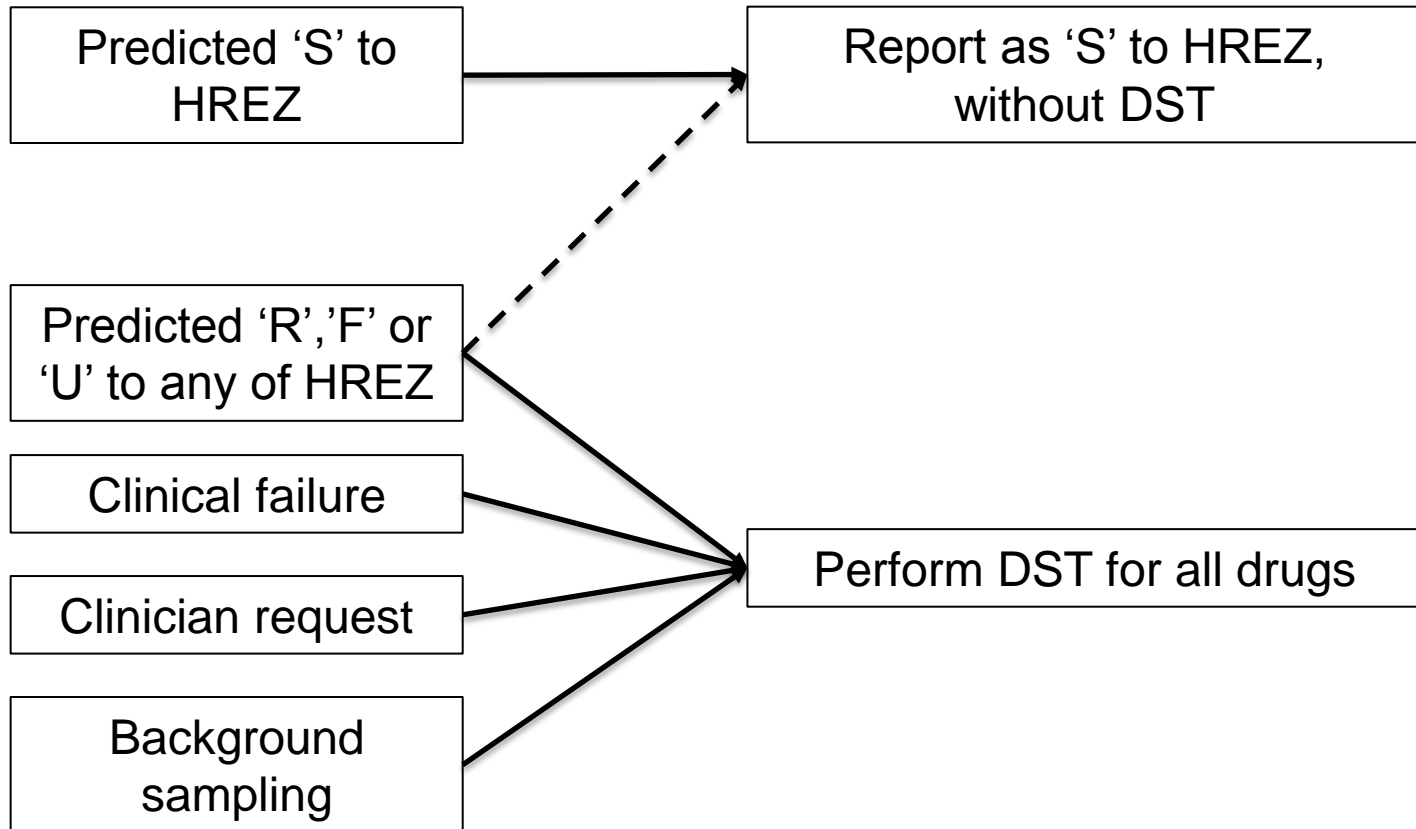
The CRyPTIC Consortium and the 100,000 Genomes Project

ABSTRACT

BACKGROUND

The World Health Organization recommends drug-susceptibility testing of *Mycobacterium tuberculosis* complex for all patients with tuberculosis to guide treatment decisions and improve outcomes. Whether DNA sequencing can be used to accurately predict profiles of susceptibility to first-line antituberculosis drugs has not been clear.

Imagined work-flow after introduction of updated resistance catalogue





Changes to Resistance Reporting

Why Change ?

- Feedback from clinical users- requesting more information on resistance mutations and implications for management.
- Confusion in interpretation of 'U' and 'F'- both may be interpreted as Resistance
- Variable reporting by laboratories-waiting until phenotypic results available before reporting to clinicians- failing to report genotypic results predicting drug resistance or susceptibility
- 70% of reports for TB complex will be 'final' with genomic prediction of 'S' to INH, RIF, ETB and PZA



Scenario 1 – Isoniazid Sensitive but one first line drug fail (Ethambutol)

Mycobacterium tuberculosis

Identified by WGS

	WGS RESISTANCE	PHENOTYPIC DST
Isoniazid	Sensitive	NOT TESTED
Rifampicin	Sensitive	NOT TESTED
Ethambutol	see comment	to follow
Pyrazinamide	Sensitive	NOT TESTED
Quinolone Group	Sensitive	
Streptomycin	Sensitive	
Aminoglycosides	Sensitive	

Ethambutol - Poor quality sequence, unable to make predictions. WGS will be repeated

If there is a clinical concern, please discuss with NMRS clinician



Scenario 2 – Isoniazid Resistant, Rifampicin Fail, Ethambutol and Pyrazinamide Unknown

Mycobacterium tuberculosis

Identified by WGS

	WGS RESISTANCE	Phenotypic DST
Isoniazid	Resistant	To Follow
Rifampicin	see comment (F)	To Follow
Ethambutol	No prediction (U)	To Follow
Pyrazinamide	No prediction (U)	To Follow
Quinolone Group	Sensitive	To Follow
Streptomycin	Sensitive	
Aminoglycosides	Sensitive	To Follow

Isoniazid mutation is KATG/INH



Scenario 2 – Isoniazid Resistant, Rifampicin Fail, Ethambutol and Pyrazinamide Unknown

- | **Rifampicin** – Depending on what has caused the FAIL, one of three comments can be used:
 - Poor quality sequence in Rifampicin gene – unable to make prediction. X work will follow **(if there are lots of ‘junk’ within the sequence)**
 - WGS has detected the possible presence of both sensitive and resistant strains. Please contact NMRS clinicians to discuss.
(Potential minority variant comment – First comment if this is present)
 - Poor quality sequence, unable to make predictions. WGS will be repeated **(if there is a poor quality sequence)**

Ethambutol & Pyrazinamide – Mutation of uncertain significance detected in Ethambutol (XXX) and Pyrazinamide (XXX) genes.

XX% of such mutations do not cause resistance

If there is a clinical concern, please discuss with NMRS



Scenario 3 – Isoniazid Sensitive, RIF / EMB / PZA unknown

Mycobacterium tuberculosis

Identified by WGS

WGS RESISTANCE

Phenotypic DST

Isoniazid

Sensitive

Rifampicin

No prediction

Ethambutol

No prediction

Pyrazinamide

No prediction

Quinolone Group

Sensitive

Streptomycin

Sensitive

Aminoglycosides

Sensitive



Scenario 3 – Isoniazid Sensitive, **RIF / EMB / PZA unknown**

Mutation not present in current catalogue detected in Rifampicin (XXX), Ethambutol (XXX) and Pyrazinamide (XXX) genes.

There is xxx% probability that this isolate is fully sensitive

No phenotypic sensitivity testing will be performed unless there is a clinical concern, and discussion with NMRS clinician

Please see supplementary appendix of (Link to Tim Walkers supplementary appendix) and (Link to slidecast / TB handbook) for further information



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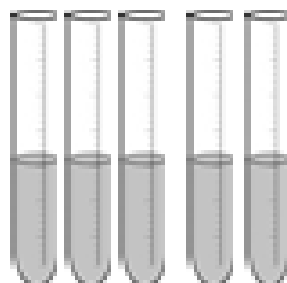
Conventional methods for Drug Susceptibility Testing

TB+ culture

Phenotypic detection

Molecular detection

Con R I E P



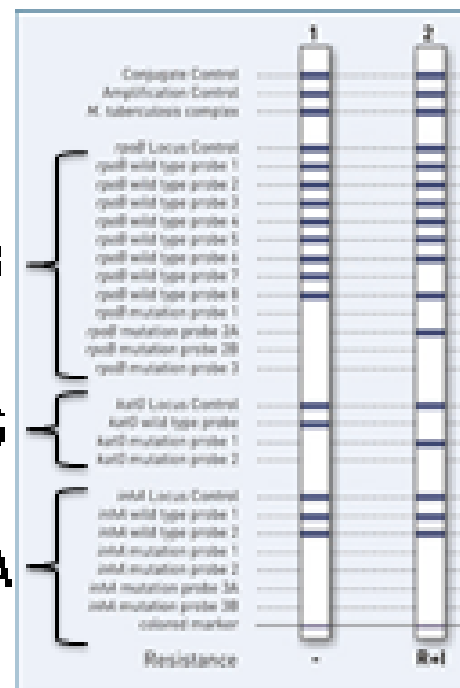
Bactec MGIT 960 system

Con = control
R = Rifampicin (RIF)
I = Isoniazid (INH)
E = Ethambutol
P = Pyrazinamide

**Rifampicin
susceptibility
testing**

**Isoniazid
susceptibility
testing**

**katG
inhA**

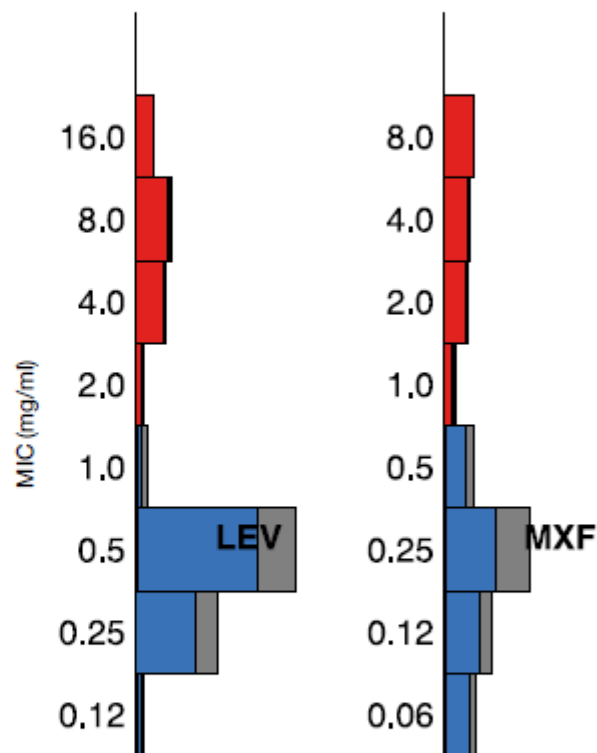
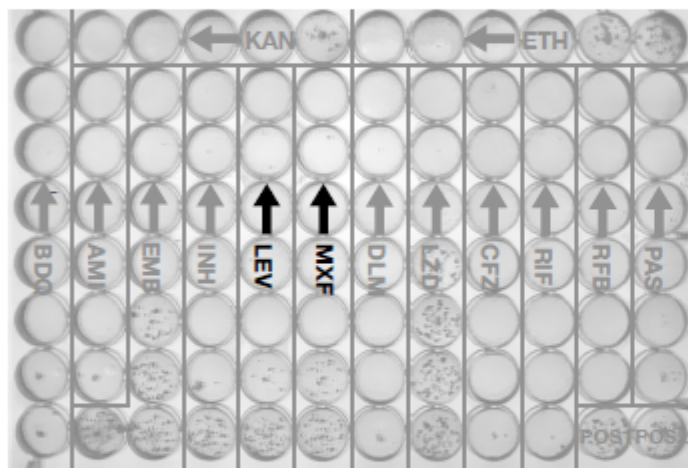


HAIN Genotype MTBDRp/lus



Microtitreplate Testing for Phenotypic Drug Susceptibility- 'TREK Plate'

-Measures MIC for multiple drugs



Improving resistance prediction

Comprehensive Resistance Prediction for Tuberculosis: an International Consortium (CRyPTIC)

Creating a catalogue of 'all' determinants conferring antituberculosis drug resistance.

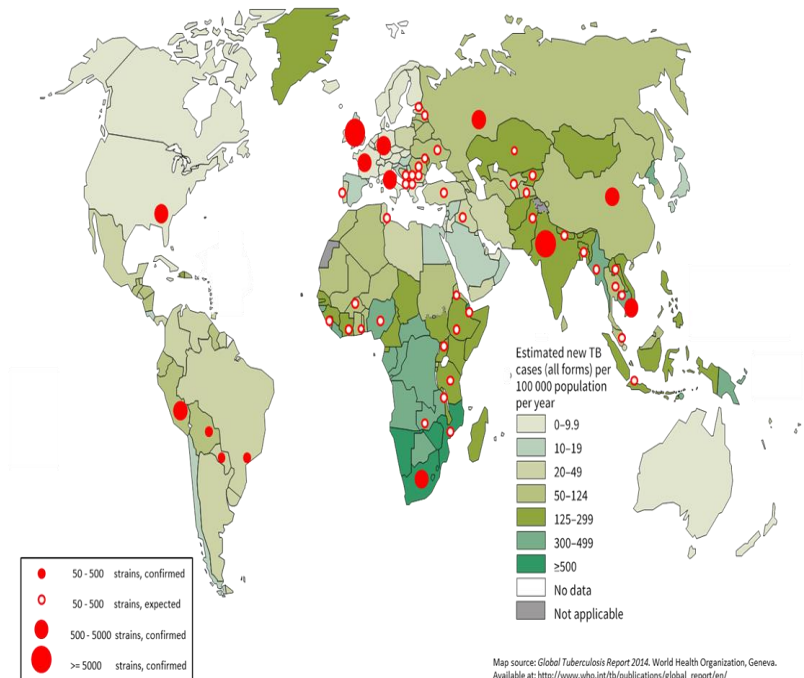
Will investigate a very large number of isolates over-sampled for resistance

Potential total 100,000

42,000 with extensive DST to 15 drugs, including bedaquiline and delamanid:

Gates Foundation funded 21,000 isolates (5,000 with extensive DST)

Wellcome Funding 80,000 isolates (37,000 with extensive DST)





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WGS for public health

Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study

Timothy M Walker*, Camilla L C Ip*, Ruth H Harrell*, Jason T Evans, Georgia Kapatai, Martin J Dedcoat, David W Eyre, Daniel J Wilson, Peter M Hawkey, Derrick W Crook, Julian Parkhill, David Harris, A Sarah Walker, Rory Bowden, Philip Monk†, E Grace Smith†, Tim E A Peto†

Assessment of *Mycobacterium tuberculosis* transmission in Oxfordshire, UK, 2007–12, with whole pathogen genome sequences: an observational study

Timothy M Walker, Maeve K Lalor, Agnieszka Broda, Luisa Saldana Ortega, Marcus Morgan, Lynne Parker, Sheila Churchill, Karen Bennett, Tanya Golubchik, Adam P Giess, Carlos Del Ojo Elias, Katie J Jeffery, Ian C J W Bowler, Ian F Laurenson, Anne Barrett, Francis Drobniewski, Noel D McCarthy, Laura F Anderson, Ibrahim Abubakar, H Lucy Thomas, Philip Monk, E Grace Smith, A Sarah Walker, Derrick W Crook, Tim E A Peto*, Christopher P Conlon*

Home / Eurosurveillance / Volume 22, Issue 2, 12/Jan/2017 / Article

Surveillance and outbreak report

Open Access

A joint cross-border investigation of a cluster of multidrug-resistant tuberculosis in Austria, Romania and Germany in 2014 using classic, genotyping and whole genome sequencing methods: lessons learnt

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Lena Fiebig^{1,2}, Thomas A Kohl^{3,4}, Odette Popovic⁴, Margarita Mühlenfeld⁵, Alexander Indra⁶, Daniela Homorodean⁷, Domnica Chiotan⁸, Elvira Richter⁹, Sabine Rüsche-Gerdes¹⁰, Beatrix Schmidgruber¹¹, Patrick Becker^{12,13}, Barbara Hauer¹, Stefan Niemann^{1,10,12}, Franz Allerberger⁵, Walter ...

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Journal of Infection
Volume 73, Issue 3, September 2016, Pages 210-218



XDR-TB transmission in London: Case management and contact tracing investigation assisted by early whole genome sequencing

Amber Arnold^{a, b, c, d, e}, Adam A. Witney^{a, b, c, d, e}, Stephanie Vergnano^{a, b, c, d, e}, Anita Roche^{a, b, c, d, e}, Catherine A. Cosgrove^{a, b, c, d, e}, Angela Houston^{a, b, c, d, e}, Katherine A. Gould^{a, b, c, d, e}, Jason Hinds^{a, b, c, d, e}, Peter Riley^{a, b, c, d, e}, Derek Macallan^{a, b, c, d, e}, Philip D. Butcher^{a, b, c, d, e}, Tom S. Harrison^{a, b, c, d, e}

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<https://doi.org/10.1016/j.jinf.2016.04.037>

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Volume 66, Issue 6
15 March 2018

Molecular Epidemiology of Tuberculosis in British Columbia, Canada: A 10-Year Retrospective Study

Jennifer L Guthrie, Clare Kong, David Roth, Danielle Jorgensen, Mabel Rodrigues, Linda Hoang, Patrick Tang, Victoria Cook, James Johnston, Jennifer L Gardy

Clinical Infectious Diseases, Volume 66, Issue 6, 5 March 2018, Pages 849–856, <https://doi.org/10.1093/cid/cix906>



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RESEARCH ARTICLE

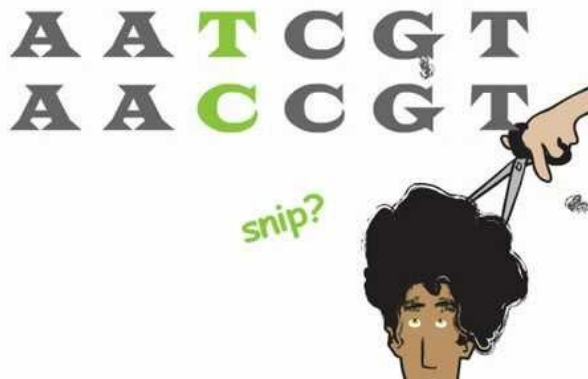
Identifying Likely Transmission Pathways within a 10-Year Community Outbreak of Tuberculosis by High-Depth Whole Genome Sequencing

Alexander C. Outhred, Nadine Holmes, Rosemarie Sadsad, Elena Martinez, Peter Jellis, Grant A. Hill-Cawthorne, Gwendolyn L. Gilbert, Ben J. Marais, Vitali Sintchenko

Published: March 3, 2016 • <https://doi.org/10.1371/journal.pone.0150550>



WGS relatedness



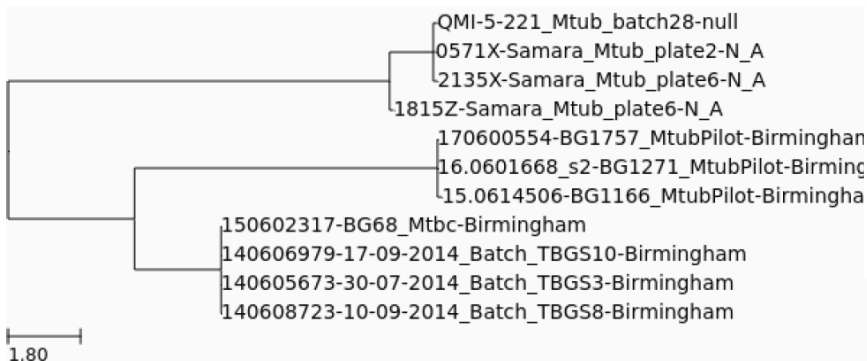
- 0-5 SNPs difference between strains, most probably linked
- 5-12 SNPs may be linked
- >12 SNPs less likely to be linked

Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study

Timothy M Walker*, Camilla L C Ip*, Ruth H Harrell*, Jason T Evans, Georgia Kapatai, Martin J Dedicoat, David W Eyre, Daniel J Wilson, Peter M Hawkey, Derrick W Crook, Julian Parkhill, David Harris, A Sarah Walker, Rory Bowden, Philip Monk†, E Grace Smith†, Tim E A Peto†

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Epidemiologically linked cases tend to have small numbers of SNPs

One can set cut offs

Epidemiologically unlinked cases tend to have large numbers of SNPs

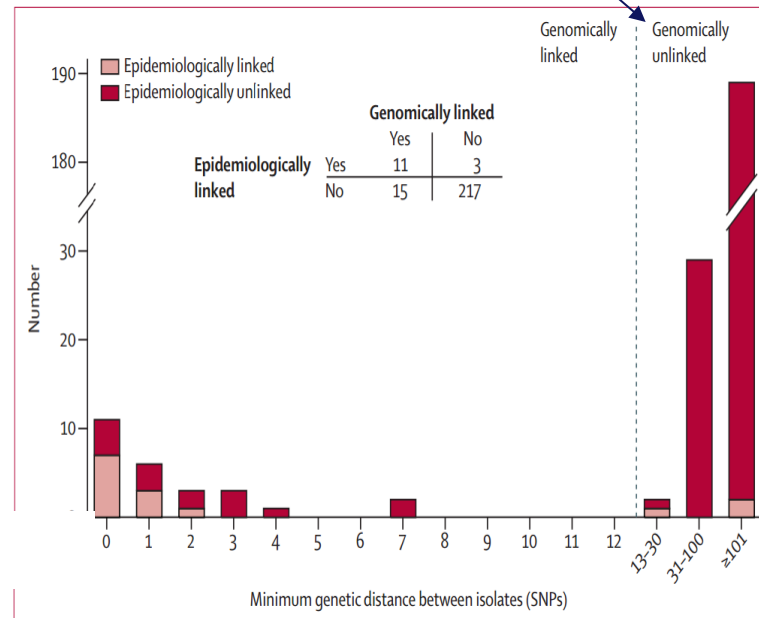


Figure 6: Minimum genetic distance between isolates



Depictions include phylogenetic trees

Cluster: AA208-4.

Clustering pipeline is TB-SNPcutoff-12 (#1) build #127.

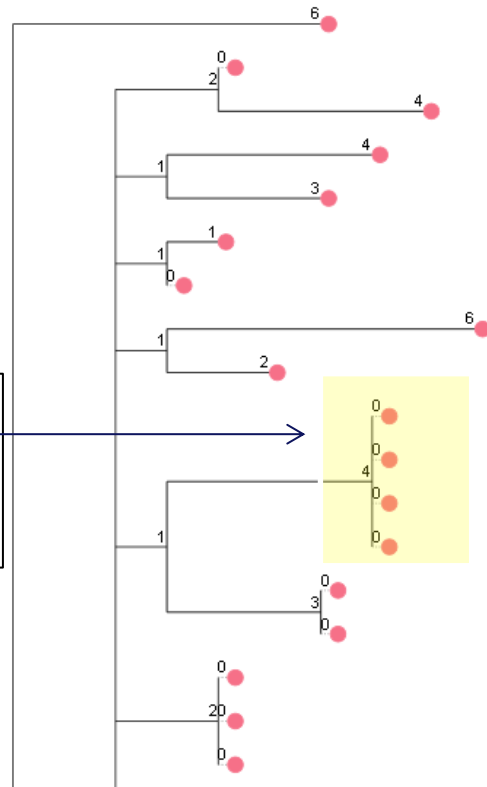
Sequencing data as of: 2018-03-15 10:04:38.697000.

Meta-data as of: 2018-03-15 16:24:11.012460.

[ref: TB-SNPcutoff-12/127/20766/182871-531]

Tree is of type iqTree

Root-to-tip distance is 12.0 SNP. Scale is ~ 33.0 px per SNP.



Cases shown
are 0 SNPs
apart

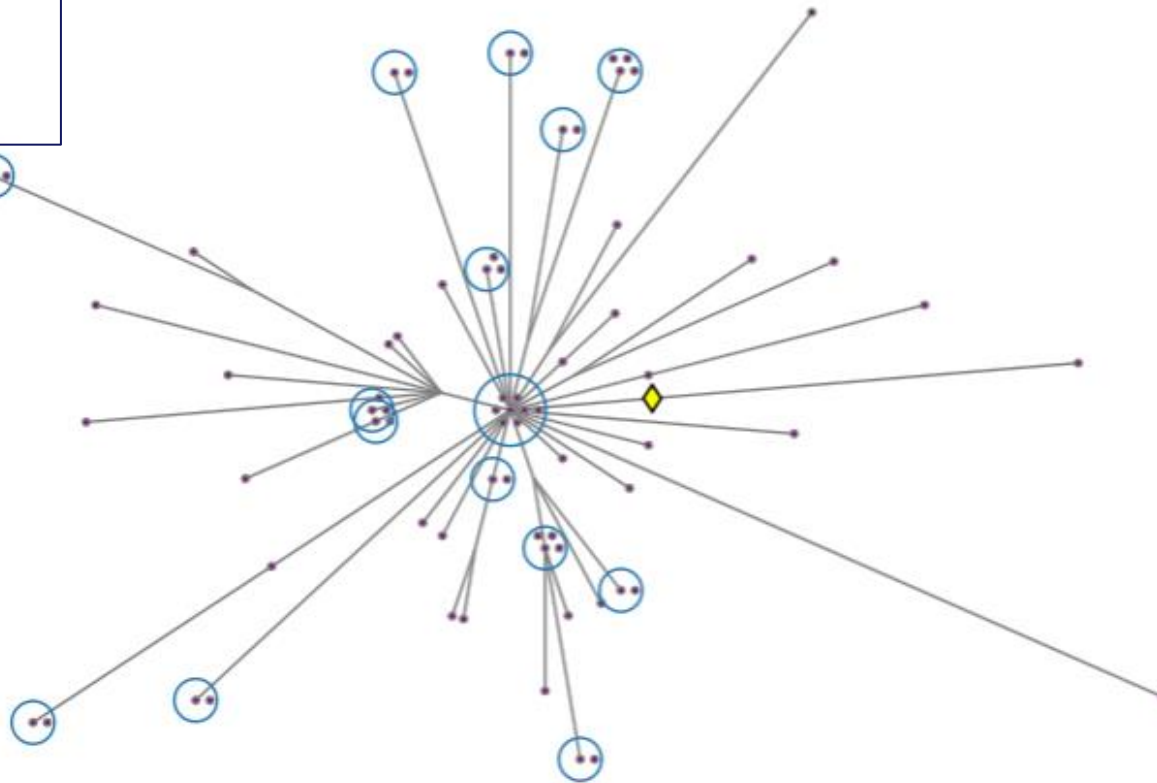
MIRUCIR ZHE FA SpecName

- R SR S SS Florence_IT-92
- S SR S SS 16.0601260
- S SR S SS 493572_H18084039003-1
- E1139 S SR S SS 404171_H163840123-1
- S SR S SS 470181_H17528044103-2
- S SR S SS 482643_H12052001402-2
- S SR S SS 481538_H13246000702-2
- S SR S SS IMRLH109
- S SR USF 481550_H13344001302-2
- S SR S SS 16.0618578
- S SR S SS 16.0617117
- S SR SF 16.0618578
- E1140 S SR SU 16.0617145
- S SR SF 15.7626611
- F SR S SS 15.7626465
- S SR S SS BIR-369_5
- S SR S SS BIR-220_4
- S SR S SS BIR-369a_6



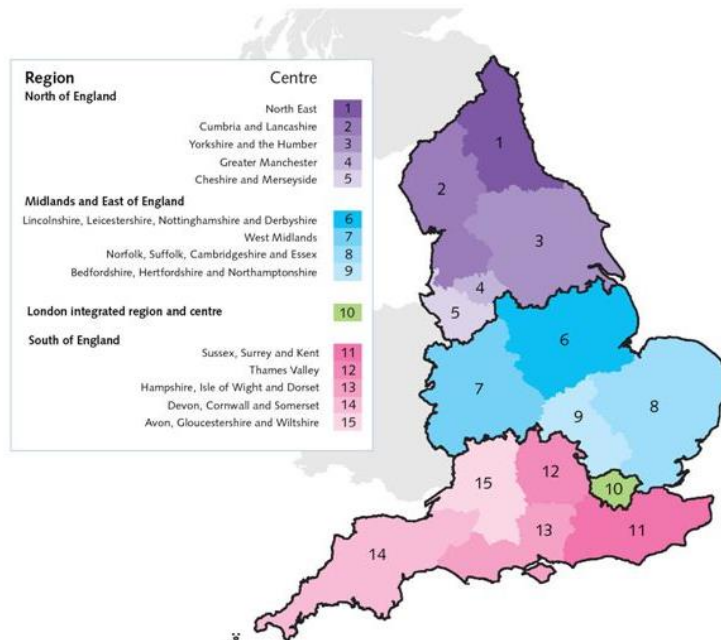
Simplified versions-responding to user feedback

Cases in
circles are 0
SNPs apart





MIRU to WGS: transition



- “Birmingham footprint” (E and W Mids, part of Y&H): parallel sequencing since 2015, extensive back-catalogue

- The North (prev sent to Newcastle): key isolates from active MIRU clusters of PH importance identified and sequenced late 2016

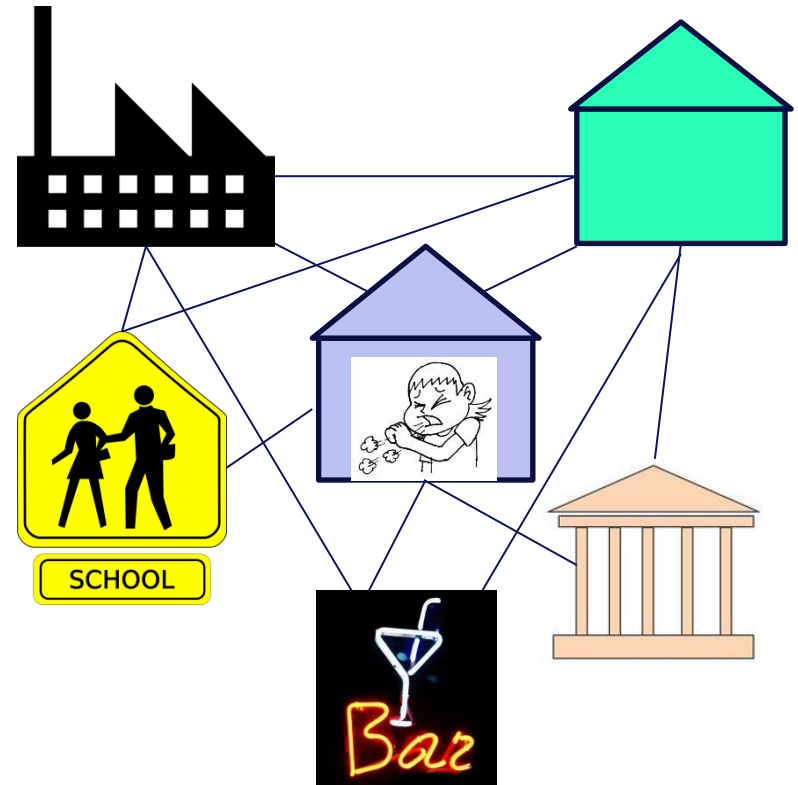
- The South: back catalogue Jan-May 2017



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TB contact tracing and cluster investigation

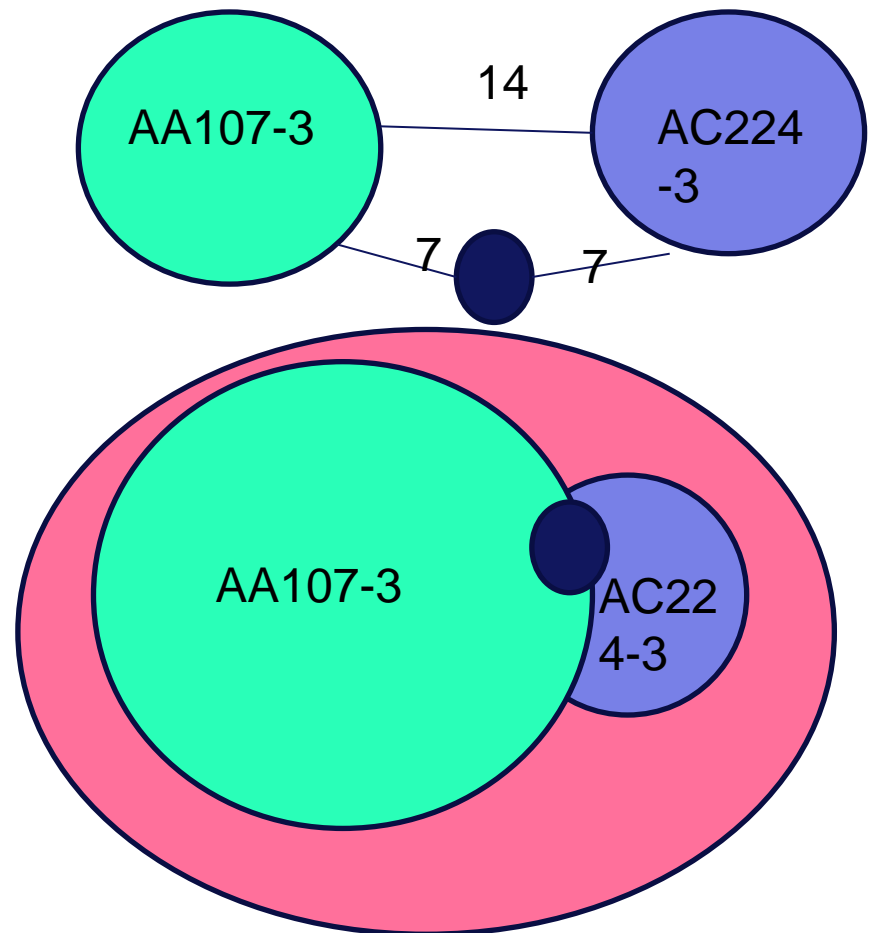
- Identify PH actions/ intervention points
- WGS data deluge and complexity threaten to overwhelm already stretched services





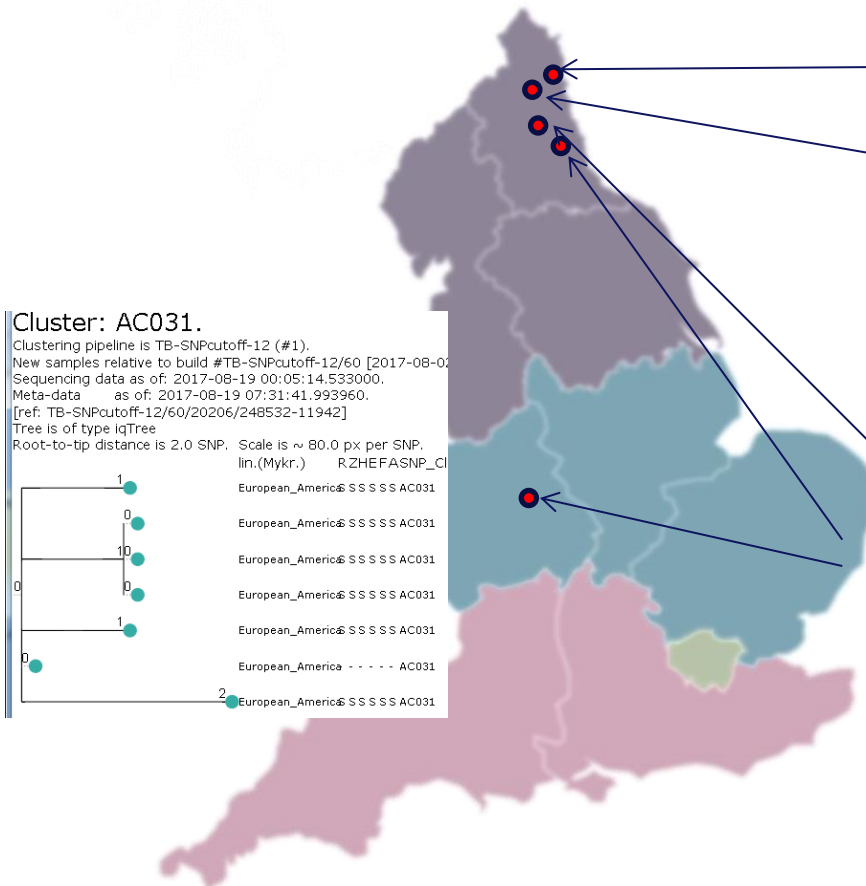
Clustering and naming

- 12 SNP cut off
 - i.e any patient who is within 12 SNP of any other patient will be put into cluster together
- Cluster names: 2 letters, 3 numbers,
- Followed by arbitrary string of letters/ numbers
- Number for TB lineage
- Clusters have much higher likelihood of epi linkage than MIRU-VNTR
- Substructure within clusters





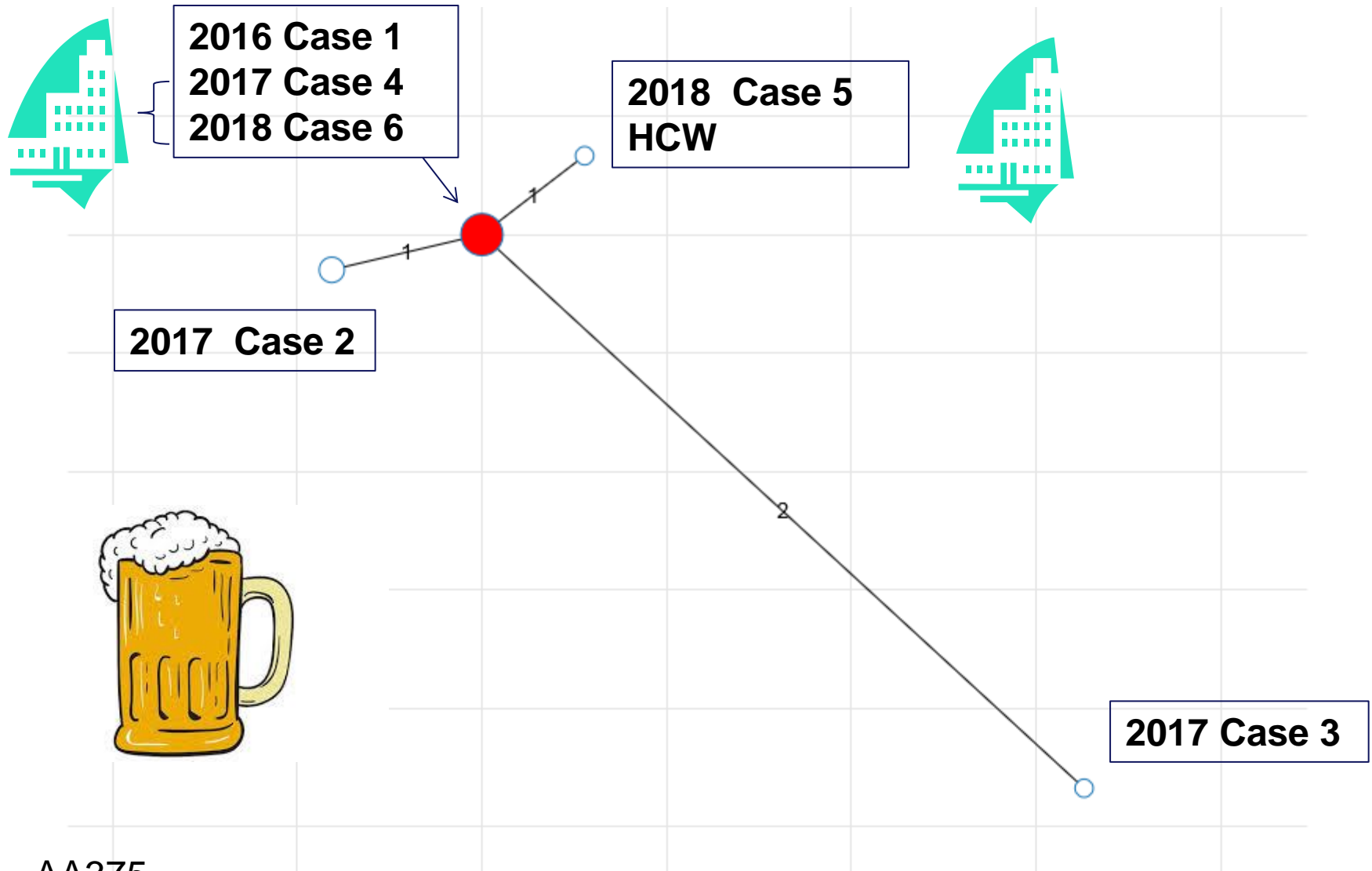
Unsuspected within-UK transmission



2016 : 2 cases NE England. Same strain type (MIRU-VNTR). No epi links; pts both recent entrants from same E African country. Presumed transmission prior to UK entry

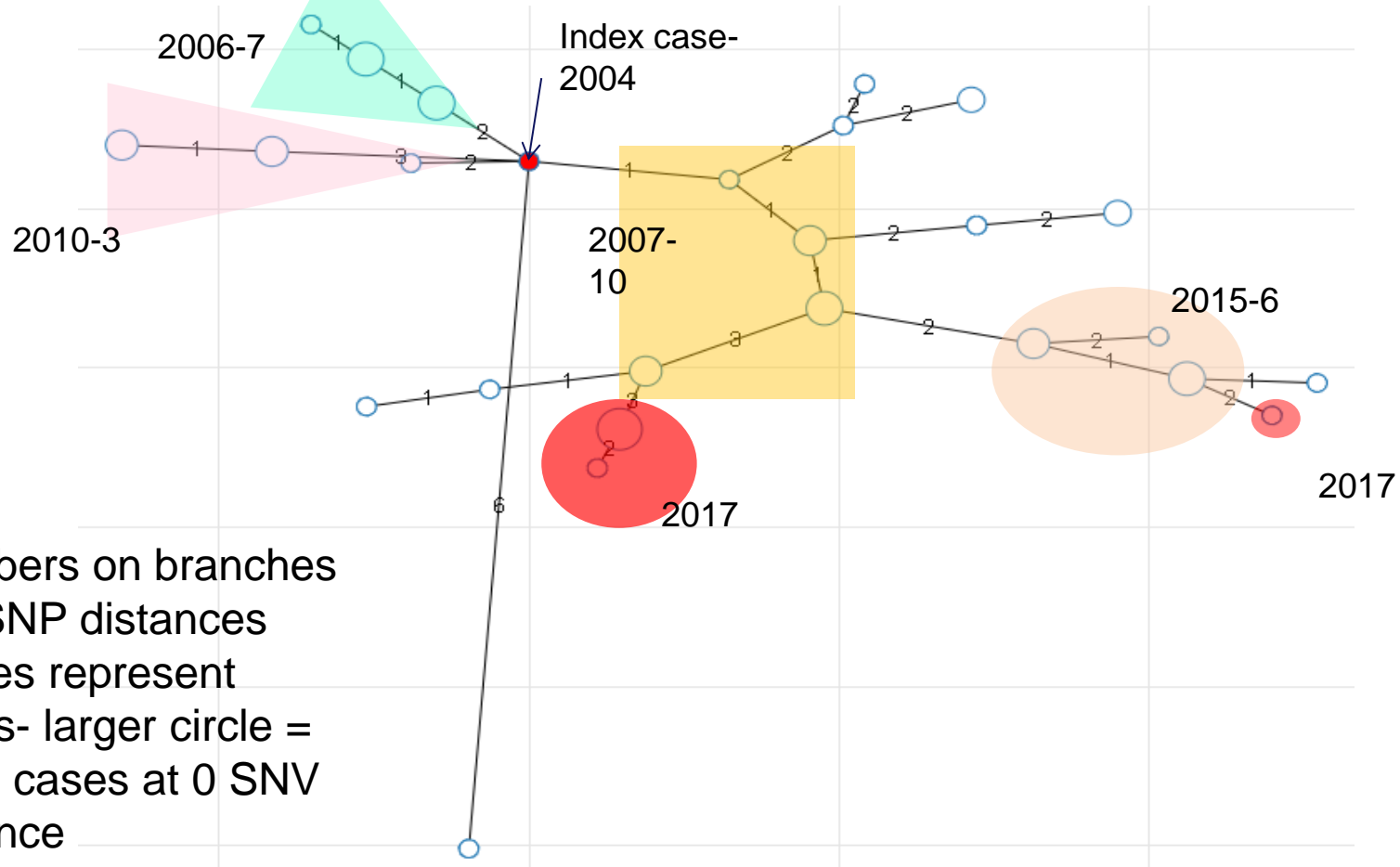
2017: 3 further cases, all now SNP typed: 0-3 SNP distance
More detailed contact tracing- find common social link for screening and latent TB treatment

Multiple TB transmissions and uncontrolled exposures in healthcare settings



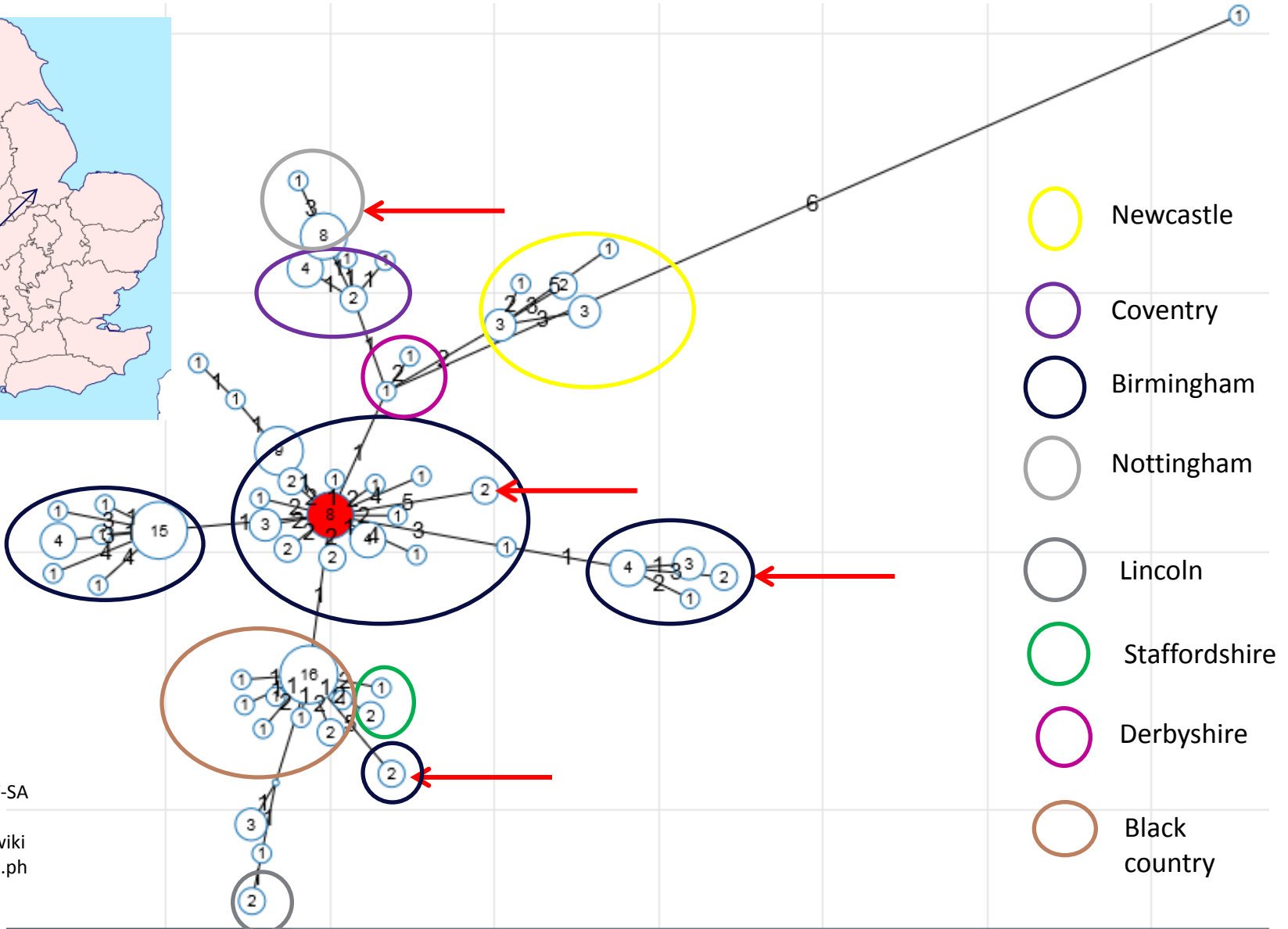


Making sense of clusters and guiding further intervention

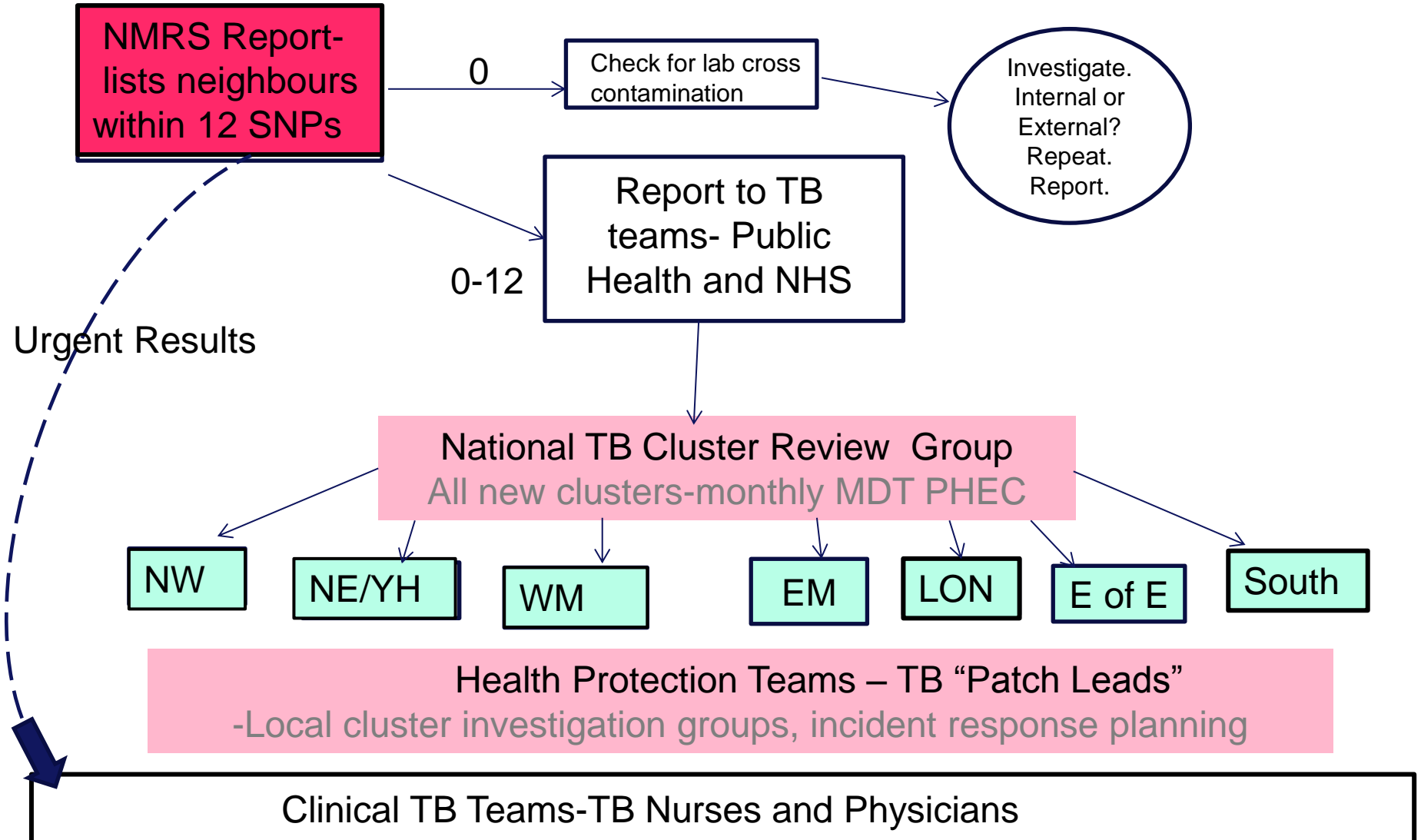


AA090-“chaotic lifestyle”

← New cases
2017/8



Reporting TB Clusters-information for action





Shining a light in dark places:

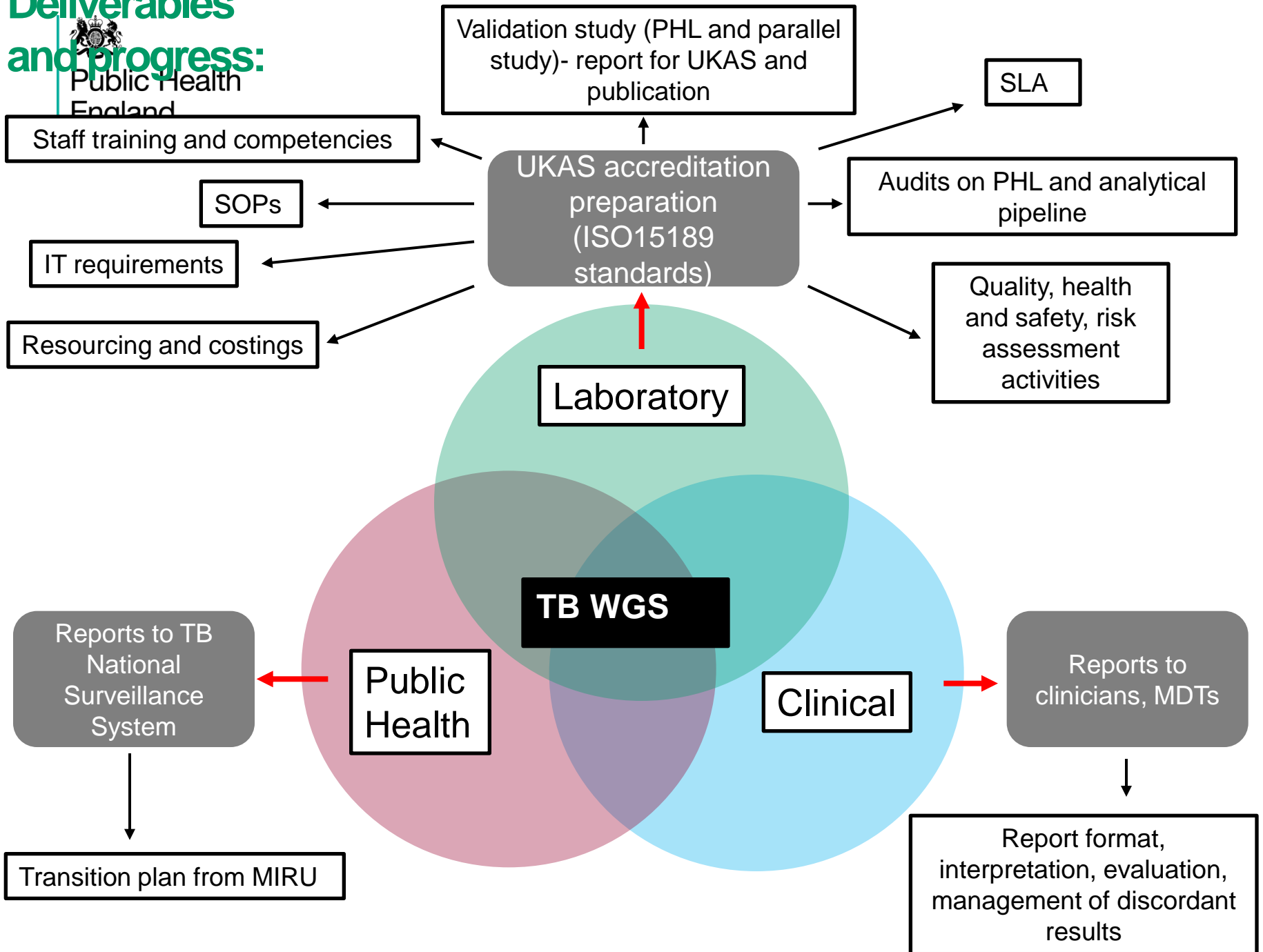


- Case 1: HCW in clinic
- Case 2: Patients in GP WR
- Case 3: bronchoscopy list
- Case 4: lab cross-contamination
- Getting the basics right!
- Changing the transmission paradigm
- Studies investigating SNPs associated with increased transmissibility
- Individualising infection control responses
- Rapid detection of laboratory cross-contamination highlights systematic issues- important for consolidation and commissioning

Deliverables and progress:



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What we've learned and where we go next

- **Translation into routine practice is hard!**
- Successful early implementation requires gathering, enthusing and educating a group of clinical and public health leaders
- Empowering local ownership
- Listening and support
- Pay attention to feedback and understand how your reports are viewed and understood
- Ongoing collaboration with involved academics is vital
- Communicating relatedness to clinicians
- Validating relatedness database, integrating into pan-PHE, pan-organism system
- Sequencing from sample
- Academic evaluation of public health and cost-effectiveness of WGS for TB (HPRU)
- Integrating into developing international systems- governance and security

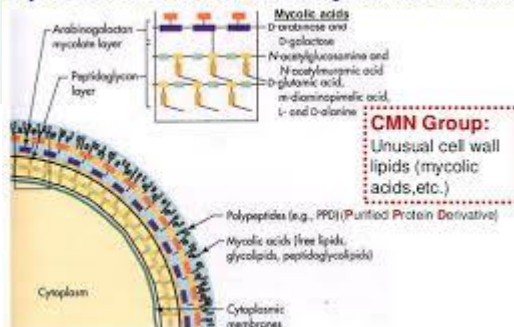


New adventures in sequencing

- Direct from sample
- Near-patient?



Lipid-Rich Cell Wall of Mycobacterium



Challenges

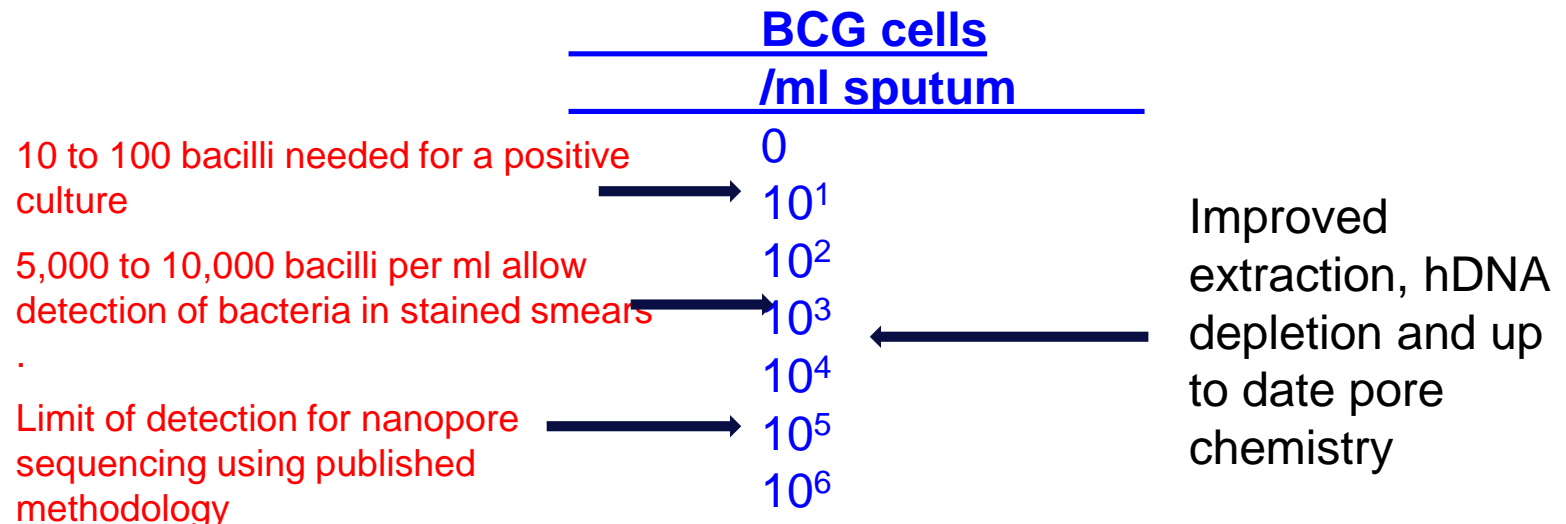
Selecting patient and setting

Sufficient DNA, getting it out

Data analysis and reporting



Investigating the possibility of further improvements to speed, cost and read length using the Nanopore MinION R9.4/R9.4.1 platform.





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NMRS-South

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