Remarkable people. Extraordinary place.

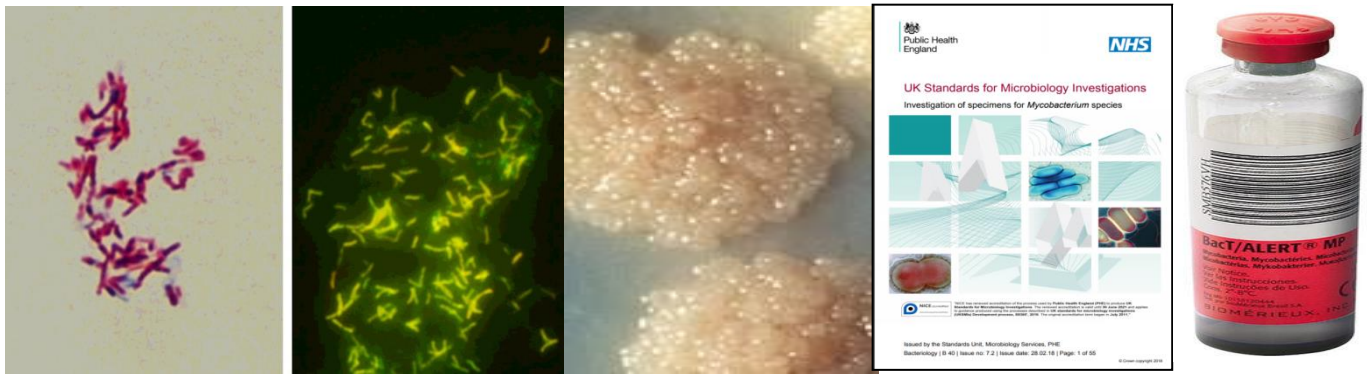
NHS Hull University Teaching Hospitals NHS Trust

Laboratory Diagnosis of Tb

Phillipa J Burns

MSc, FIBMS, ANSHCS, CSci, PGDip Mgt, FRCPath

Principal Clinical Scientist, Hull University Teaching Hospitals – SHYPS Network



Remarkable people. Extraordinary place.

Tb

NHS Hull University Teaching Hospitals NHS Trust

***Mycobacterium tuberculosis* complex (MTBC)**

though less often by other members of the complex:
*Mycobacterium bovis**,
Mycobacterium africanum,
Mycobacterium canettii or
Mycobacterium caprae

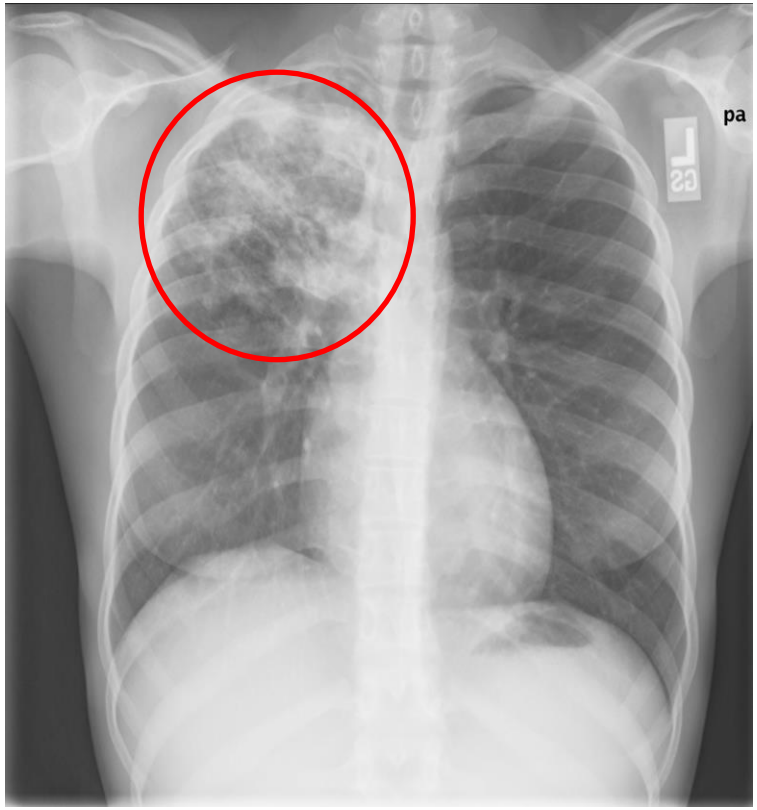
Mycobacterium microti and *Mycobacterium pinnipedii (almost always mammalian hosts, but a few cases have been reported in patients who are* immunocompromised

*Strains of the live vaccine *Mycobacterium bovis* BCG, which are also used intra-vesically in the treatment of bladder cancer, can occasionally cause disease in patients who are immunocompromised.

Remarkable people. Extraordinary place.

Primary Infection

NHS Hull University Teaching Hospitals NHS Trust



Initial infection in a person by MTBC organisms is termed primary tuberculosis, usually site of entry, usually the lung.

Lymph nodes will also be infected at this stage (primary complex)

The tuberculin skin test (TST, Mantoux test) becomes positive at 3-8 weeks after infection, and marks the development of cellular immunity and tissue hypersensitivity.

This test is useful in detecting latent disease.

Interferon gamma release assays are also available.

Remarkable people. Extraordinary place.

Primary Infection

NHS
Hull University Teaching Hospitals
NHS Trust

- Bronchoalveolar lavage/bronchial washings
- Gastric washings (Not all labs accept these)
- Sterile site body fluids(CSF>6ml needed)
- Urine specimens (x3 EMU)
- Skin, bone, and tissue including post mortem specimens (ideally caseous, not in formalin)
- Pus or pus swabs (Mycobacteria stick to flocking, so swabs not ideal)
- Faecal samples (not recommended)

Remarkable people. Extraordinary place.

Primary Infection

NHS Hull University Teaching Hospitals NHS Trust

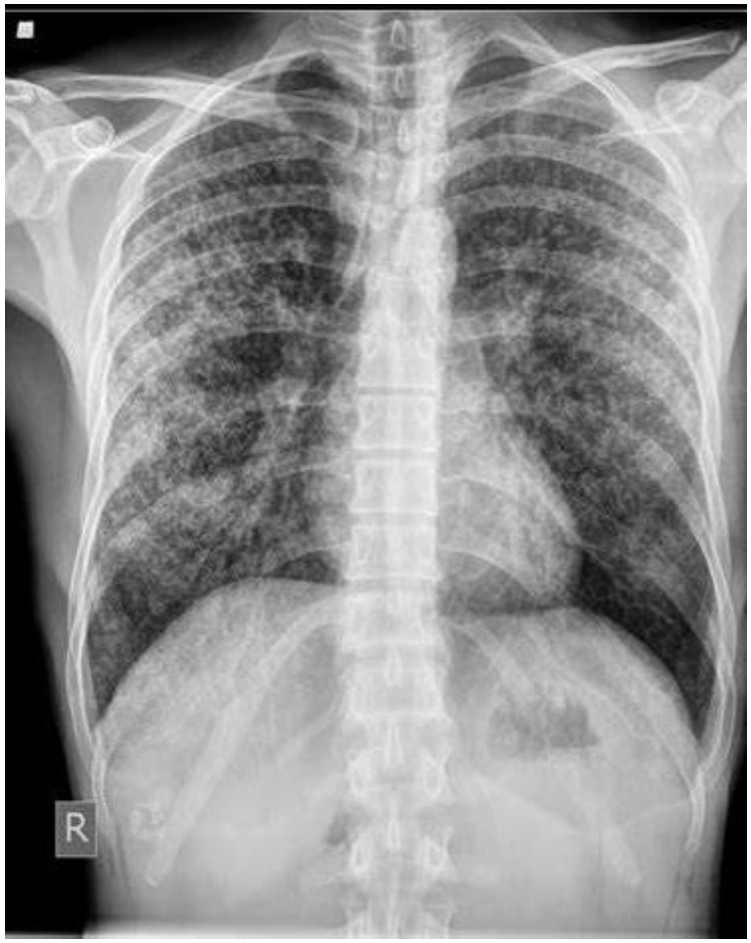
- Blood
- Bone Marrow
- Pus
- Sterile Fluids

Can all be added directly to a Tb Culture Bottle

Remarkable people. Extraordinary place.

Post Primary

NHS Hull University Teaching Hospitals NHS Trust



Foci developing in the endothelium of blood vessels may rupture leading to disseminated or miliary tuberculosis.

Remarkable people. Extraordinary place.

Post Primary

Hull University Teaching Hospitals NHS Trust

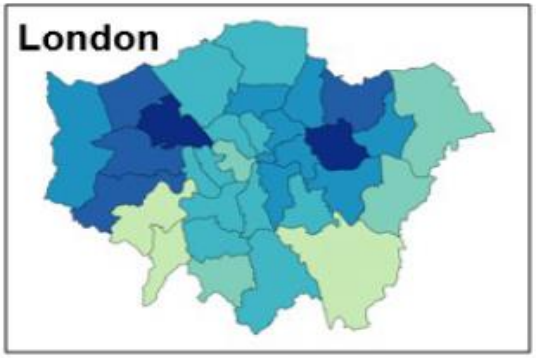
Post-primary tuberculosis develops either as a result of reactivation of organisms in a ‘healed' primary lesion or because of exogenous re-infection.

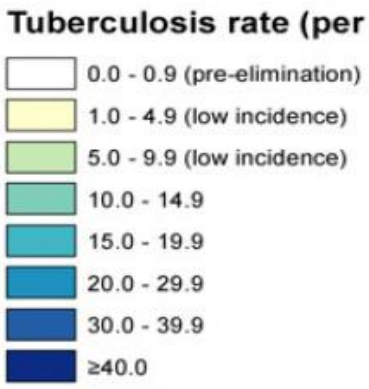
Post-primary tuberculosis usually occurs five or more years after the primary infection and may affect children as well as adults. Infection with *M. tuberculosis* only progresses to clinical disease in a minority of cases.

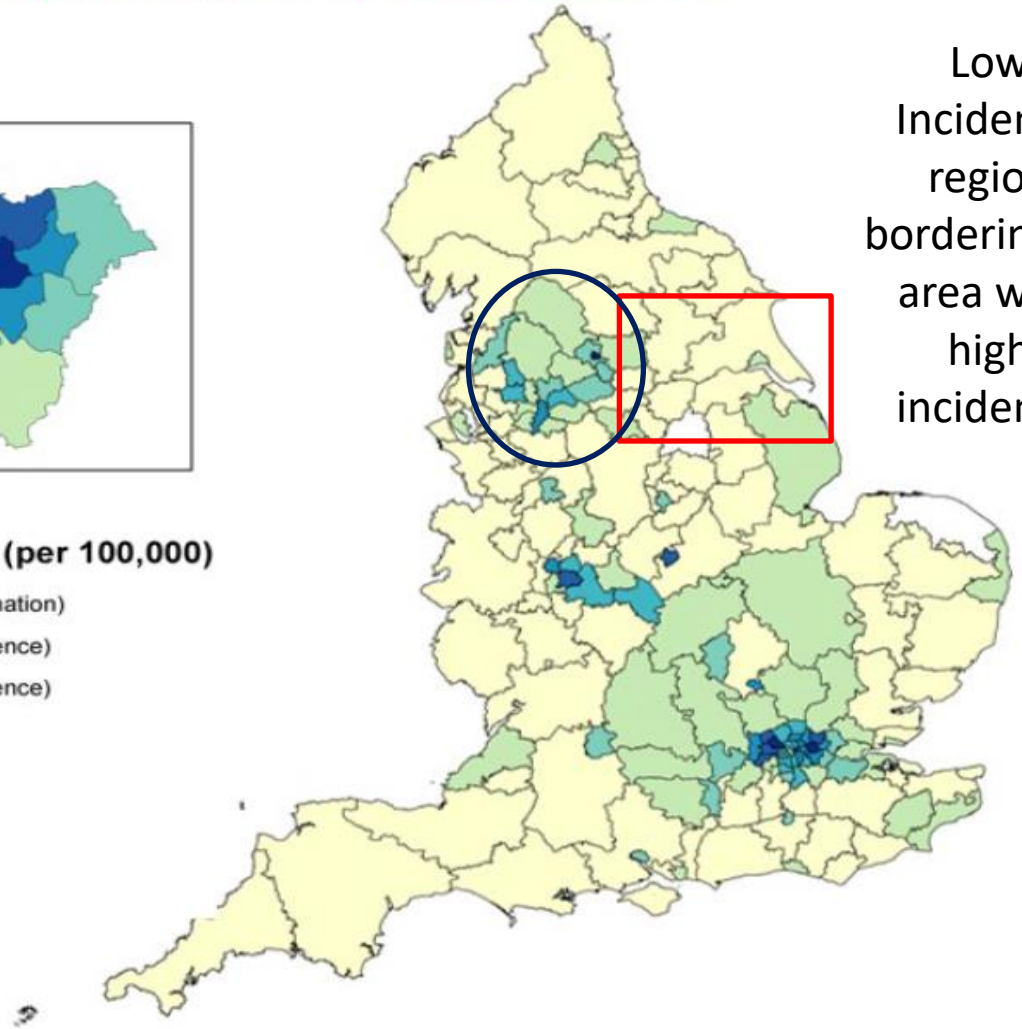
Patients who are infected with HIV are predisposed to reactivation of latent TB infection, and also to a rapid progression of recently acquired infection.

Other predisposing factors may be vitamin D deficiency

Three-year average TB rates by clinical commissioning group, England, 2016-2018







Low Incidence region bordering an area with high incidence

Remarkable people. Extraordinary place.

Laboratory Testing: Sputum

Sputum specimens should be relatively **FRESH** (less than 1 day old) to minimise contamination. **PURULENT** specimens are best.
Two to three samples of ≥5mL should be collected approximately 8-24 hours apart with at least one from early morning.

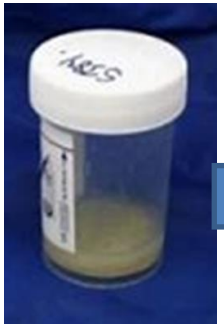
Samples taken **EARLY MORNING** (that is, shortly after patient waking) have the greatest yield. When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline ('sputum induction') before expectoration may be helpful.

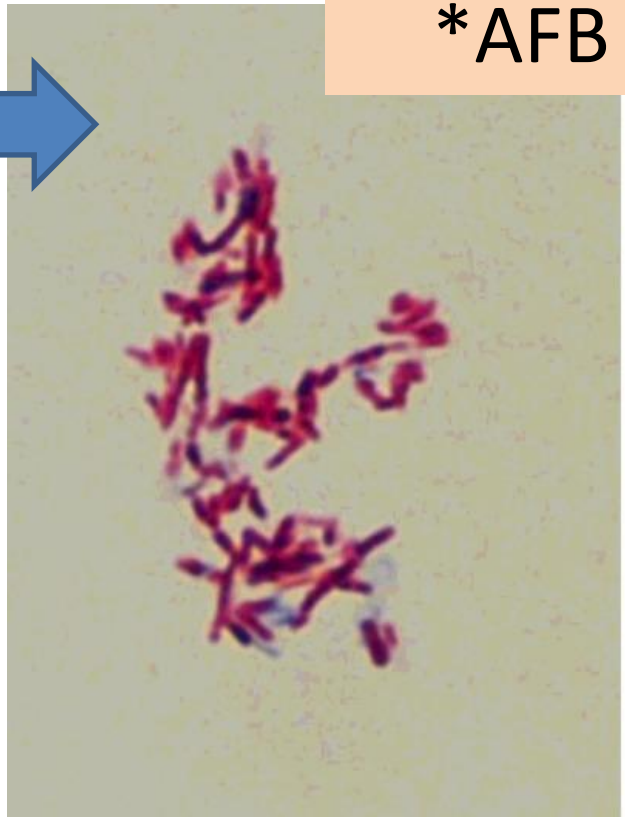
NHS Hull University Teaching Hospitals NHS Trust

Remarkable people. Extraordinary place.

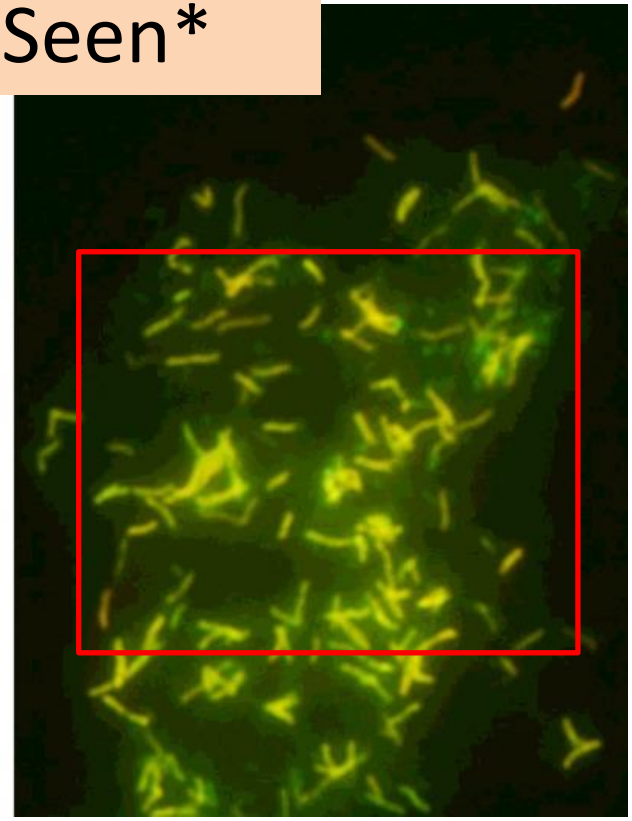
Laboratory Testing: Direct Smear Microscopy

NHS Hull University Teaching Hospitals NHS Trust





AFB Seen

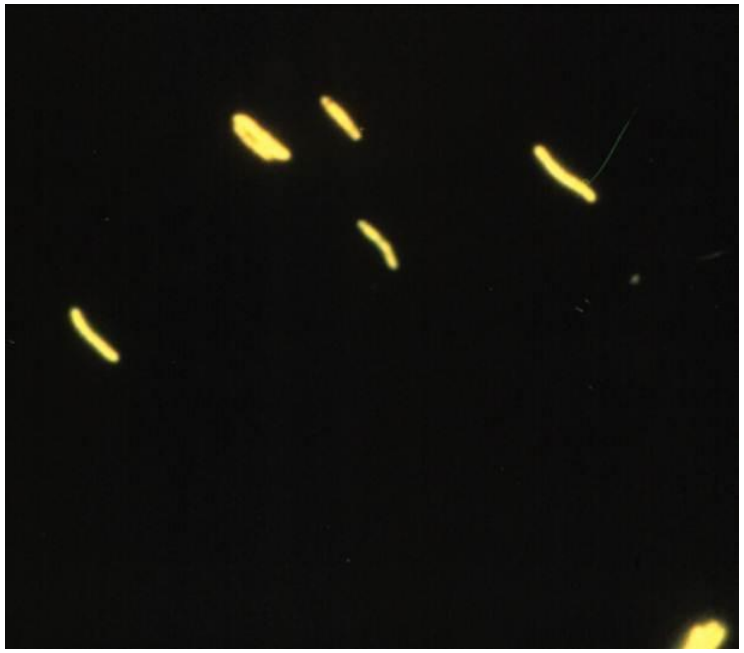


ZN Stain

PA Stain

Remarkable people. Extraordinary place.

Hull University Teaching Hospitals NHS Trust



Auramine-phenol staining is more sensitive than that by the Ziehl-Neelsen method, and is therefore more suitable for assessment of smears from clinical specimens: variable sensitivity & specificity reported across journals – UK recommended method

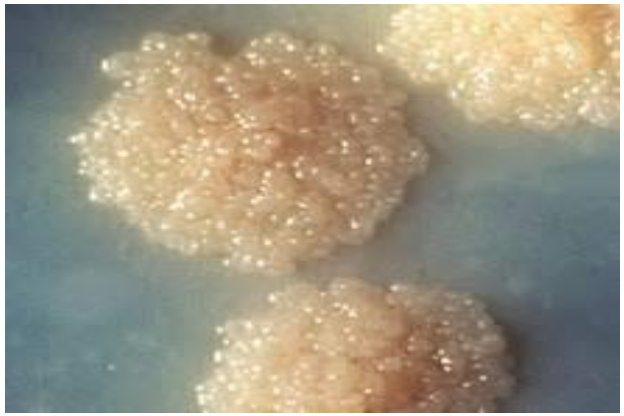
UK SMI: B 40 Investigation of specimens for Mycobacterium species (publishing.service.gov.uk)

Microscopy result should be reported within one working day of receipt of the specimen

Remarkable people. Extraordinary place.

Culture Detection

NHS Hull University Teaching Hospitals NHS Trust

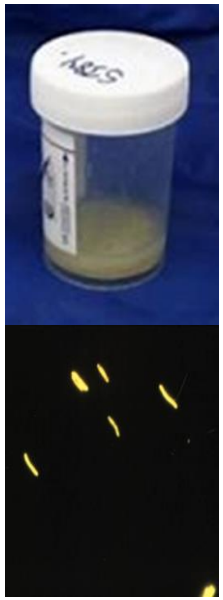


Refer to Birmingham

Remarkable people. Extraordinary place.

Molecular Detection

NHS Hull University Teaching Hospitals NHS Trust



High clinical suspicion of MTb OR PA Smear Positive

Direct Cepheid

Result: 2hrs

Direct inoculation of the cartridge, ultrasonic lysis of the organisms to release DNA

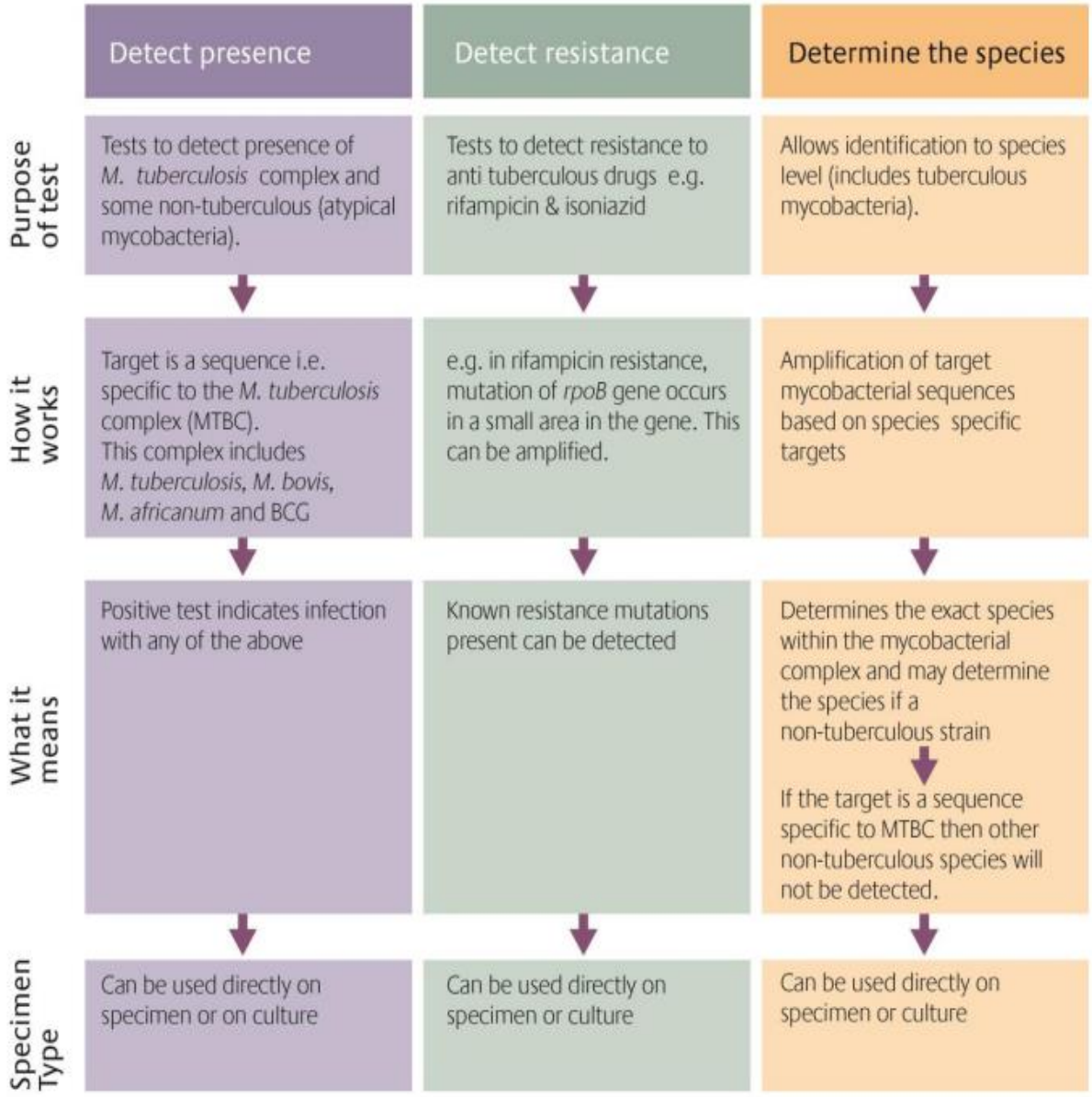
DNA mixed with dry PCR reagents

Realtime Semi-Nested PCR

Rifampicin Resistance gene *RpoB* detected

The Xpert MTB/RIF assay (Xpert), the first point-of-care assay for tuberculosis (TB), was endorsed by the World Health Organization in December 2010

Local Molecular Testing – Early classification of MTb Vs MDR MTb



Hull University Teaching Hospitals NHS Trust

PRESENCE

↓

RESISTANCE

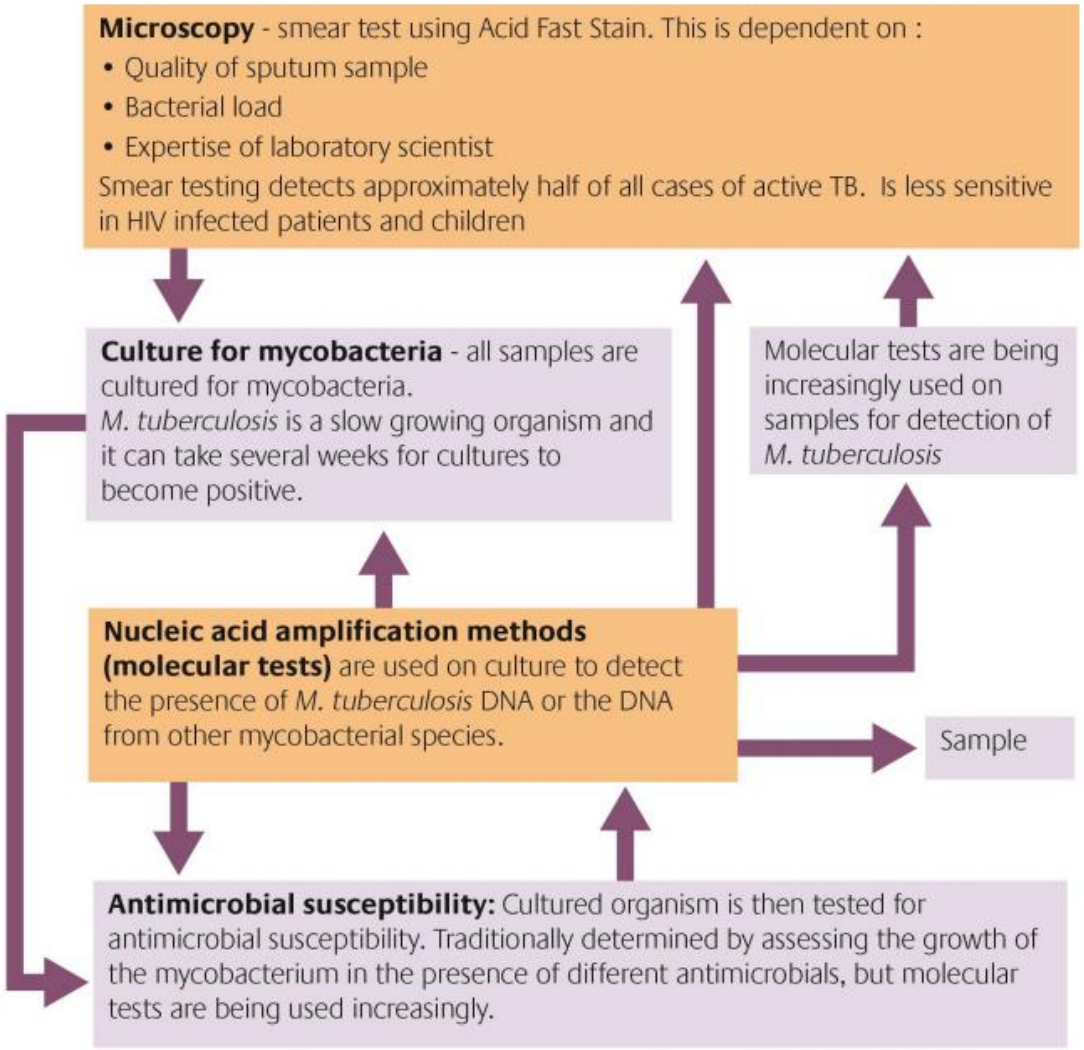
MDR TB

↓

SPECIES

Laboratory Mixed Methods Approach

Figure 2. Microbiology tests available for the diagnosis of active TB



Microscopy: Fast, Cheap & indicates bacterial load

Molecular Tests: expensive, enable MTB detection before culture

Culture is needed to be able to perform sensitivity testing & whole genome sequencing

Remarkable people. Extraordinary place.

Lab Testing Summary

NHS Hull University Teaching Hospitals NHS Trust

AFB Not Seen on Smear

AFB not seen but the CLINICAL suspicion is high: children, HIV, if the ID would alter patient care or if the need for large contact tracing is being explored

AFB Seen on Smear

Cultures Prepared:
Solid Culture
Liquid Culture

PCR Cepheid Done from direct specimen

Cultures Prepared:
Solid Culture
Liquid Culture

POS
(8-12 weeks)

NEG
(8-12 weeks)

NEG for MTB

POS

NEG
(8-12 weeks)

PCR Cepheid From the Culture**

NTM

PCR Cepheid From the Culture**

Indication that the treatment is working

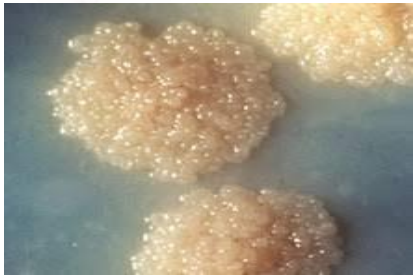
Liquid Culture sent to the ref lab for ID & SENS (isoniazid, rifampicin**, pyrazinamide, ethambutol) - Our nearest lab is Birmingham.

Remarkable people. Extraordinary place.

Susceptibility Testing

Hull University Teaching Hospitals NHS Trust

M. tuberculosis is usually detected and identified within 7 to 21 days depending upon biomass, which is also reflected in the smear result.



Drug susceptibility testing has 3 main goals:

- to facilitate the management of individual patients, particularly if drug resistance is likely
- to provide data on which to plan drug combinations for treatment
- to provide a surrogate measure of the relative effectiveness of tuberculosis control programmes

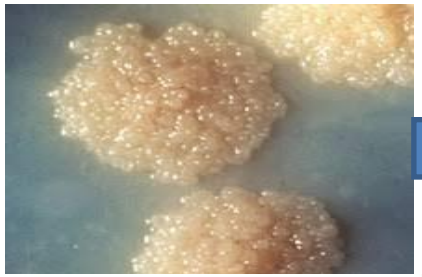
Results of tests for primary therapeutic agents should be completed within 14 days of receipt

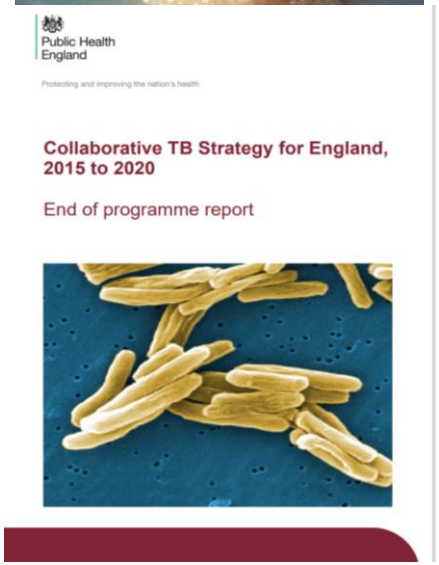
AST mostly performed by WGS
Standard AST can take up to 40 days

Remarkable people. Extraordinary place.

WGS

Hull University Teaching Hospitals NHS Trust





WGS based identification service from liquid or solid culture media provided free to the NHS approx. 7d

Drug susceptibility testing and genotypic resistance prediction for M. tuberculosis complex:

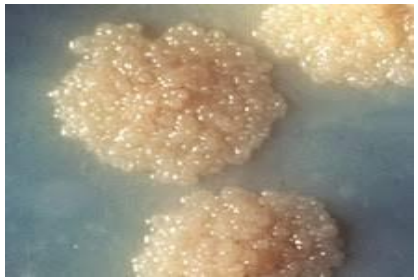
Genotypic drug susceptibility predictions are made for all isolates. Routine phenotypic susceptibility testing for first line agents (isoniazid / rifampicin / pyrazinamide / ethambutol) is **no longer performed**.
If WGS predicts resistance or if WGS data is not sufficiently clear to accurately make a prediction, phenotypic testing of first line agents will be performed. Testing for second and third line agents will be performed for multi-drug resistant isolates when clinically indicated (Early information linking XDR cases is vital)

[England world leaders in the use of whole genome sequencing to diagnose TB - GOV.UK (www.gov.uk)]

Remarkable people. Extraordinary place.

WGS

Hull University Teaching Hospitals NHS Trust



Determination of M. tuberculosis isolate relatedness – based on **SNP differences** determined by WGS, provided free to the NHS and for the support of outbreak investigations, detection of laboratory cross-contamination events

Drug	Mutation	Nucleotides	Support (A/C/G/T)	Source	Prediction
AK	rss_*1484*	G->N	(0/0/4/0)	Line-probe	F
AK	rrs_*1401*	A->N	(2/0/0/0)	Line-probe	F
CAP	rrs_*1402*	C->N	(0/3/0/0)	Line-probe	F

Drug	Mutation	Nucleotides	Support (A/C/G/T)	Source	Prediction
EMB	embB_D354A	GAC->GCC	(0/0/26/0) (1/31/0/1) (0/27/0/0)	derived-(3/5)	R
INH	katG_S315T	AGC->ACC	(41/0/0/0) (0/44/0/0) (0/43/0/0)	Line-probe/derived-(471/480)	R
KAN	rrs_C517T	C->T	(0/0/0/49)	derived-(1/6)	R
RIF	rpoB_*449*	CTG->CNG	(0/39/0/0) (3/0/0/32) (0/0/36/0)	Line-probe	F

SNPs result from mutations at a single position in the DNA sequence. Because SNPs gradually accumulate over time, the number of SNPs that differ between isolates (SNP distance) can provide information about whether the TB cases could be the result of recent transmission

BSAC-SC2018-Day2-GraceSmith.pdf

Remarkable people. Extraordinary place.

NTM

NHS
Hull University Teaching Hospitals
NHS Trust

Non-tuberculous mycobacteria (NTM) are also increasingly encountered as a cause of disease in humans.

Unlike *M. tuberculosis*, isolation of an NTM species from specimens such as sputum does not equate to disease – the microbiology results need to be interpreted in conjunction with clinical and radiological findings.

Remarkable people. Extraordinary place.

Rapid Growers

NHS Hull University Teaching Hospitals NHS Trust

- *Mycobacterium abscessus, Mycobacterium chelonae, Mycobacterium fortuitum*
- *These grow in routine culture SA bottles*

– D3 onwards

- *M. abscessus* more so than the other non-tuberculous mycobacteria are an increasing problem for the cystic fibrosis patient group. Testing should be considered in cystic fibrosis patients who show deteriorating lung function but where no clear pathogen has been identified. The Cystic Fibrosis Trust microbiology standards recommend routine screening of NTM at least once a year for all patients able to produce sputum and all *M. abscessus* positive isolates should be referred to the appropriate reference laboratory for strain typing (VNTR).

Remarkable people. Extraordinary place.

Slow Growers

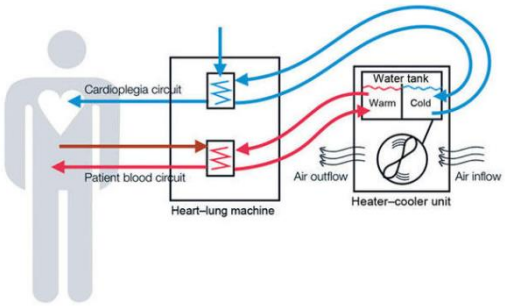
NHS Hull University Teaching Hospitals NHS Trust

***Mycobacterium avium – intracellulare* group (MAI)**

There are currently three species within the MAC and they are *M. avium, M. intracellulare and M. chimaera*. Additionally, there are now three valid named subspecies of *M. avium: M. avium* subsp. *avium; M. avium* subsp. *paratuberculosis; and M. avium* subsp. *Silvaticum.*

M. chimaera, a slow growing NTM found in the environment has been implicated recently in several cases of endocarditis or deep infection following cardiac surgery involving the use of cardiac bypass equipment.

Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium ulcerans
Mycobacterium xenopi

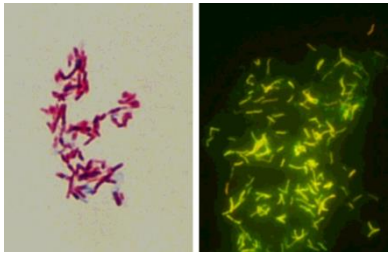


Remarkable people. Extraordinary place.

Conversion

S-H-Y-P-S

NHS Hull University Teaching Hospitals NHS Trust



Smear Positive → Smear Negative

Once treatment is initiated we repeat cultures until the patient becomes culture & smear negative

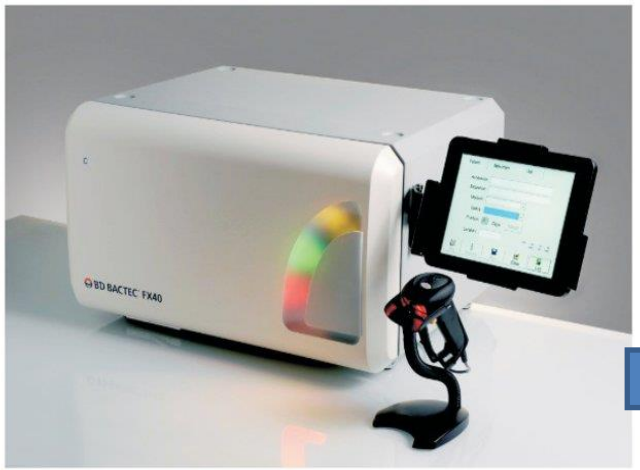
Persistent Smear positive occurs when patients have extensive cavities – this can influence length of treatment or instigate a change in treatment.

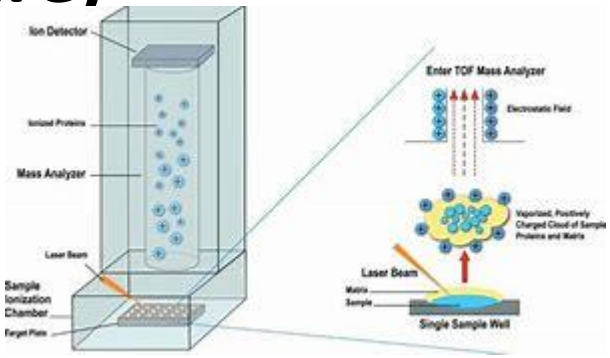
We will re-refer for WGS derived AST

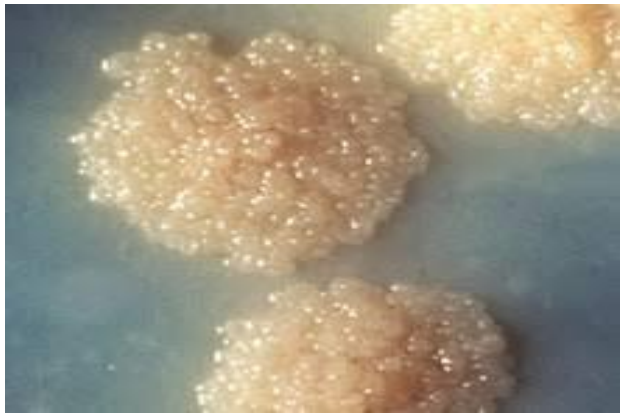
Remarkable people. Extraordinary place.

Culture Detection
MALDI-TOF (Near Future)

NHS
Hull University Teaching Hospitals
NHS Trust







Refer to Birmingham

Remarkable people. Extraordinary place.

Resources

NHS
Hull University Teaching Hospitals
NHS Trust

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/489198/Molecular_diagnosis_of_tuberculosis_for_healthcare_professionals.pdf

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/684543/B_40i7.2.pdf