DRAFT FOR CONSULTATION

Laboratory Guidelines for the Diagnosis of *Mycobacterium tuberculosis* Infection

Developed by
Department of Health
TB Monitoring and Lab Services Working Group
Dr. Grace Smith - Chair

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☐ NHS Trust CEs	☐ NHS Trust Board Chairs
☐ SHA CEs	☐ Special HA CEs
☐ Care Trust CEs	☐ Directors of HR
☐ Foundation Trust CEs	☐ Directors of Finance
☐ Medical Directors	☑ Allied Health Professionals
☑ Directors of PH	☑ GPs
☐ Directors of Nursing	☐ Communications Leads
☐ Local Authority CEs	☐ Emergency Care Leads
☐ Directors of Adult SSs	☐ Directors of Children's SSs

☑ Other

please specify: **Laboratory staff**

7 Additional Circulation List:

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☐ Directors of Adult SSs	☐ Directors of Children's SSs
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13	Contact Details:	**Dr. Irene Gonsalvez** **Expert Advice Support Office** **Corporate Services** **2E09, Health Protection Agency** **61 Colindale Avenue, London NW9 5EQ** **0208 327 6687**

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Membership of the guidelines development sub group of the Monitoring and Lab Services Working Group

Prof Brian Duerden	**Inspector of Microbiology, Department of Health**
Dr Grace Smith	**Chair of Working Group, Deputy Director, HPA, Birmingham**
Prof Francis Drobniewski	**HPA, Mycobacterium Reference Unit**
Dr John Magee	**Regional Centre for Mycobacteriology, Newcastle**
Dr. Ruth Gelletlie	**HPA, Local and Regional Services**
Dr Helen Jones	**NHS microbiologist - high incidence area , Wolverhampton**
Dr Bob Wall	**NHS microbiologist - high incidence area , London**
Dr Tim Collyns	**NHS microbiologist - interest in path modernisation, Leeds**
Dr Margaret Logan	**NHS microbiologist - interest in path modernisation, Gloucester**

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Dr John Watson	**HPA, Monitoring and Lab Services Working Group**
Dr Pam Sonnenberg	**HPA, Monitoring and Lab Services Working Group**
Prof Peter Davies	**TB clinician/outcome monitoring, Liverpool, Monitoring and Lab Services Working Group**
Dr Maggie Barker	**London, RO, Monitoring and Lab Services Working Group**
Dr. Andrew Stacey	**NHS, Royal Berkshire**
Dr. Richard Cooke	**NHS, Aintree**
Members of the Department of Health TB Delivery Services Working Group	
Members of the Department of Health Commissioning Services Working Group	

Laboratory Guidelines for the Diagnosis of *Mycobacterium tuberculosis* Infection

Introduction

These guidelines are designed to support the diagnosis of tuberculosis by appropriate laboratory tests. The guidelines address the needs and expectations of patients and their clinicians. The aim of these guidelines is to ensure the rapid, specific diagnosis of *M. tuberculosis* infection; to support the early confirmation of appropriate treatment and instigation of suitable measures to reduce transmission; and to provide timely evidence to help identify and investigate possible outbreaks. Many microbiology laboratories will only perform some of the investigations themselves, because confirmation of identity, antimicrobial susceptibility testing and molecular typing will be done at only a few specialist centres, but each laboratory must fulfil the appropriate standard(s) for the procedure(s) they perform. The time guidelines are given in terms of working days on the basis of services being provided six days each week with local arrangements for public holidays to minimise delays.

These guidelines are designed to complement the information and recommendations published in other national guidance documents and in particular should be read in conjunction with the guidelines on tuberculosis recently issued by the National Institute for Health and Clinical Excellence (NICE) and the National Bacteriology Standard Operating Procedure (BSOP) for the microbiological investigation of specimens for *Mycobacterium* species.

Detailed guidance for the selection of specimens and their collection are set out in the NICE guidelines on tuberculosis and in the national BSOP
Click here to access the NICE Guidelines, Quick Reference Version

Click here to access the NICE Guidelines, full version

For further information on NICE, go to www.nice.org.uk

http://www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop40.pdf
www.evaluations-guidelines.org.uk

1. **Samples that may be examined**

(a) *Types of sample*

Laboratories should be prepared for the examination of a wide variety of specimen types, including:
Sputum or other respiratory samples; CSF, spinal / para-spinal / intra-cerebral material; gastric washings; lymph node or other tissue samples or tissue fluids; blood or bone marrow; bone; urine; faeces.

(b) *Number of samples*

Sputum: three fresh, purulent samples (ideally 5 ml or greater) from the lower respiratory tract collected on consecutive days. Most other specimen types will be single samples.

(c) Documentation

Full personal identification and clinical details must be provided with the samples to comply with local specimen labelling policies and minimum data set requirements (see RCPath guidance).

2. Transfer to the laboratory

Specimens should be received in the laboratory ideally within one working day, (maximum 48h) of collection. This is necessary to prevent increased overgrowth by commensal flora and the deterioration of mycobacterial cell walls which may not impact on viability but can lead to the failure to retain stain and risk a false negative smear test. Laboratories that do not perform any mycobacteriological investigations on site must transfer specimens to their processing laboratory within one working day.

Transport on potentially infected clinical samples, see item **6**.

3. Initial investigations

Microscopy - auramine fluorescent staining
At least a six-day service for smear examination on appropriate samples should be provided during the normal working day. Out-of-hours smear testing for *M. tuberculosis* may compromise quality guidelines and is not recommended.

Microscopy should be performed and the result issued within one working day of receipt of the specimen by the processing laboratory. New positive results should be telephoned to a member of the clinical team responsible for the patient's care. It should be ensured that the lead TB nurse, lead clinician for TB and the CCDC are also informed within one working day, in accordance with locally agreed arrangements.

The laboratory must be accredited for this work, have an internal quality control (IQC) programme in place and show satisfactory performance in an external quality assurance (EQA) proficiency scheme. Laboratory staff should maintain proficiency in interpretation of smears through CPD and peer review (e.g., by an interpretative QA programme).

Molecular tests for *M. tuberculosis* complex may be used in appropriate circumstances; see item **8**.

4. Culture, isolation and identification

These should be completed within 21 days of the receipt by the source laboratory of a specimen which is subsequently shown to be positive for *M .tuberculosis* complex for at least 90% of specimens. (Although most non-tuberculous species will grow in this time, some are slower, e.g. *M. malmoense, M. xenopi*. Definitive identification of some of these species may also be more protracted.)

a) *Culture*

- Automated liquid culture should be done on all samples being processed for mycobacterial culture.
- This should be set up within one working day of receipt of the specimen (six day service)
- Conventional solid culture should also be set up on samples for tuberculosis investigation (see BSOP)

b) *Positive cultures*

- Acid fast bacilli isolates for identification and susceptibility testing should be sent to the appropriate Regional Centre for Mycobacteriology (RCM) within one working day of the culture becoming positive. If the Mycobacterial Growth Indicator Tube (MGIT), BD culture system is used, the culture should be incubated for a further 48h before despatch to achieve suitable biomass. The culture should reach the regional centre within one working day of despatch (Transport: see item **6**). At least one AFB isolate from each new patient should be identified to complex / species level, and suitable susceptibility tests performed if *M. tuberculosis* complex (MTBC).

c) *Identification*

- A nucleic acid amplification test (NAAT) or a hybridisation gene probe for MTBC should be done within one working day of a culture being shown to be positive or within one working day of receipt of a positive culture by the RCM.
- As necessary, other hybridisation probes and phenotypic identification tests will be done in the RCM.

d) *Reporting*

- The RCM should report receipt of the isolate and initial identification results to the source laboratory within one working day. The source laboratory should inform a member of the clinical team responsible for the patient's care, and ensure that the lead TB nurse, the lead clinician for TB and the CCDC are informed of new positive culture results and identification results from the RCM within one working day of the results being received, in accordance with locally agreed arrangements.

5. Laboratory facilities and expertise

- Safety: all culture work in primary diagnostic laboratories and RCMs should be done in a Category 3 containment facility which has HSE approval for the purpose; has a contingency plan for containment in the case of accidental dispersal; and has a continuity plan for service support in the event of Category 3 laboratory closure
- Laboratories must be accredited for mycobacteriology culture, have an IQC programme in place, and show satisfactory performance in an EQA proficiency scheme for every level of service provided, i.e., microscopy,

culture, identification, and susceptibility testing. Identification should be done in a laboratory with sufficient throughput to offer a daily service and maintain competence

- Consultant Medical Microbiologists/Clinical Scientists and BMS staff in laboratories providing *M. tuberculosis* culture should maintain their expertise and competence in laboratory testing and in the provision of advice on diagnosis, management and infection control aspects of tuberculosis.

Details of laboratory procedures for processing individual specimen types are given in the national BSOP (http://www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop40.pdf)

6. Transport of samples and cultures

(a) Patient samples

These should be transported by a system conforming to the requirements for potentially infected samples. This should be routine for general bacteriology samples.

(b) Positive cultures

Under current international transport regulations, these are category A cultures but an exemption clause allows them to be transported as category B material for clinical and diagnostic purposes if transported by road or rail. These cultures are assigned to UN 3373 (Diagnostic or Clinical specimens) and must bear the marking "Diagnostic Specimens" or "Clinical Specimens", and be packed to Packing Instructions P650. Substances packed and marked in accordance with Packing Instruction P650 are not subject to any other requirements in the regulations – thus there is no requirement for additional transportation documentation. Such specimens should not be transported via Royal Mail because any mail may be transported by air, which carries additional requirements.

7. Susceptibility testing

(a) Results

Results of susceptibility tests to primary therapeutic agents should be available within 30 days of the initial receipt in the source laboratory of a clinical sample from which *M. tuberculosis* complex is isolated; for each new patient case. The primary agents to be tested are:

- *isoniazid, rifampicin, pyrazinamide, ethambutol*
- these tests should be completed within 14 days of receipt of the isolate by the RCM
- The results should be reported to the source laboratory within one working day. If a new isolate of MTBC is found to be resistant to *isoniazid* and / or *rifampicin*, this should be telephoned by the RCM to the source laboratory.

- The source laboratory should inform a member of the clinical team responsible for the patient's care, and ensure that the lead TB nurse, the lead clinician for TB and the CCDC are informed of the results within one working day of the results being received, in accordance with locally agreed arrangements.

(b) Molecular detection

Molecular detection of resistance gene markers for *rifampicin* is useful in identifying possible MDR TB (see NICE guidance). These tests should be available within one working day of a test being agreed between the source laboratory and the RCM or another testing laboratory. The results (including the confirmation of the presence of *M. tuberculosis* complex) should be available within three working days of receipt of the specimen or isolate at the testing laboratory. Susceptibility testing should be done in an RCM with appropriate accreditation, IQC and EQA in place.

The laboratory requirements are set out in item **5**.

8. Molecular fingerprinting/typing

All new isolates of MTBC should undergo 15-loci MIRU-VNTR typing and the results entered in the national database within 14 days of receipt of the isolate at the RCM. Other molecular techniques may be used for particular investigations as appropriate.

These tests will be done at an RCM.

- The results should be reported to the source laboratory within one working day.
- The source laboratory should inform a member of the clinical team responsible for the patient's care, and ensure that the lead TB nurse, the lead clinician for TB and the CCDC are informed of the results within one working day of them being received, in accordance with locally agreed arrangements.

9. Reporting to HPA surveillance system

The laboratory that first isolates *M. tuberculosis* from a sample should report this to the HPA as part of CoSURV reporting to CDR. The RCM will also report to CDR all positive cases within one working day of confirming the positive results.

The RCM will report culture details including susceptibility results to *MycobNET* within one working day of the report being sent to the source laboratory.

10. Direct NAAT detection of *M. tuberculosis*

This is not part of the routine investigation of samples for *M. tuberculosis* but should be available for particular samples where there is a high suspicion of infection and a definitive diagnosis of *M. tuberculosis* is deemed to be urgent in clinical terms or for

health protection purposes (see NICE guidance). This test should be arranged between the requesting clinician and a suitably experienced local Medical Microbiologist, Clinical or Biomedical scientist who will liaise with the RCM or other laboratory providing the service. A result should be available within three working days of receipt of the sample by the testing site laboratory.

11. Immunodiagnostic tests

Two types of immunodiagnostic laboratory tests that can be performed on blood samples are currently commercially available – the detection of Interferon γ release from activated lymphocytes by ELISA and the detection of activated specific T-cells by ELISPOT. Currently available evidence indicates a potential role in the detection of latent tuberculosis; their role in the diagnosis of active disease is not established (see NICE guidance). Laboratories should seek access to these tests when clinicians consider the results would be helpful. There is insufficient evidence at present to set guidelines for laboratory provision, but any laboratory providing the tests should be accredited and have an IQC programme in place. An EQA scheme is not yet available. Generally, it would be expected that a clinician should receive a result within two weeks of submitting a sample for these tests.

12. Histopathology – of lymph nodes and other tissues samples

- Report within three days of receiving the sample when tuberculosis is suspected
- A member of the clinical team responsible for the patient's care should be informed, along with the local microbiology service of the relevant findings. It should be ensured that the lead TB nurse, the lead clinician for TB and the CCDC are informed of the results within one working day, in accordance with locally agreed arrangements.
- PCR may be useful for confirmation of identity of infection with *M. tuberculosis*, in particular if no suitable material has been sent, or is still available, for culture. However, there is currently no CE marked commercial kits available. (Also of NICE Guidelines).
- if *M. tuberculosis* infection is suspected at autopsy, samples should be sent for microbiological investigation before flooding the site with formalin

13. Audit trail

All laboratories involved in the provision of diagnostic services for tuberculosis should be able to show that they fulfil the criteria listed above for timeliness and completeness of reporting and quality assurance in reports for commissioners, SHA performance managers, the Healthcare Commission and Clinical Pathology Accreditation (CPA UK Ltd).

Brian Duerden
1 June 2006

Glossary

BMS	Biomedical Scientist
CCDC	Consultant in Communicable Diseases Control
CDR	Communicable Diseases Report
CPA	Clinical Pathology Accreditation
CPD	Continuing Professional Development
CSF	Cerebro-spinal Fluid
ELISA	Enzyme-Linked Immunosorbent Assay
EQA	External Quality Assurance
HSE	Health and Safety Executive
IQC	Internal Quality Control
MDR TB	Multi Drug Resistant Tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats
MTBC	*M. tuberculosis* complex
NAAT	Nucleic Acid Amplification Test
NBSOP	National Bacteriology Standard Operating Procedure
NICE	National Institute for Health and Clinical Excellence
RCM	Regional Centre for Mycobacteriology
RC Path	Royal College of Pathologists
PCR	Polymerase Chain Reaction
SHA	Strategic Health Authority