Supplementary appendix

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Methods

Sample collection
All newly-positive MGIT cultures were collected and processed for this investigation, including duplicate specimens from the same patient. All specimens were collected between September 2013 and April 2014 (Table S1). No other selection criteria were imposed (except Borstel where only the second positive primary culture from MTBC patients was available for processing).

DNA extraction
Inactivated BACTEC™ MGIT™ (Beckton Dickinson, USA) aliquots (1-2 mL) were pelleted through centrifugation at 16100 rcf for 15 minutes. Supernatant was removed and the pellet re-suspended in 1 mL sterile saline before centrifugation at 16100 rcf for 15 minutes to re-pellet the aliquot, and removal of the supernatant. The pellet was then re-suspended in 700 µL of molecular grade water and the suspension mechanically disrupted in Lysing Matrix B (MP Biomedicals, USA) using the FastPrep-24 tissue homogeniser (MP Biomedicals, USA) with three cycles at 6 m/s for 40s. Following disruption the aliquot was centrifuged at 16100 rcf for 10 minutes, and 450 µL of supernatant transferred to a new 1·5 mL tube.

DNA was isolated through precipitation in the presence of 1:10 volumes of 3 M sodium acetate (45 µL) and 1:1 volumes of ice-cold ethanol (minimum 96%; 1 mL) and incubated at -20°C for one hour. The DNA was pelleted by centrifugation at 16100 rcf for 15 minutes. Supernatant was removed, and the DNA pellet washed twice with 70% ethanol before complete removal of the supernatant and air drying at room temperature for 10-15 minutes. The DNA pellet was re-suspended in 50 µL 1x Tris Ethylenediaminetetraacetic acid (TE) buffer at 55°C. 45 µL of the final supernatant was cleaned using Solid Phase Reversible Immobilisation (SPRI) beads (AMPure XP, Beckman Coulter, USA) in a volume of 1·8x beads to eluate (81 µL). Following manufacturer’s protocols the bead pellet was washed twice with 70% ethanol and dried at room temperature for 10-15 minutes. DNA was eluted from the SPRI beads in 26 µL 1x TE buffer with 25 µL transferred to a new 1·5 mL tube and stored at -20°C.

Sequencing
Samples were normalised to 0·2 ng/µL following quantitation using the Qubit dsDNA High Sensitivity kit on the Qubit 2·0 Fluorometer (LifeTechnologies, USA). Sequencing libraries were prepared for MiSeq (Illumina, USA) sequencing using the Nextera XT protocol (Illumina, USA, Part #15031942 rev. C, October 2012). Manufacturer’s instructions were followed with the following modifications: limited-cycle PCR amplification program was extended from 12 to 15 cycles, and libraries were manually normalised to 2-10 nM based on DNA concentration, or DNA concentration and average library size as measured by the Qubit dsDNA High Sensitivity kit on the Qubit 2-0 Fluorometer and the D1K High Sensitivity Screentape on the 2200 TapeStation (Agilent Technologies, USA). Libraries were pooled in equal volume, denatured according to the manufacturer’s protocols and diluted to a sequencing concentration of up to 20 pM. Finally, 12·5 pM PhiX (Illumina, USA) was added at 1% of the loading volume.

Sequence processing
Sequence processing was blinded to clinical information and routine laboratory results. Completed sequencing runs were shared via Illumina BaseSpace and data downloaded to Oxford for semi-automated analysis by a bespoke bioinformatic pipeline (Figure S2). Reads were deposited in the
Firstly, species were identified for each isolate using a gene presence/absence algorithm, developed as follows. The whole genome sequences of 169 commercially available mycobacterium type species were gathered; either through sequencing and assembly with Velvet v1.0.18 or NCBI. Using the assembled genomes, genes were annotated or predicted (Prokka v1.8) and clustered. Unique representatives of clusters were identified using cd-hit (4.5.4) and used to construct a mycobacterium pangenome. Raw reads from the 169 isolates were then mapped against the pangenome (BWA v0.7.5a) to detect genes that were present only in one species. Those genes were used to generate a catalogue of unique genes for each mycobacterial species and/or cluster. Isolates sequenced throughout this investigation were mapped (Bowtie2 v2.0.0-beta7) to this catalogue of unique genes to identify species. A minimum read-depth of 5 and coverage of 80% was required to affirm the presence of a gene.

If the sequenced isolate was identified as belonging to MTBC, it was subsequently mapped to the H37Rv (GenBank NC000962·2) reference genome with Stampy (v1.0.22; mapped files available on request). Self-self BLAST was used to define repetitive regions, which were masked. Nucleotide calls were made with SAMtools mpileup (v0.1.08) and required a minimum depth of five reads with at least one read in each direction. Median read-depth, based on read length and number of reads mapping to the reference genome, was 73, IQR 36-99. Mapped read depth was assessed using bedtools v2.16.1 and the genomecov option, used to analyse the bam files generated by through mapping (Figure S3). Each mapped MTBC complex isolate was examined for mutations known to confer a resistant phenotype (Table S2). A minimum sequencing depth of five base calls was required for phenotype to be predicted based on a specific resistance-conferring variant being identified; where minority variant mutations were present with a depth of ≥5 and comprising ≥10% of the total base calls no single base was called and a mixed phenotype was predicted.

Genomic matches were identified using a rapid nearest neighbour finding algorithm. A maximum likelihood tree (R v3.1.2 with ape v3.2) was created from a set of 2191 previously sequenced isolates and the single nucleotide polymorphism (SNP) differences between adjacent nodes of the tree were stored in a database. Previously sequenced isolates were collected and sequenced as part of separate investigations from the Regional Mycobacterial Reference Laboratory, Birmingham, between 1996 and 2012 and Oxford University Hospitals between 2007 and 2012. Also included were previously published sequences from Gardy et al (2011) with a minority of sequences obtained from other available European samples processed between 2011-2013. Newly sequenced isolates could then be queried against the database in real-time, with the algorithm reporting all matches within 20 SNPs of the queried isolate or the single closest match if all differences were >20 SNPs. Isolates within or equal to five SNPs were considered compatible with recent direct or indirect transmission, given within host evolution and observed genetic differences within known household outbreaks, with isolates 6-12 SNPs distant possibly compatible with transmission. The SNP differences between each queried isolate and the adjacent node were stored in a ‘bucket’ at the node, allowing new isolates to be iteratively added to the database. This system avoided the high computational cost of tree reconstruction with the addition of each new isolate. Subclades of the
tree would need to be recalculated when buckets became overpopulated, but because the number of isolates included in this study was relatively small, no subclade or tree reconstruction was required during the course of this investigation. (Identifying when a subclade or tree would need to be reconstructed is an important question for future work.) All isolates reported as nearest neighbours were compared to the queried isolate by pairwise alignment and maximum-likelihood trees (PhyML v3·0) constructed from concatenated variable sites.

**Analysis**

Following the full WGS report being issued, data from routine and reference laboratory processing of the same isolate were gathered by local laboratory staff and returned to Public Health England (PHE) Oxford for analysis. All data were fully anonymised.

Data collected from routine and reference laboratories included: the date of sample collection from the patient, or when the sample was received by the routine laboratory; whether the sample was a duplicate (from the same patient) of a previously processed sample; the date that the MGIT became positive; the date that an aliquot of the MGIT was sent to the reference laboratory; the date on which species information was obtained from the reference and/or local laboratory; reference and/or local laboratory species result for the isolate; and for isolates identified as MTBC by the routine laboratory, the date on which drug sensitivity profiles were obtained from the reference laboratory; the drugs tested by the reference laboratory; the drug sensitivity profile of the isolate; the date on which MIRU-VNTR data were obtained from the reference laboratory; and the MIRU-VNTR profile of the isolate.

Data collected throughout WGS processing and analysis included: the date of sample extraction; the date WGS was performed; the date WGS data were shared via BaseSpace; the date of species identification; the date of drug sensitivity prediction; the date of nearest neighbour matching; and the date a full WGS report was returned to the participating centre.

Data were compiled at the John Radcliffe Hospital, Oxford, UK, and analysed in Stata 13·1 (StataCorp, USA). Routine species identification and drug sensitivity profiles were treated as the reference standard for comparison to WGS results; discrepancies were re-tested using routine methods where possible but isolates were not re-sequenced. As specimens were anonymised, primary analysis including comparisons with species and resistance results from routine laboratories was performed for all specimens. Identification of duplicate MTBC specimens was performed by each participating centre, and data regarding the duplication of non-MTBC specimens were not collected. MTBC de-duplication was performed by selecting the first sample taken from each patient (where multiple samples taken from the same patient), or by selecting the second sample where WGS had been re-performed due to sequencing technical failure. Both the full dataset (all specimens) and de-duplicated data set were utilised for outbreak analysis and statistical analysis of drug-resistance. The time for each stage of the diagnostic workflow to complete was compared across routine and WGS processing, excluding isolates where routine processing was not performed in full (for example where, clinical diagnosis was performed based on previously processed isolates from the same patient, as identified by participating centres). Multivariable fractional polynomial logistic regression was performed to identify which quality control measures contributed to WGS sequence processing failure (Stata mfp; exit p=0·05).
**Costs**

A micro-costing questionnaire based on standard operating procedures and diagnostic algorithms was completed by a regional reference laboratory (the Regional Centre for Mycobacteriology, Birmingham, UK), and a local clinical laboratory performing WGS (Oxford, UK). The local laboratory did not perform the full routine mycobacterial workflow. Other participating centres in the UK declined to participate. Questionnaires were completed by clinical scientists and financial managers. Accuracy of the questionnaire, clinical and WGS workflows, and the associated costs were ensured through expert consultations and interview with clinical scientists performing the tests, and financial managers. The questionnaire gathered bottom-up costs for the full procedure from the clinical sample being received by the routine laboratory to data interpretation and reporting; this included consumables, hardware (computing and laboratory equipment; initial cost, maintenance and proportion of time used for MTBC diagnostics), staff time, staff training time, annual staff turnover, equipment calibration, service contracts, and reported error rates (Table S3). For second-line drug phenotyping, only costs based on staff time, consumables and equipment were gathered from the National Mycobacterial Reference Laboratory, London, UK, via interview with clinical scientists. Throughput, used to annualise costs of both diagnostic workflows, was based on reported sample numbers in Birmingham for 2014.

Routine processing of clinical samples was based on procedures at Birmingham, and included sample receipt, MGIT culture, Cepheid Xpert MTB/RIF assay, species identification for MGIT cultures using either the Hain GenoType MTBC or Hain GenoType Mycobacterium CM/AS assays, MIRU-VNTR, phenotyping for first-line drug susceptibility testing (DST) (MGIT culture), Hain MTBDRplus for first-line DST, secondary phenotyping for first-line DST (Lowenstein-Jensen (LJ) media), Hain MTBDRsl for second line DST and sending off drug-resistant isolates for full second-line phenotyping at an external laboratory.

Staff salaries published by the NHS Agenda for Change were taken from the year 2014. Hardware costs were attached to equipment from laboratory price listings (Table S3). As per guidance of the National Institute for Health and Care Excellence, cost figures do not include VAT. For capital items the cost was spread over the item’s predicted lifetime and depreciated using equivalent annual costing with a discount rate of 3.5%. Of the total costs, a 20% rate was added for overheads including items such as general hospital administration, cleaning and electricity. In addition, a rate of 20% for national insurance and superannuation was included in the analysis and it was assumed that staff work 37.5 hours per week and 46 weeks per year.

A sensitivity analysis was performed to assess how pricing changes would affect the cost of overheads, WGS equipment and WGS major consumables. Alterations in the annual throughput of specimens and the specimen batch size of WGS were included, alongside changes in the staff grade performing laboratory work, error rates, and the lifetime of routine and WGS laboratory equipment (Tables S9 and S10).
**Figure S1:** Flow diagram of suspected Mycobacterium sample through the current diagnostic pathway and the WGS diagnostic pathway used during this investigation. Timings for current diagnostic pathway are typical for routine and Public Health England (PHE) reference laboratories in the UK and may vary. Ziehl-Neelsen (ZN) positive samples typically become BACTEC Mycobacterial Growth Indicator Tube (MGIT) positive 7-10 days from inoculation. ZN negative samples can become BACTEC MGIT positive up to 35 days from inoculation. See Figure S2 for detail of data analysis. Green shading = routine and reference laboratory processing; blue shading = WGS processing; purple shading = reporting.
Figure S2: Flow diagram of WGS data through processing pipeline. Blue shading = WGS processing; purple shading = reporting; no shading = manual processing steps. QC = Quality control.
Figure S3: Histogram of the mapped read depth of MTBC samples found during this investigation.

Note: One observation per reference genome site per mapped sample.
Figure S4: Example of prototype quality checking report completed and returned to sequencing centres for each isolate.

QC Report

| Sample Details |     |
|----------------|-----|
| Sequencing Location | Oxford |
| Local LIMS Specimen ID | M5979 |
| GUID | N/A |

| Sequencing run Statistics |     |
|----------------------------|-----|
| GC Content | 65% |
| Median Insert Size | 302 |
| Reads For Mapping | 4091106 |
| Reads Mapped to TB Reference | 3995924 |
| Percentage Mapped to TB Reference | 97.7% |
| Coverage of TB Reference | 91.7% |
| Percentage Mapped to Human Genome | 0% |
| QC comment |     |
Figure S5: Example of prototype sequencing report completed and returned to sequencing centres for each isolate. GUID = Global Unique Identifier; ‘Ambiguous’ resistotype = mixed phenotype prediction.

### Whole Genome Sequencing Report from MGIT Positive Samples

Not for diagnostic use

| Sample Details                  |          |          |          |
|--------------------------------|----------|----------|----------|
| **Sequencing Location**        | Oxford   |          | 4th Nov 2013 |
| **Local UMS Specimen ID**      | M5979    | Run Date | 6th Dec 2013 |
| **GUID**                       | N/A      |          |          |

| Organism Identification        |          |
|--------------------------------|----------|
| **Mycobacterium tuberculosis** |          |

| Resistotype                  | Prediction | HAIN Mutation | Extended Catalogue | Ambiguous |
|------------------------------|------------|---------------|--------------------|-----------|
| **Isoniazid**                | Sensitive  |               |                    |           |
| **Rifampicin**               | Sensitive  |               |                    |           |
| **Ethambutol**               | Sensitive  |               |                    |           |
| **Pyrazinamide**             | Sensitive  |               |                    |           |
| **Streptomycin**             | Sensitive  |               |                    |           |
| **Moxifloxacin**             | Sensitive  |               |                    |           |
| **Amikacin**                 | Sensitive  |               |                    |           |

| Relatedness                  |          |          |
|------------------------------|----------|----------|
| **Nearest neighbour(s)**     |          |          |
| **GUID**                     | No. of SNPs Apart | Centre |
| C0002321033                  | 2         | Oxford   |
| C00023214 (M8076)            | 0         | Oxford   |
| C00023211 (M5992)            | 0         | Oxford   |

| Authorised                   |          |          |
|------------------------------|----------|----------|
| **Signature:**               |          |          |
| **Position:**                |          | Date: 12th December 2013 |
| **Print name:**              | Timothy Walker |          |
Figure S6: Total number of sequencing reads generated per sample; samples categorised according to WGS result using routine laboratory results as reference standard. Successful WGS species identification defined as complete concordance, loss/gain of NTM in co-infection, identification of subspecies. Failed WGS species identification defined as no species identified, loss of MTBC and discordance. The total number or reads were truncated at $7 \times 10^6$. Inverse association between total number of reads and failure (power -0.5); $p=0.005$. *WGS & routine lab failed: WGS failed owing to low read numbers and/or high levels of contamination with non-mycobacterial DNA; no mycobacterial genes found by species presence/absence algorithm.
**Figure S7:** The percentage GC content of each sample; samples categorised according to WGS result using routine laboratory results as reference standard. Successful WGS species identification defined as complete concordance, loss/gain of NTM in co-infection, identification of subspecies. Failed WGS species identification defined as no species identified, loss of MTBC and discordance. Inverse association between GC content and failure (power 1); p<0.001. *WGS & routine lab failed: WGS failed owing to low read numbers and/or high levels of contamination with non-mycobacterial DNA; no mycobacterial genes found by species presence/absence algorithm.*
**Figure S8:** The predicted probability of WGS failing to identify or incorrectly identifying species depended independently on the number or reads available for mapping (p=0.005) and GC content (p<0.001) based on a multivariable fractional polynomial logistic regression model. This allows the effect of each predictor to vary non-linearly, i.e. for each unit increase in the predictor to have a different impact on the odds of success across the range of values taken by the predictor. Area under the receiver-operating curve =0.90.
**Figure S9:** Workflow at Regional Centre for Mycobacteriology (Birmingham, UK) used for costing analysis with costs for each diagnostic step and cumulative costs of diagnosis by both routine methods, and WGS if WGS was to replace all routine diagnostic steps, or species identification and MIRU-VNTR only. *Performed at regional centre. ‡Performed at National Centre for Mycobacteriology (London, UK)*

**Workflow**

| Step | Details |
|------|---------|
| Sample reception and entry into database | N = 15,265 |
| Auramine microscopy, preparation of specimen and inoculation into MGIT |
| Positive growth | N = 2,027 |
| ZN microscopy |
| Appearance typical of MTBC |
| Appearance atypical of MTBC |
| DNA extraction for Hain testing |
| Hain GenoType MTBC | Positive | Identified as MTBC | N = 606 |
| | Negative | Identified as NTM | N = 1,341 |
| Hain GenoType CM | Positive | Identified as MTBC |
| | Negative | Identified as NTM |
| Hain GenoType A5 | Positive |
| | Negative |

**Cost**

| Cost scenarios (per specimen) |
|-------------------------------|
| Culture (culture negative samples) | £52.35 |
| Cepheid Xpert MTB/RIF | £99.66 |
| Whole Genome Sequencing | £118.55 |
| Culture + WGS (NTM) | £170.94 |
| Culture + WGS + First-line DST (MTC) | £306.41 |
| Culture + WGS + First-line DST + second-line DST (drug-resistant MTC) | £500.68 |

**Species identification**

| Identification (overall) | £55.05 |
| -------------------------|-------|
| Cost of individual Hain assays: |
| MTBC £9.54 per specimen |
| CM £9.75 per specimen |
| AS £28.89 per specimen |

**MIRU-VNTR**

| MIRU-VNTR |
|-----------|
| £107.75 per specimen |

| DST scenarios (per specimen) |
|------------------------------|
| 1st-line DST |
| £135.47 per specimen |
| (MGIT phenotyping for: pyrazinamide, isoniazid, rifampicin, ethambutol and a control tube; plus Hain MTBDRplus (£27.30) for resistant isolates) |
| Partial 2nd-line DST* |
| £93.00 per specimen |
| (LJ slope phenotyping for: pyrazinamide, isoniazid, rifampicin, ethambutol and a control slope; plus Hain MTBDRsk (£34.77) and postage) |
| 2nd-line DST† |
| £101.27 per specimen |
| (MGIT phenotyping for first-line drugs plus 7 second-line drugs and a control MGIT) |

*Performed at regional centre. ‡Performed at National Centre for Mycobacteriology (London, UK)
Figure S10: Percentage of total cost split by cost category
Table S1: Sample collection at each participating centre; dates of MGIT collection and summary of routine laboratory species identification results.

| Participating centre                               | MGIT collection           | Routine laboratory species identification (N) |  |
|---------------------------------------------------|---------------------------|----------------------------------------------|---|
|                                                   | Start date                | End date                                     | Mycobacterium tuberculosis complex | Non-tuberculous mycobacteria | Co-infection (MTBC+NTM) | Failed to identify | Total |
| John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford (UK) | 10th October 2013          | 22nd February 2014                           | 33                                      | 59                          | 1                         | 5                  | 98    |
| Leeds Teaching Hospitals NHS Trusts (UK)          | 11th October 2013          | 25th January 2014                            | 42                                      | 41                          | 0                         | 4                  | 87    |
| Brighton and Sussex University Hospitals NHS Trust (UK) | 6th September 2013         | 8th January 2014                             | 10                                      | 27                          | 0                         | 1                  | 38    |
| Birmingham Heartlands Hospital NHS Foundation Trust (UK) | 31st January 2014          | 14th April 2014                              | 32                                      | 19                          | 0                         | 1                  | 52    |
| St James’ Hospital, Dublin (Ireland)              | 2nd October 2013           | 9th March 2014                               | 13                                      | 9                           | 1                         | 0                  | 23    |
| Forschungs Institute, Borstel (Germany)           | 3rd February 2014          | 14th April 2014                              | 34                                      | 0                           | 0                         | 0                  | 34    |
| University Hospital Lille (France)                | 25th December 2013         | 28th February 2014                           | 6                                       | 6                           | 1                         | 0                  | 13    |
| British Columbia Centre for Disease Control, Vancouver (Canada) | 27th November 2013         | 6th December 2013                            | 5                                       | 5                           | 1                         | 0                  | 11    |
Table S2: Catalogue of mutations examined for phenotypic resistance prediction. Mutations selected from Hain line probe assays and in-house catalogues based on literature searches, as previously described.6

| Drug      | Catalogue | Mutation | Mutation |
|-----------|-----------|----------|----------|
| Isoniazid | Hain      | fabG1_-15* | fabG1_-16* |
|           |           | fabG1_-8* | katG_-315* |
|           | Extended catalogue | ahpC_C-39T | ahpC_G-46A |
|           |           | katG_G279D | katG_T180K |
|           |           | katG_T302R | katG_V473F |
|           |           | katG_Y300C |          |
| Rifampicin| Hain      | rpoB_-425* | rpoB_-426* |
|           |           | rpoB_-427* | rpoB_-428* |
|           |           | rpoB_-429* | rpoB_-430* |
|           |           | rpoB_-431* | rpoB_-432* |
|           |           | rpoB_-433* | rpoB_-434* |
|           |           | rpoB_-435* | rpoB_-436* |
|           |           | rpoB_-437* | rpoB_-438* |
|           |           | rpoB_-439* | rpoB_-440* |
|           |           | rpoB_-441* | rpoB_-442* |
|           |           | rpoB_-443* | rpoB_-444* |
|           |           | rpoB_-445* | rpoB_-446* |
|           |           | rpoB_-447* | rpoB_-448* |
|           |           | rpoB_-449* | rpoB_-450* |
|           |           | rpoB_-451* | rpoB_-452* |
| Ethambutol| Hain      | embB_-306* |          |
|           | Extended catalogue | embB_G406D |          |
| Pyrazinamide | Extended catalogue |
|--------------|--------------------|
| embB_G406S   | pncA_A-11G         |
| embB_Q1002R  | pncA_A102V         |
| embB_Q497R   | pncA_A134V         |
| embB_T506N   | pncA_A161P         |
| embB_W332R   | pncA_A171E         |
|              | pncA_A46V          |
|              | pncA_C138R         |
|              | pncA_C138Y         |
|              | pncA_C14R          |
|              | pncA_C72R          |
|              | pncA_D12A          |
|              | pncA_D63G          |
|              | pncA_D8G           |
|              | pncA_G162D         |
|              | pncA_G17D          |
|              | pncA_G78C          |
|              | pncA_G97D          |
|              | pncA_G97S          |
|              | pncA_H137R         |
|              | pncA_H51P          |
|              | pncA_H57D          |
|              | pncA_H57Y          |
|              | pncA_H71R          |
|              | pncA_H82R          |
|              | pncA_K96N          |
|              | pncA_K96T          |
|              | pncA_L116R         |
|              | pncA_L159P         |
|              | pncA_L172E         |
|              | pncA_L172P         |
|              | pncA_L19P          |
|              | pncA_L27P          |
|              | pncA_L35R          |
|              | pncA_L4S           |
|              | pncA_L85P          |
|              | pncA_P54T          |
|              | pncA_Q10P          |
|              | pncA_Q141P         |
|              | pncA_R121P         |
| Streptomycin | Extended catalogue |
|-------------|-------------------|
|             | gid_A138V         |
|             | gid_A200E         |
|             | gid_A200E         |
|             | gid_G34A          |
|             | gid_G71R          |
|             | gid_H48N          |
|             | gid_L91P          |
|             | gid_S100F         |
|             | gid_V65G          |
|             | gid_V88A          |
|             | rpsL_K43R         |
|             | rpsL_K88Q         |
|             | rpsL_K88R         |
|             | rrs_A514C         |

| Ciprofloxacin, Ofloxacin and Moxifloxacin (Fluroquinolones) | Hain |
|---------------------------------------------------------------|------|
|                                                              |      |
|                                                              | gyra_*85* |
|                                                              | gyra_*86* |
|                                                              | gyra_*87* |
|                                                              | gyra_*88* |
|                                                              | gyra_*89* |
|                                                              | gyra_*90* |
|                                                              | gyra_*91* |
|                                                              | gyra_*92* |
|                                                              | gyra_*93* |
|                                                              | gyra_*94* |
|                                                              | gyra_*96* |
| Extended catalogue | gyrA_*97* |
|--------------------|-----------|
|                    | gyrA_A74S |
|                    | gyrA_P102H |
| Amikacin, Capreomycin, Kanamycin (Aminoglycosides) | Hain |
|                    | rrs_*1401* |
|                    | rrs_*1402* |
|                    | rrs_*1484* |
| Extended catalogue | eis_G-10A |
Table S3: Input parameters for costing estimates. *Items over £10,000

| Equipment*                                                                 | Total cost (GBP) | Annual maintenance (GBP) | Lifetime (Years) |
|--------------------------------------------------------------------------|------------------|--------------------------|------------------|
| MiSeq (Illumina, USA)                                                    | 83,282           | 9750                     | 10               |
| Tapestation 2200 (Agilent, USA)                                          | 12,000           | 0                        | 5                |
| Fluorescent microscope                                                   | 11,914           | 0                        | 10               |
| GeneXpert (Cepheid, USA)                                                 | 44,734           | 6576                     | 10               |
| QIAgility (Qiagen, USA)                                                  | 26,000           | 0                        | 10               |
| WAVE System (Transgenomic, USA)                                          | 31,000           | 6593                     | 10               |
| MGIT 960 (Beckton Dickinson, USA)                                         | 25,000           | 2974                     | 10               |
| BeeBlot (Bee Robotics, UK)                                               | 18,500           | 0                        | 10               |

Key variables

| Variable                                                   | Value        |
|------------------------------------------------------------|--------------|
| National Insurance/Superannuation Multiplier               | 1·2          |
| Weeks worked per year                                     | 46           |
| Hours worked per week                                     | 37·5         |
| Overheads (%)                                              | 20           |
| Discount rate (%)                                          | 3·5          |

| Error rates                                               | %            |
|-----------------------------------------------------------|--------------|
| Microscopy                                                | 1            |
| MGIT culture                                              | 2            |
| Cepheid Xpert MTB/RIF                                     | 10           |
| Species identification (Hain ID)                          | 0·05         |
| DNA extraction for Whole Genome Sequencing                | 13           |
| Whole Genome Sequencing                                   | 4            |
| Whole Genome Sequencing data analysis                     | 1·4          |
| MIRU-VNTR                                                  | 10           |
| Drug susceptibility testing                               | 0·05         |
| Initial routine identification | WGS identification | WGS: Unique genes available | WGS: Unique genes identified | Re-testing performed | Re-testing result | Summary |
|-------------------------------|---------------------|-----------------------------|-----------------------------|---------------------|-----------------|---------|
| M. tuberculosis complex & M. avium complex | M. tuberculosis complex & M. avium complex | 100 & 33 | 99 & 29 | No | N/A | Unable to re-test WGS isolate. |
| M. avium complex | M. tuberculosis complex & M. avium complex | 100 & 33 | 4 & 33 | Yes. Re-sequenced new library preparation | M. avium complex | Re-testing supports routine identification. Potential contamination during original library preparation. |
| M. avium complex | M. tuberculosis complex & M. avium complex | 100 & 33 | 84 & 33 | Yes. Hain testing of WGS DNA extraction | M. tuberculosis complex & M. avium complex | Re-testing supports WGS identification. Potential contamination during original sample extraction. |
| M. avium complex | M. tuberculosis complex & M. avium complex | 100 & 33 | 34 & 33 | Yes. Re-sequenced new library preparation | M. avium complex | Re-testing supports routine identification. Potential contamination during original library preparation. |
| M. abscessus complex | M. abscessus complex & M. avium complex | 40 & 33 | 40 & 32 | Yes. Hain testing of WGS DNA extraction | M. abscessus complex | Re-testing supports routine identification. Potential contamination during original sample preparation. |
| M. tuberculosis complex & M. avium complex | M. tuberculosis complex | 100 | 97 | Yes. Hain testing of WGS DNA extraction | M. tuberculosis complex | Re-testing supports WGS identification. Co-infection was confirmed in patient; suggesting M. avium in extract used for WGS was below detection limits. |
| M. tuberculosis complex & M. avium complex | M. avium complex | 33 | 2 | No | N/A | Patient with confirmed co-infection; poor quality sequencing (88% human DNA) |
| M. fortuitum-acetamidolyticum | M. avium complex | 33 | 24 | No | N/A | Unable to re-test WGS isolate. |
| Undescribed mycobacterial species | M. avium complex | 33 | 33 | Yes (automated scanning of Hain and correspondence with Hain Lifescience) | Hain invalid | Initially identified as M. avium complex/M. malmoense co-infection, re-examination of the Hain test result and correspondence with Hain Lifescience led to identification of an undescribed mycobacterium bearing close relation to M. avium |
| M. fortuitum | M. fortuitum-acetamidolyticum | 100 | 33 | No | N/A | Poor quality sequencing, with only 33% M. fortuitum-acetamidolyticum genes identified by WGS. |
| Routine identification | WGS identification | WGS: Unique genes available | WGS: Unique genes identified | Re-testing performed | Re-testing result | Summary |
|-------------------------|--------------------|-----------------------------|-----------------------------|---------------------|-----------------|---------|
| *M. kansasii*           | *M. avium* complex | 33                          | 33                          | Yes                 | *M. avium* complex | Routine identification based on testing of previous isolate from this patient. Patient confirmed to be infected with *M. avium* complex after testing of same isolate used for WGS. |
| *M. avium* complex     | *M. scrofulaceum*  | 72                          | 1                           | No                  | N/A             | Poor quality sequencing data (500,000 reads available for analysis) |
| *M. tuberculosis* complex | *M. abscessus* complex | 40                          | 38                          | No                  | N/A             | Unable to re-test WGS isolate. |
Table S6: Drug-resistance predicted by WGS and reference laboratory DST by MTBC specimen (N=168). S = Sensitive; R = Resistant; F = Failed prediction; M = Mixed reads with both wild-type and resistance-conferring mutation; . = Not tested.

| Sample ID    | Isoniazid | Rifampicin | Ethambutol | Pyrazinamide | Streptomycin | Fluoroquinolones | Aminoglycosides | Isoniazid | Rifampicin | Ethambutol | Pyrazinamide | Streptomycin | Fluoroquinolones | Aminoglycosides |
|--------------|-----------|------------|------------|--------------|--------------|------------------|-----------------|-----------|------------|------------|--------------|--------------|------------------|------------------|
| 140602981    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602172    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602480    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602321    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602881    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602189    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602173    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602632    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602055    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140601636    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602933    | F         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | F               |
| 140602863    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603011    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140600095    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603272    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603568    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602707    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603170    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603683    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603704    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603310    | R         | R          | S          | S            | R            | S                | S               | S         | S          | M          | S            | R            | S                | S               |
| 140603751    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140604083    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603858    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602314    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603921    | R         | S          | R          | R            | R            | M                | S               | R         | S          | S          | S            | R            | S                | S               |
| 140604540    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | M               |
| 140604530    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140602969    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 140603481    | R         | R          | R          | S            | R            | R                | R               | R         | R          | R          | R            | R            | R                | R               |
| 140600470    | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 566-14       | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 307-14       | S         | S          | S          | S            | S            | S                | S               | S         | S          | R          | S            | S            | S                | S               |
| 570-14       | S         | S          | S          | S            | R            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 965-14       | S         | S          | S          | S            | S            | S                | S               | S         | S          | R          | S            | S            | S                | S               |
| 966-14       | S         | S          | S          | S            | S            | S                | S               | S         | S          | R          | S            | S            | S                | S               |
| 222-14       | S         | S          | S          | S            | S            | S                | S               | S         | S          | R          | S            | S            | S                | S               |
| 2202-14      | S         | S          | S          | S            | S            | S                | S               | S         | S          | R          | S            | S            | S                | S               |
| 2238-14      | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 1710-14      | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 2385-14      | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
| 2234-14      | S         | S          | S          | S            | S            | S                | S               | S         | M          | S          | S            | S            | S                | S               |
| 2223-14      | S         | S          | S          | S            | S            | S                | S               | S         | S          | S          | S            | S            | S                | S               |
1964-14 S S S S . . . S F S S S S S S
2602-14 S S S S . . . S S S S S S S S
2536-14 S S S S . . . S S S S S S S S
2525-14 R S S S . S S R S S S S S S
2714-14 R S S S . S S R S S S S S S
2740-14 S S S S . . . S S S S S S S S
2535-14 S S S S . . . S S S S S S S S
2741-14 S S S S . . . S S S S S S S S
2928-14 S S S S . . . S S S S S S S S
2985-14 S S S S . . . S S S S S S S S
2921-14 S S S S . . . S S S S S S S S
2880-14 S S S S . . . S F S S S S S S
2970-14 S S S S . . . S S S S S S S S
2984-14 S S S S . . . S S S S S S S S
2713-14 R S S S . S S R S S S S S S
2929-14 S S S S . . . S S S S S S S S
3533-14 S S S S . . . S S S S S S S S
3230-14 S S S S . . . S S S S S S S S
3271-14 S S S S . . . S S S S S S S S
3231-14 S S S S . . . S S S S S S S S
13U178650 S S S S S S . . . S S S S S S S S
13U179862 S S S S S S . . . S S S S S S S S
13U175523 S S S R . . . S S S R S S S S
13U180137 R S S S . . . R S S S S S S S S
13U187526 R S S S . . . R S S S S S S S S
13U190202_E2 S S S S . . . S S S S S S S S
13U180134_E2 R S S S . . . S S R S S S S S S
IMRL1 S S S S S S . . . S S S S S S S M
IMRL3 S S S S S S . . . S S S S S S S S
IMRL4 S S S S S S . . . S S S S S S S S
IMRL5 S S S S S S . . . S M S S S S S S
IMRL7 S S S S S . . . S S S S S S S S
IMRL8 S S S S S . . . S S S S S S S R
IMRL9 S S S S S . . . S S S S S S S M
IMRL10 S S S S S . . . S S S S S S S S
IMRL11 S S S S S . . . S S S S S S S S
IMRL14 S S S S S . . . S S S S S S S F
IMRL15 R R S S R S S . R R R S S S S
IMRL16 R S S R R S . . . S S S R S S S S
IMRL24 R S S S S . . . R S S S S S S M
7626433 S S S S . . . S S S S S S S S
7626690 S S S S . . . S S S S S S S S
7626487 S S S S . . . S S S S S S S S
7625776 S S S S . . . F F F F F F F F
7626555 S S S S . . . S S S S S S S S
7626713 S S S S . . . S S S S S S S S
7626878 S S S S . . . S S S S S S S S
7626930 R R R S R S R S R R R S S S S
7626418.s2 S S S S . . . S S S S S S S S
7626586.s2 S S S S . . . F F F F F F F F
7626944.s2 S S S S . . . F F F F F F F F
7626833.s2 S S S S . . . S S S S S S S S
7626987.s2 S S S S . . . F F F F F F F F
7626571 S S S S . . . S S S S S S S S
| 7626675 | R | R | R | S | R |
| 7626694 | S | S | S | S | . . . |
| 7626725 | S | S | S | S | . . . |
| 7627124 | S | S | S | S | . . . |
| 7627311 | S | S | S | S | . . . |
| 7627400 | S | S | S | S | . . . |
| 7627652 | S | S | S | S | . . . |
| 7627716 | S | S | S | S | . . . |
| 7627226 | R | R | R | S | R |
| 7627572 | S | S | S | S | . . . |
| 7627746 | S | S | S | S | . . . |
| 7627886 | S | S | S | S | . . . |
| 7627900 | S | S | S | S | . . . |
| 7628147 | S | S | S | S | . . . |
| 7628143 | S | S | S | S | . . . |
| 7628121 | S | S | S | S | . . . |
| 7620009 | S | S | S | S | . . . |
| 7620018 | S | S | S | S | . . . |
| 7620179 | S | S | S | S | . . . |
| 7620215 | R | S | S | S | . . . |
| 7627873 | S | S | S | S | . . . |
| 7620149 | S | S | S | S | . . . |
| 7620040 | S | S | S | S | . . . |
| 7620602 | S | S | S | S | . . . |
| 762071 | S | S | S | S | . . . |
| 762073 | S | S | S | S | . . . |
| 555710 | F | F | F | F | . . . |
| Li66 | S | S | S | S | . . . |
| Li68 | S | S | S | S | . . . |
| W35519 | S | S | S | S | . . . |
| H27078 | R | S | S | S | . . . |
| M3785 | S | S | S | S | . . . |
| L42182 | S | S | S | S | . . . |
| L42183 | S | S | S | S | . . . |
| L44277 | S | S | S | S | . . . |
| F24817 | S | S | S | S | . . . |
| H29889 | S | S | S | S | . . . |
| M5992 | S | S | S | S | . . . |
| F24816 | S | S | S | S | . . . |
| L46596 | S | S | S | S | . . . |
| M6076 | S | S | S | S | . . . |
| L49781 | S | S | S | R | . . . |
| M5979 | S | S | S | S | . . . |
| L48766 | S | S | S | S | . . . |
| W37690 | S | S | R | R | . . . |
| H32592.12 | S | S | S | S | . . . |
| W41861.12 | S | S | S | S | . . . |
| L11705.12 | S | S | S | S | . . . |
| T62839 | S | S | S | R | . . . |
| W41274 | S | S | S | S | . . . |
|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| W41858   | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| W41856   | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | F   | S   | S   | S   |
| W41856_I2| S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| W41860   | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| W41858_I2| S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| F28999   | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| H34227   | S   | S   | S   | R   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | R   | S   |
| L17046   | S   | S   | S   | R   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | R   | S   |
| L17643   | S   | S   | S   | R   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | R   | S   |
| T64606   | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| W44412_I2| S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| W44411   | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| H4354_s2 | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| H4167_s2 | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| T3020_s2 | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| W4037_s2 | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
| W1592_s2 | S   | S   | S   | S   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | S   | S   | S   | S   |
Table S7: Summary of the eight occasions across six patients with discordant sensitivity results and subsequent screening for additional mutations

| Drug            | Sample ID | DST (Phenotype) | WGS prediction | Candidate mutation | Summary                                                                 |
|-----------------|-----------|-----------------|----------------|--------------------|-------------------------------------------------------------------------|
| Isoniazid       | 140603310 | Resistant       | Sensitive      | katG_W328L         | Candidate mutation reported in resistant isolates in another study⁷     |
|                 | IMRL16    | Resistant       | Sensitive      | None               | BCG isolate where low-level intrinsic resistance is reported⁸          |
| Rifampicin      | 140603310 | Resistant       | Sensitive      | rpoB_V170F         | Candidate mutation reported in resistant isolates in another study⁹     |
| Ethambutol      | W37690    | Resistant       | Sensitive      | embB_G406A         | Hain testing confirmed no Hain mutation conferring resistance was present. Candidate mutation reported in resistant isolates in another study¹⁰ |
|                 | IMRL15    | Sensitive       | Resistant      | embB_M306V         | Candidate mutation known to confer resistance but has previously been associated with sensitive phenotypes¹¹ |
| Pyrazinamide    | 140603921 | Resistant       | Sensitive      | pncA_V7L           | No support in available literature for this mutation. Other mutations at this codon have previously been reported in resistant isolates¹²,¹⁴ |
| Streptomycin    | IMRL15    | Resistant       | Sensitive      | gidB_S100F         | Mutation seen in both sensitive and resistant isolates¹⁵                |
| Fluroquinolones| 140603751 | Resistant       | Sensitive      | gyrB_S313R, gyrA_E21Q | gyrB_S313R not reported as resistance-conferring in available literature. gyrA_E21Q reported in sensitive isolates¹⁶,¹⁷ |
Table S8: Outbreak clusters identified during this investigation (UK MTBC only).

| Pilot cluster ID | Known to HPU | Published cluster ID | Description | Number of previous database isolates | During this pilot study |
|------------------|--------------|----------------------|-------------|--------------------------------------|-------------------------|
|                  |              |                      |             | ≤5 SNPs from pilot isolate | 6 – 12 SNPs from pilot isolate | Number of isolates sequenced | From individuals (N) | From contributing centres (N) |
| 1                | Yes          | Cluster 7\(^1\)      | Substance misuse\(^1\) | 8 | 19 | 1 | 1 | 1 |
| 2                | Yes          | Cluster 4\(^1\)      | School\(^1\) | 1 | 63 | 2 | 2 | 1 |
| 3                | Yes          | Cluster 3\(^1\)      | School\(^1\) | 7 | 0 | 1 | 1 | 1 |
| 4                | Yes          | Cluster 9\(^1\)      | Substance misuse\(^1\) | 21 | 9 | 1 | 1 | 1 |
| 5                | Yes          | Lifestyle            | Lifestyle (interregional, isoniazid-resistant) | 7 | 1 | 1 | 1 | 1 |
| 6                | Yes          |                      | European MDR-TB | 2 | 1 | 3 | 2 | 1 |
| 7                | No           | G5/E3\(^2\)         | Lifestyle | 11 | 0 | 4 | 2 | 1 |
| 8                | Yes          |                      | Social group | 8 | 0 | 4 | 2 | 1 |
| 9                | Yes          |                      | Social group | 8 | 0 | 4 | 2 | 1 |

Note: Available MIRU-VNTR in pilot clusters 6, 7 and 9 were compatible with the genomic clusters
Table S9: Total cost per sample by process accounting for error rates. Error rates; 1% microscopy, 2% MGIT culture, 10% Cepheid Xpert MTB/RIF, 0.05% species identification (Hain ID), 13% DNA extraction for WGS, 4% WGS, 1.4% WGS data analysis, 10% MIRU-VNTR, DST 0.05%.

| Process | Costs by resource category (GBP) | Total per sample (GBP) |
|---------|---------------------------------|------------------------|
|         | Staff | Consumables | Equipment | Overheads | Miscellaneous |                  |
| WGS and routine clinical workflows |       |             |           |           |                |                  |
| MGIT culture | 18.83 | 21.04 | 3.34 | 8.73 | 0.45 | 52.39 |
| Cepheid Xpert MTB/RIF | 12.32 | 50.49 | 19.79 | 16.61 | 0.45 | 99.66 |
| WGS workflow only |       |       |       |       |                |                  |
| WGS | 20.02 | 66.84 | 11.20 | 19.76 | 0.73 | 118.55 |
| Routine clinical workflows only |       |             |           |           |                |                  |
| Identification assays |       |             |           |           |                |                  |
| Hain MTBC | 24.38 | 19.78 | 1.71 | 9.18 | 0.00 | 55.05 |
| Hain CM/AS |       |       |       |       |                |                  |
| MIRU-VNTR | 15.95 | 50.32 | 23.52 | 17.96 | 0.00 | 107.75 |
| First-line DST | 58.91 | 41.62 | 12.36 | 22.58 | 0.00 | 135.47 |
| Limited second-line DST† | 39.42 | 36.37 | 1.71 | 15.50 | 0.00 | 93.01 |
| Second-line DST‡ | 56.67 | 5.53 | 22.19 | 16.88 | 0.00 | 101.27 |

*For routine processing includes: annual staff turnover (10%), anemometer calibration and maintenance contract for category 3 laboratory. For WGS includes: annual staff turnover (10%) and staff training.
†Performed at the Birmingham clinical laboratory
‡Performed at a second reference laboratory (London, UK). Based on staff time, consumables and equipment only.
Table S10: Staff time required, per specimen, for each workflow process and associated costs

|                   | Grade 3 | Grade 6 | Grade 7 | Grade 8 | Total  |
|-------------------|---------|---------|---------|---------|--------|
| **Cost per hour (GBP)** | 12:36   | 20:98   | 24:81   | 42:04   | N/A    |
| **MGIT Culture**   |         |         |         |         |        |
| Hands-on time (minutes) | 6:13    | 35:44   | 11:99   | N/A     | 53:56  |
| Cost of error (GBP)  | 0:02    | 0:18    | 0:02    | N/A     | 0:22   |
| Total cost per sample (GBP) | 1:28    | 12:57   | 4:98    | N/A     | 18:83  |
| **Cepheid Xpert MTB/RIF** |         |         |         |         |        |
| Hands-on time (minutes) | 1:54    | 18:90   | 11:99   | N/A     | 32:43  |
| Cost of error (GBP)  | 0:00    | 0:41    | 0:02    | N/A     | 0:43   |
| Total cost per sample (GBP) | 0:32    | 7:02    | 4:98    | N/A     | 12:32  |
| **Whole Genome Sequencing** |         |         |         |         |        |
| Hands-on time (minutes) | N/A     | 24:67   | 22:85   | 2:00    | 49:52  |
| Cost of error (GBP)  | N/A     | 0:50    | 0:05    | 0       | 0:55   |
| Total cost per sample (GBP) | N/A     | 9:12    | 9:50    | 1:40    | 20:02  |
| **Hain GenoType MTBC/CM/AS** |         |         |         |         |        |
| Hands-on time (minutes) | N/A     | 95:92   | 3:00    | N/A     | 68:92  |
| Cost of error (GBP)  | N/A     | 0:07    | 0:02    | N/A     | 0:09   |
| Total cost per sample (GBP) | N/A     | 23:12   | 1:26    | N/A     | 24:38  |
| **MIRU-VNTR**       |         |         |         |         |        |
| Hands-on time (minutes) | 34:50   | 18:30   | 3:00    | N/A     | 55:80  |
| Cost of error (GBP)  | 0:71    | 0:47    | 0:02    | N/A     | 1:20   |
| Total cost per sample (GBP) | 7:82    | 6:87    | 1:26    | N/A     | 15:95  |
| **First-line DST**  |         |         |         |         |        |
| Hands-on time (minutes) | N/A     | 164:61  | 3:00    | N/A     | 167:61 |
| Cost of error (GBP)  | N/A     | 0:10    | 0:02    | N/A     | 0:12   |
| Total cost per sample (GBP) | N/A     | 57:65   | 1:26    | N/A     | 58:91  |
| **Limited second-line DST** |         |         |         |         |        |
| Hands-on time (minutes) | N/A     | 108:92  | 3:00    | N/A     | 111:92 |
| Cost of error (GBP)  | N/A     | 0:08    | 0:02    | N/A     | 0:10   |
| Total cost per sample (GBP) | N/A     | 38:16   | 1:26    | N/A     | 39:42  |
| **Second-line DST (phenotyping)** |         |         |         |         |        |
| Hands-on time (minutes) | N/A     | N/A     | 137:00  | N/A     | 137:00 |
| Cost of error (GBP)  | N/A     | N/A     | 0:03    | N/A     | 0:03   |
| Total cost per sample (GBP) | N/A     | N/A     | 56:67   | N/A     | 56:67  |
Table S11: Cost sensitivity analysis for routine clinical and WGS workflows. Shaded rows are current costs.

| Variable                            | Adjustment value | MiSeq Culture (GBP) | Cost change (%) | Cost change (%) | Cost change (%) | Cost change (%) | Cost change (%) | Cost change (%) | Cost change (%) |
|-------------------------------------|------------------|---------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Overheads                           | 10%              | 48.02               | -8.33           | 91.34          | -8.33          | 108.68         | -8.33          | 50.47          | -8.33          |
|                                     | 20%              | 52.39               | 0               | 99.66          | 0              | 118.55         | 0              | 55.05          | 0              |
|                                     | 30%              | 56.75               | 8.33            | 107.95         | 8.33           | 128.44         | 8.33           | 59.64          | 8.33           |
| Annual throughput                   | 90%              | 52.90               | 0.97            | 102.35         | 2.71           | 120.16         | 1.34           | 55.28          | 0.41           |
|                                     | 100%             | 52.39               | 0               | 99.66          | 0              | 118.56         | 0              | 55.05          | 0              |
| Equipment lifetime (years)          | 5                | 54.32               | 3.55            | 106.51         | 6.44           | 123.72         | 4.17           | 56.85          | 3.17           |
|                                     | 10               | 52.39               | 0.00            | 99.66          | 0.00           | 118.56         | 0.00           | 55.05          | 0.00           |
| Cost of MiSeq                       |                   |                     |                 |                |                |                |                |                |                |
| Cost change (%)                     | -20%             | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | This study†       | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | +20%             | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
| Cost of WGS                        |                   |                     |                 |                |                |                |                |                |                |
| library preparation                | -20%             | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | This study†       | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | +20%             | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
| Cost of sequencing                  |                   |                     |                 |                |                |                |                |                |                |
| cartridge                           | -20%             | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | This study†       | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | +20%             | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
| Sequencing                          |                   |                     |                 |                |                |                |                |                |                |
| batch size                          | 15                | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | 17                | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
| Staff grade                         | Grade 6           | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |
|                                     | Grade 7           | N/A                 | N/A             | N/A            | N/A            | N/A            | N/A            | N/A            | N/A            |

Note: The table includes various cost components such as staff grade, sequencing throughput, and other elements relevant to clinical and WGS workflows, with specific focus on cost changes and percentages.
| Total lower ǂ | N/A | 47·04 | -10·22 | 82·25 | -17·47 | 104·02 | -12·26 | 50·15 | -8·91 | 86·71 | -19·53 | 122·61 | -9·49 | 84·93 | -8·69 | 90·36 | -10·77 |
|--------------|-----|-------|--------|-------|--------|--------|--------|-------|-------|-------|--------|--------|-------|-------|-------|-------|-------|
| Total upper ǂ | N/A | 57·26 | 9·3    | 110·66| 11·04  | 130·01 | 9·67   | 59·86 | 8·74  | 119·86| 11·24  | 148·41 | 9·55  | 100·99| 8·58  | 112·66| 11·25 |

*Error rates reported during this study were - 1% microscopy, 2% MGIT culture, 10% Cepheid Xpert MTB/RIF, 0·05% species identification (Hain ID), 13% DNA extraction for WGS, 4% WGS, 1·4% WGS data analysis, 10% MIRU-VNTR, DST 0·05%.
†Cost of MiSeq £83,282·00; Cost of Nextera XT library preparation kit £1,649·06; Cost of 300bp v2 MiSeq sequencing cartridge £560·11.
‡Total values are the summation of percentage cost change for the cheapest (lower) and most expensive (upper) of the costs generated during the sensitivity analysis.
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