Wales Centre for Mycobacteria: Information for Patients and Users

Contents

1 Laboratory Location

The Public Health Wales Centre for Mycobacteria is located at:

Public Health Wales Microbiology Cardiff,
University Hospital Llandough,
Penlan Road,
Llandough,
CF64 2XX.

Telephone Number: 029 2071 6408
029 2181 6408

Out of hours: 029 2074 7747
029 2184 7747

Fax: 029 2071 5134
029 2181 5134

Email: WCM@wales.nhs.uk

DX Address:

Wales Centre for Mycobacteria
Cardiff Public Health Wales (Llandough)
DX 6070400
Penarth 90 CF

This User Manual is a list of current tests offered by the WCM or referral tests that we send to other laboratories. Please contact the WCM for current clinical guidance on appropriate selection and use of tests for detecting Mycobacterial infection.

2 Clinical Services offered by the WCM and Referrals to other Laboratories

Clinical Services offered by the WCM:

- Microscopy on primary respiratory and non-respiratory samples – auramine and Ziehl-Neelsen.
- Primary sample processing of respiratory and non-respiratory samples for mycobacterial culture.
- Direct PCR for *Mycobacterium tuberculosis* complex DNA and mutations conferring resistance to rifampicin in respiratory and CSF samples.
- Culture – liquid media, solid media, and mycobacterial blood cultures.

- Identification of *M. tuberculosis* complex isolates and non-tuberculous mycobacteria by HAIN LifeScience GenoType CM and/or Whole Genome Sequencing (WGS).
- Sensitivity testing of *M. tuberculosis* complex isolates – first and second-line by WGS and culture.
- DNA fingerprinting of *M. tuberculosis* complex strains.
- Latent TB infection detection using QuantiFERON-TB Gold Plus IGRA test.

Referrals to other Laboratories:

- *M. tuberculosis* complex isolates for third line sensitivity testing by culture are referred to NMRS-South at PHE Colindale.
- PCR on fresh/fixed tissue samples are referred to NMRS-South at PHE Colindale or Microbiology Department at Leeds Teaching Hospitals NHS Trust.
- *Mycobacterium abscessus* isolates for further sub-speciation are referred to Great Ormond Street Hospital.
- Non-tuberculous mycobacteria for sensitivity testing are referred to NMRS-South at UKHSA Colindale.
- Blood samples for T-SPOT.*TB* testing are referred to Oxford Diagnostic Laboratories.
- *Mycobacterium bovis* isolates are referred to the Animal and Plant Health Agency at Weybridge for spoligotyping.
- Direct PCR for *pan-Mycobacterial* DNA and mutations conferring resistance in non-respiratory samples are referred to Leeds Teaching Hospitals NHS Trust
- Blood samples for anti-mycobacterial antibiotic level monitoring are either referred to the Cardiff Toxicology Laboratory at University Hospital Llandough or the Antimicrobial Reference Laboratory at Southmead Hospital, Bristol.

3 Opening Hours of the WCM

The full services of the WCM are available 08:45 AM to 16.45 PM Monday to Friday. The WCM is closed on Public Holidays.

4 Examinations offered by the WCM

4.1 AFB Microscopy and Culture

The WCM is the TB Reference laboratory for Wales and carries out culture specifically for mycobacteria for samples from all laboratories in Wales. Most referring laboratories in Wales carry out smear microscopy on-site before referring the rest of the sample to the WCM for culture and identification.

Samples Required	**Preferable Primary Sample Volumes**	**Special Precautions**
Sputums	Three samples of ≥5 ml in sterile universal containers	Three samples should be collected approximately 8-24 hours apart with at least one from early morning shortly after waking.
Bronchoalveolar lavage/bronchial washings	≥5 ml in a sterile universal container	Contamination of the bronchoscope with tap water, which may contain environmental *Mycobacterium* species, should be avoided.
Gastric washings	Three consecutive days with ≥5 ml in sterile universal containers	Direct microscopy is not done. Collect samples early in the morning before breakfast on three consecutive days. Aspirates should be promptly delivered and processed to avoid acidic deterioration of organisms
Sterile site body fluids - CSF, pleural fluid, joint fluid etc.	As much as possible. ≥6 ml in adults in a sterile universal container	Collect aseptically.
Urines	Early Morning Urines are preferable in sterile universal containers **(N.B. NOT containing Boric acid)**	Direct microscopy is not done. Should be collected in the early morning on three consecutive days in a CE marked leak proof container that does not contain boric acid. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.
Skin, bone, and tissue including post mortem samples	As much as possible in sterile universal container (NO preservatives)	Collect aseptically and placed in a CE Marked leak proof container without preservatives but with sterile distilled water added to prevent desiccation. A

Samples Required	Preferable Primary Sample Volumes	Special Precautions
		caseous portion should be selected if possible.
Faeces	Maximum of 20 ml in a single container.	Direct microscopy is not done.
Pus or pus swabs	As much as possible in sterile universal container	Collect aseptically. Pus is the sample type of choice. Swabs are less preferable.
Bone marrow	Maximum of 5 ml in a mycobacterial blood culture bottle.	Direct microscopy is not done. Aspirate and add directly to a mycobacterial blood culture bottle.
Blood	Maximum of 5 ml in a mycobacterial blood culture bottle.	Direct microscopy is not done. Inoculate directly into a mycobacterial blood culture bottle.

Special Precautions:

- Please see Appendix B for packaging instructions.

Turnaround times:

- All smear positive microscopy results are phoned within 24 hours of receipt.
- A final culture result is issued after seven weeks.

Biological Reference Intervals: None

Clinical Decision Values: None

4.2 Species Identification of Mycobacterial Isolates

The WCM is the TB Reference laboratory for Wales and carries out culture specifically for Mycobacteria on samples from all laboratories in Wales. The WCM also accepts positive cultures referred from other laboratories. Please see Appendix A for instructions of sending aliquots from BD MGITs.

All positive cultures grown within the WCM or referred to WCM, undergo species identification using WGS and/or the HAIN LifeScience GenoType CM assay as the first line assay. After this, all subsequent examinations are carried out on the cultures used for species identification. Separate aliquots of positive cultures do not have to be sent for each test unless stated.

Samples Required	Sample Volumes
Positive liquid culture	≥3 ml aliquot
LJ slope with positive growth	Entire LJ slope
Positive mycobacterial blood culture	≥1 ml aliquot

Other tests available within the WCM or at other laboratories may be used depending on the sample received, results obtained, and any discussions with users.

Special Precautions:

- Please see Appendix A for recommendations of sending MGIT aliquots to the WCM.
- Please see Appendix B for packaging instructions.

Turnaround times:

- All new positive cultures grown within the WCM are telephoned to the referring laboratory within two days of detection.
- For current estimated turnaround times for this test, please contact the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

4.3 Sensitivity Testing of *M. tuberculosis* complex Isolates

Sensitivity testing is carried out by WGS and culture methods.

WGS is used on all new *M. tuberculosis* complex isolates to detect antibiotic resistance conferring mutations in genes known to confer resistance to isoniazid, rifampicin, ethambutol, pyrazinamide, quinolones, streptomycin, and aminoglycosides.

M. tuberculosis complex isolates that are not *M. bovis* and are WGS pyrazinamide resistant, unknown, or failed are sent to NMRS-South for confirmation of pyrazinamide resistance by phenotypic methods.

Isolates that are mono-resistant to, unknown WGS mutation, or failed WGS result against any of the first-line agents are then tested by phenotypic methods against that agent, and also against two second-line agents at the WCM – ciprofloxacin and streptomycin.

Isolates that are resistant to two or more of the first-line agents are referred to NMRS-South for third-line sensitivity testing.

Special Precautions:

- Please see Appendix B for packaging instructions.

Turnaround times:

- For current estimated turnaround times for this test, please contact the WCM.
- Any new resistant MTBC isolates will be telephoned to the referring laboratory or user.

Biological Reference Intervals: None

Clinical Decision Values: None

4.4 DNA fingerprinting of *M. tuberculosis* complex strains

DNA fingerprinting using WGS is done on all new *M. tuberculosis* complex isolates cultured in the WCM and new cultures referred from other laboratories. Duplicate isolates may also be analysed by WGS. WGS Strain Typing results are shared with Public Health Wales Health Protection Leads and UKHSA.

Special Precautions:

- Please see Appendix B for packaging instructions.

Turnaround time:

- For current estimated turnaround times for this test, please contact the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

4.5 Phenotypic testing of clinically significant non-tuberculous mycobacteria on request

Within the WCM, sensitivity testing is not carried out routinely for all non-tuberculous mycobacteria isolates. Sensitivity testing is carried out in line with 2016 European CF NTM and 2017 BTS NTM Guidelines or can be requested for clinically significant isolates. Testing is currently carried out at NMRS-South.

Special Precautions:

- Please see Appendix B for packaging instructions.

Turnaround times:

- For current estimated turnaround times for this test, please contact the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

4.6 Identification of Mycobacterial Cultures

The WCM uses HAIN LifeScience GenoType CM assay routinely for mycobacterial species identification.

The WCM uses WGS routinely for further identification of members of the *Mycobacterium avium/intracellulare* complex, *Mycobacterium abscessus* and *Mycobacterium chelonae* isolates, and resistance detection and DNA fingerprinting of *M. tuberculosis* complex isolates from positive cultures.

WGS results are obtained from two bioinformatics pipelines – one hosted in Public Health Wales and a second pipeline hosted by UKHSA.

The PHW pipeline provides interim results for mycobacterial speciation and isoniazid and rifampicin resistance in *M. tuberculosis* complex isolates.

The UKHSA pipeline provides full results for mycobacterial speciation, resistance detection in *M. tuberculosis* complex isolates for seven antibiotic classes, and *M. tuberculosis* complex strain typing.

Selection: Positive cultures grown in the WCM or referred isolates are analysed by HAIN LifeScience GenoType CM assay/WGS without the need for specific requests.

Special Precautions: Please see Appendix B for packaging instructions.

Turnaround time: For current estimated turnaround times for this test, please contact the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

4.7 Direct Detection of *M. tuberculosis* complex DNA and molecular resistance to Rifampicin in primary samples by PCR

The WCM performs Direct PCR for the detection of *M. tuberculosis* complex DNA and molecular resistance to Rifampicin on primary sputum and CSF samples using the Cepheid Xpert MTB/RIF Ultra assay.

Samples Required	Sample Volumes
Sputum	≥1 ml for culture and PCR.
Broncho-Alveolar Lavage	
Tracheal aspirates	

Non-directed bronchial lavage	
CSF	

At least 1 ml is preferable for culture and PCR on respiratory samples. At least 1 ml is absolutely required for both culture and PCR on CSF samples. If <1 ml of CSF is received, then only culture will be done.

Special Precautions:

- Please see Appendix B for packaging instructions.
- A positive PCR result can be used to rule in the diagnosis of TB but a negative PCR result cannot exclude TB.

Turnaround times:

- PCR results are reported within two days of receipt of sample and/or request in the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

5 Examinations referred by the WCM to other laboratories

5.1 Additional Tests on Positive Cultures

The WCM will send an aliquot of liquid media or a LJ slope to the respective laboratory for:

- *M. tuberculosis complex* third-line sensitivity testing for all isolates resistant to two or more antibiotics.
- *Mycobacterium abscessus* complex sub-speciation.
- Non-tuberculous mycobacteria sensitivity testing on request.
- *S*poligotyping on all *M. bovis* isolates.
- Phenotypic pyrazinamide testing on M. tuberculosis isolates that are resistant, unknown mutation, or have failed by WGS.

Turnaround times:

- For current estimated turnaround times for this test, please contact the WCM.
- Results for MDR-TB isolates that have resistance to additional antibiotics identified will be telephoned to the referring laboratory when results are received from the NMRS.

Biological Reference Intervals: None

Clinical Decision Values: None

5.2 Blood samples for T-SPOT.*TB* testing

Blood samples may be collected from children and immunocompromised individuals for Latent TB Infection detection and tested using the T-Spot.*TB* assay. The WCM refers whole blood samples received for this test to Oxford Diagnostic Laboratories.

ODL can be contacted Monday to Friday from 8:30 AM to 4:30 PM for further information:
Telephone: 01235 433164
Email: odllabstaff@oxfordimmunotec.com

Samples Required	Sample Volumes
Whole blood samples collected in lithium heparin, sodium heparin, or sodium citrate tubes for T-SPOT®.TB IGRA testing will be accepted.	Typically, in immunocompetent patients, sufficient peripheral blood mononuclear cells (PBMCs) to perform the T-SPOT.TB test can be obtained with the following age-dependent guidelines: Adults and children ≥ 10 years of age: 6 mL Children ≥2 to <10 years of age: 4 mL Children <2 years of age: 2 mL It may be advisable to collect double the recommended blood volume for immunocompromised patients

Special Precautions:

- Collect a blood sample according to the instructions supplied with the collection device.
- The tube contents must be inverted (8-10 times) to ensure that the whole blood is mixed thoroughly with the anticoagulant.
- Store collected blood at room temperature (18-25°C).
- Do not refrigerate or freeze.
- Blood samples must arrive at the local laboratory before 3:30 PM on the day of collection. This is so that the sample can be transported to Oxford Diagnostic Laboratories and will arrive no later than 2:00 PM the day after venepuncture.

Turnaround times:

- For current estimated turnaround times for this test, please contact the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

5.3 TB PCR on Fresh/Fixed Tissue Sample

Samples for the detection of *M. tuberculosis* complex or Mycobacterial species DNA are referred to the Microbiology Department Leeds Teaching Hospitals NHS Trust.

Two PCR tests can be done in Leeds:

- *M. tuberculosis* DNA and Rifampicin resistance testing without Mycobacterial species DNA detection
- *M. tuberculosis* DNA and Mycobacterial species DNA detection without Rifampicin resistance testing.

Samples Required	Sample Volumes
Fresh or fixed tissue	As much as possible

Special Precautions:

- Fresh tissue samples are preferable to fixed tissue samples.
- PCR on fresh or fixed tissue has an increased yield if the sample is known to be smear positive.

Turnaround times:

- For current estimated turnaround times for this test, please contact the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

6 Latent TB Infection Detection by Interferon Gamma Release Assays (IGRA)

QuantiFeron is used routinely and T-Spot.TB is used in specific patients such as immunocompromised patients and children.

Within the WCM, the QuantiFERON®-TB Gold Plus assay on the DiaSorin Liaison XL analyser is used on blood samples received in QuantiFERON-TB Gold Plus Blood Collection Tubes. Blood samples for T-SPOT.TB tests are also received and referred to Oxford Diagnostic Laboratories.

Both assays show broadly similar performance characteristics using different laboratory processes. T-SPOT.TB may be less affected by weakened immune systems such as immunocompromised patients but

obtaining definitive results in immunocompromised patients may not always be possible for both tests.

6.1 Latent TB Infection Detection using QuantiFERON®-TB Gold Plus (QFT®-Plus)

Clinical users and local laboratories can now order QuantiFERON-TB Gold Plus blood collection tubes directly from Qiagen via Oracle. The WCM does hold a small stock of tubes.

Samples Required	Sample Volumes
Blood in four Qiagen QFT tubes for each patient: • QuantiFERON Mitogen Tubes (purple cap with white ring • QuantiFERON TB2 Tubes (yellow cap with white ring) • QuantiFERON TB1 Tubes (green cap with white ring) • QuantiFERON Nil Tubes (gray cap with white ring)	0.8 to 1.2 ml blood in each tube. Ideally 1.0 ml in each tube.

Special Precautions:

- Please see Appendix B for packaging instructions.
- A definitive result may not be possible to obtain in immunocompromised individuals or patients with other infections present at the time of testing. Please contact the WCM for further advice.
- The WCM advises that patient sampling is only done Monday to Thursday and by special request on Friday.
- QFT Blood Collection Tubes can be used up to an altitude of 810 meters above sea level.
- QFT tubes should be between 17-25 °C at the time of blood filling.
- Prior to incubation, maintain the tubes at room temperature (17-27 °C).
- QFT tubes must be transferred to a 37 °C incubator as soon as possible and within 16 hours of collection.
- Incubate the tubes UPRIGHT at 36-38 °C for 16 to 24 hours. The incubator does not require CO_2 or humidification.
- Users should ensure that the incubation for 16 to 24 hours ends at a time where there will be someone available to remove the samples from the 37 °C incubator. If this is not possible, incubation should be delayed until an appropriate time. Incubation must always commence within 16 hours of collection.

- If the blood is not incubated immediately after collection, re-mixing of the tubes by inverting 10 times must be performed immediately prior to incubation.
- After incubation at 36-38°C, blood collection tubes may be stored at 4-27°C for up to three days prior to centrifugation.

Turnaround times:

- Quantiferon results are reported within two days of receipt in the WCM.

Biological Reference Intervals: None

Clinical Decision Values: None

7 Examinations outside the scope of our accreditation

Public Health Wales Microbiology Division has been awarded UKAS accreditation for compliance with ISO 15189:2012.

The following tests in the WCM are currently outside of our accredited scope:

1. All veterinary samples.
2. Environmental water sampling.
3. QuantiFeron testing at University Hospital Llandough.
4. *M. tuberculosis* complex Strain Typing by WGS using the PHE pipeline.

Ongoing assurance of quality in the meantime is maintained by internal and external quality assurance, quality control and training.

Please refer to Public Health Wales Microbiology UKAS Schedule of Accreditation (Medical Laboratory No. 9510) for the full scope, including tests referred to specialist and reference units within the Public Health Wales Microbiology Division.

8 Instructions for Completion of Request Forms

Both the request form and sample container must be labelled with at least two patient identifiers, such as:

- Patient's full name.
- Patient's date of birth.
- Patient's postcode.
- Hospital/clinic number or NHS number.

- Date of sample collection.

Minimum information required on the request form is:

- Full Name of Patient.
- Patient Identity number.
- Date of birth.
- Gender.
- Sending Laboratory's Reference Number.
- Date sample was collected.
- Tests or investigations required.
- Laboratory Findings.
- Address of requester.
- Relevant clinical details.

Relevant clinical details that should be included:

- Travel history.
- Previous infection with TB.
- Previous treatment for TB.
- Contact of infectious TB patient.
- Animal contact.

For samples or isolates referred to the WCM:

- Each sample and request form should have a unique identifier that is usually a Laboratory Number.
- The unique identifier should be labelled clearly on the sample and form.
- The unique identifier on the sample and corresponding request form should match.

For samples from patients in Wales referred to the WCM, the following criteria should preferably be met:

- Sending hospital should be stated clearly on the form.
- AFB microscopy "done" or "not done" should be stated clearly on the form.
- Forms should be scanned into TrakCare Lab LIMS at the originating laboratory.
- A5 forms only.
- One episode number per one sample.
- Samples should be packed individually.

The WCM will attempt to process all primary samples and referred cultures as much as possible. Any required information on the sample or request form that is missing could result in a delay in sample processing.

Please provide full details of where to send the result and who to contact if we need to report an urgent, significant result.

9 Instructions for preparation of Patients

Collect samples before antimicrobial therapy is started where possible.

10 Instructions for Patient-Collected Samples

Sputum Samples

Sputum samples should be relatively fresh to minimise contamination. Purulent samples are best. Three samples of ≥5 ml should be collected approximately 8-24 hours apart with at least one from early morning.

Samples taken early morning (i.e. 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.

Urine Samples

Urine samples should be collected in the early morning on three consecutive days in a CE marked leak proof container (that does not contain boric acid), and placed in a sealed plastic bag. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.

11 Requirements for Patient Consent

Tuberculosis is an infectious disease. It may be necessary for healthcare professionals to obtain information about individuals for contact tracing even if they are not currently symptomatic.

12 Criteria for Acceptance and Rejection of Samples received by the WCM

The WCM aims to process all primary respiratory and non-respiratory samples received. There is no minimum required volume for primary samples. Direct microscopy may not be done if a sample has a very low volume.

Criteria for Acceptance of Samples received by the WCM

The following samples will be accepted by the WCM:

- Primary respiratory or non-respiratory samples should have ≥5 ml volume or as much as possible.
- All bone marrow samples and/or inoculated blood culture bottles will be accepted.

- Referred cultures with AFB seen on ZN microscopy at the WCM will be accepted for identification, sensitivity testing, and DNA fingerprinting.
- Primary respiratory samples for direct PCR should preferably have ≥1 ml volume.
- CSF samples requested for direct PCR that are ≥1 ml will be tested by culture and PCR.
- Qiagen QFT tubes that contain 0.8-1.2 ml volume of blood in each tube and have been received within 16 hours of collection will be accepted for incubation.
- Qiagen QFT tubes that have 0.8-1.2 ml volume of blood in each tube and have been incubated within 16 hours of collection at 37 °C for between 16 and 24 hours at a local laboratory will be accepted
- Whole blood samples collected in lithium heparin, sodium heparin, or sodium citrate tubes for T-SPOT®.*TB* IGRA testing will be accepted.

Criteria for Rejection of Samples received by the WCM

The following samples will be rejected by the WCM:

- Urine samples received in red-topped boric acid containers.
- QuantiFERON tubes not incubated at 37 °C and not received within 16 hours of collection.
- QuantiFERON tubes that have been incubated at 37 °C but not for between 16 and 24 hours.
- QuantiFERON tubes where times of collection and incubation have not been entered on the request form.
- Requests for QuantiFERON testing on blood samples in containers other than Qiagen QFT tubes.
- CSF samples <1 ml for PCR will only have culture done.
- Inappropriate samples or those that are inadequately labelled, damaged or leaking are liable to be discarded.
- Referred cultures that have no AFB seen on ZN microscopy at the WCM. A repeat culture will be requested.
- EDTA-containing blood collection tubes (purple top) for T-SPOT®.*TB* IGRA testing.

The user will be contacted if the sample is urgent or if it may be difficult to collect a second sample. A hard copy report will be issued stating that the sample has been rejected, reason, and suggested repeat samples that will be accepted or contact details for further information.

13 Factors known to significantly affect the performance of examinations or interpretation of results carried out in the WCM

The likelihood of obtaining a positive culture may be reduced if:

- Samples are collected after the initiation of antibiotic therapy.
- Only one respiratory sample is obtained from a patient.

14 Clinical Advice – Ordering of examinations and interpretation of examination results

Dr Jason Evans (Lead Scientist) and Dr Matt Backx (Laboratory Director) are available for clinical advice on ordering of examinations and interpretation of examination results between 08:45 AM and 16.45 PM Monday to Friday. Kay Parry and Rhiannon Woodman (Senior Biomedical Scientists) are available for technical advice.

Dr Matt Backx	029 2074 4515 029 2184 4515
Dr Jason Evans	029 2071 6408
Kay Parry	029 2181 6408
Rhiannon Woodman	

Additional tests (i.e. PCR) can be requested verbally by telephone or by email.

15 Protection of Personal Information

The WCM and Public Health Wales comply with the Data Protection Act. This requires all organisations which handle personal information to comply with a number of important principles regarding privacy and disclosure.

The Act states that anyone who processes personal information must comply with eight principles. These state that information must be:

- Fairly and lawfully processed.
- Processed for limited purposes.
- Adequate, relevant and not excessive.
- Accurate and up to date.
- Not kept for longer than is necessary.
- Processed in line with individuals' rights.
- Secure.
- Not transferred to other countries without adequate protection.

The Act also allows people to find out what personal information is held about them. This could be on computer or on paper records.

16 Complaint Procedure

Complaints are handled following the advice given in the Wales Government document "Putting Things Right". Complaints are reviewed at local management meetings and may be escalated as appropriate. All complaints

are also reported to the Public Health Wales Service User Engagement Group and Quality and Safety Group.

17 Informed Consent

The laboratory can provide information to patients and users that explains the exact clinical procedure to be performed to enable informed consent.

18 Appendix A

Preferred procedure for sampling MGIT 960 tubes for sending to WCM

1. Remove MGIT tube from MGIT 960 without disturbing the sediment at the bottom of the tube.

2. Film small quantity of sediment to confirm presence of Acid Fast bacilli.

3. Using a sterile pipette, remove approximately 3 ml sediment from the bottom of the tube and place in a sterile universal container

4. Pack the vial according to the regulations governing transport of infectious material.

19 Appendix B

Packaging of Category III Samples for Sending to WCM

1. Clinical samples and/or microbiological cultures must be packed in a triple packaging system to 620 (602) standard as follows:

2. The primary container must be watertight and leak-proof and may be glass, metal or plastic. The container must be wrapped in sufficient absorbent material to absorb the contents of the container in the event of breakage or spillage.

3. The primary container must be placed into a secondary container that is leak proof and watertight. Several wrapped primary containers may be placed in the secondary container (not exceeding 50 ml or 50g). Sufficient absorbent material must be placed between the samples and the secondary container to prevent rattling and also to absorb any spillage or leakage.

4. The secondary container must be placed in an outer package for shipment. Include the request forms with the secondary container and not inside the secondary container. Include the following details on the outer package:

    - Name and address of Sender.
    - Name and address of receiver (see Section 1 for WCM address).
    - Contents of Package.
    - Package orientation label/ THIS SIDE UP if liquids are being sent.

5. In order to comply with the new regulations for transfer of mycobacteria you are required to notify us of cultures being forwarded. Our preferred option is that you contact us by E-mail using WCM@wales.nhs.uk and Jason.evans@wales.nhs.uk, kay.parry@wales.nhs.uk, rhiannon.woodman@wales.nhs.uk. Please do not send the patients name, only the initials, date of birth and your laboratory number. When the culture arrives we will then confirm receipt by returning the message. If no parcel arrives within two working days we will contact you.

20 Appendix C

Prices for external services

Please contact the WCM for the latest updates on prices.

21 Appendix D

WCM REQUEST FORMS

- WCM Sample Referral Form

- Public Health Wales QuantiFERON-TB Gold Plus Request forms are available on the following links:

  QuantiFeron Request Form – Electronic Test Requesting

  https://phw.nhs.wales/services-and-teams/reference-laboratories-and-specialist-services/wales-centre-for-mycobacteria-wcm/quantiferon-request-form-electronic-test-requesting/

  QuantiFeron Request Form – Non-Electronic Test Requesting

  https://phw.nhs.wales/services-and-teams/reference-laboratories-and-specialist-services/wales-centre-for-mycobacteria-wcm/quantiferon-request-form-non-electronic-test-requesting/

- Public Health Wales QuantiFERON-TB Gold Plus blood sampling instructions

Public Health Wales Centre for Mycobacteria
Microbiology Cardiff, Llandough Hospital, Penlan Road, Penarth, Vale of Glamorgan, CF64 2XX
DX: Public Health Wales Cardiff (Llandough), DX6070400, Penarth 90 CF. Tel 029 2071 6408 / 029 2181 6408

Source Lab: _____	WCM Laboratory No:
Source Lab No: _____	_____

Sample Details

Positive culture ☐ Primary Sample ☐

Date collected: _____ Original Smear: Positive / Negative Not Done / Unknown

Sample Type:_____

CF Patient: Yes / No

Please Indicate Test Required:

Identification ☐

Sensitivities ☐

NTM sensitivities will be carried out in line with the 2016 European CF and 2017 BTS Guidelines.

Culture ☐

Direct PCR for
MTBC/NTM DNA and Resistance Markers ☐

Non-processed sputum ☐
Non-processed BAL ☐
CSF ☐

Direct PCR is a chargeable service, please contact the WCM for the current price.
Smear negative samples should be discussed with the WCM before referral.

Patient Details

Surname:_____ Forename (s) _____

Male / Female / Unknown Date of Birth:_____

Address:_____ Post Code: _____

Other comments/requests:

Previous TB **yes / no / unknown**. If yes, when and site of previous TB _____

Previous Anti-TB Therapy **yes / no / unknown**. If yes, when _____

Drugs used, if known _____ Year Arrived in UK _____
Is the culture related to a possible outbreak? Yes ☐ No ☐

Index Case (if known) _____

Place of contact: _____

Why do you think this is an outbreak? _____

Do you agree to provide further information to the WCM if needed? Yes ☐ No ☐

Is the culture a lab contaminant: Yes ☐ No ☐
Is the culture a bronchoscope contaminant Yes ☐ No ☐

If yes, please give details:

GUIDELINES ON BLOOD COLLECTION, STORAGE AND TRANSPORTATION

QuantiFERON-TB Gold Plus uses the following collection tubes:

1. QuantiFERON Nil Tubes (grey cap with white ring)
2. QuantiFERON TB1 Tubes (green cap with white ring)
3. QuantiFERON TB2 Tubes (yellow cap with white ring)
4. QuantiFERON Mitogen Tubes (purple cap with white ring)

Blood collection tubes should be kept at room temperature (DO NOT REFRIGERATE). Never use blood collection tubes after the expiry date (printed on the tube label).

Antigens have been dried onto the inner wall of the blood collection tubes so it is essential that the contents of the tubes be thoroughly mixed with the blood.

The following procedures should be followed for optimal results:

1. For each subject collect 1mL of blood by venepuncture directly into each of the QuantiFERON-TB Gold Plus blood collection tubes (with grey, green, yellow, and purple caps).
    - As 1mL tubes draw blood relatively slowly, keep the tube on the needle for 2-3 seconds once the tube appears to have completed filling, to ensure that the correct volume is drawn.
    - The black mark on the side of the tubes indicates the 1mL fill volume.
    - QuantiFERON-TB Gold Plus blood collection tubes have been validated for volumes ranging from 0.8 to 1.2mL. If the level of blood in any tube is not close to the indicator line, it is recommended to obtain another blood sample.
    - If a "butterfly needle" is being used to collect blood, a "purge" tube should be used to ensure that the tubing is filled with blood prior to the QuantiFERON-TB Gold Plus tubes being used.
2. Mix the tubes by turning the tube end-over-end 8 to 10 times or shaking the tube for 5 seconds ensuring that **the entire inner surface of the tube** has been coated with the blood. Thorough mixing is required to ensure complete mixing of the blood with the tube's contents.
3. Label tubes appropriately (Patient's name, surname, DoB, Hospital No)
4. After the blood collection and mixing, keep tubes in upright position in the rack at room temperature. DO NOT REFRIGERATE OR FREEZE THE BLOOD SAMPLES.

After blood collection, bottles with blood can be:

- either delivered to your local microbiology laboratory on the same day of collection (before 5 pm).
- OR, where practical, incubated overnight in the incubator at 37°C. There is no need for CO_2 supply in the incubator. The overall time of incubation at 37°C should not be less than 16 hours and not exceed 24 hours. After incubation at 37°C, blood specimens should be sent to your local microbiology laboratory by DX in appropriate biosafety containers.
- Users should ensure that the incubation for 16 to 24 hours ends at a time where there will be someone available to remove the samples from the 37 °C incubator. If this is not possible, incubation should be delayed until an appropriate time. Incubation must always commence within 16 hours of collection.

In the laboratory:

- Please ensure the QFT®-Plus blood tubes have been centrifuged at 3,000 g for 15 minutes within 3 days post incubation.
- QFT®-Plus tubes can then be stored upright at 2-8°C for up to 28 days following centrifugation.
- The sample is to be transported via DX courier service.