

Health Protection Agency

**RESPIRATORY AND SYSTEMIC INFECTION LABORATORY
(RSIL)**

Colindale

USER MANUAL

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Section 1.0 INTRODUCTION

The Respiratory and Systemic Infection Laboratory (RSIL) is situated in the Health Protection Agency's Microbiology Services Colindale, London. RSIL is a national and international reference laboratory for a number of bacteria responsible for respiratory and systemic infection and receives bacterial isolates and clinical samples from National Health Service, HPA and commercial laboratories throughout the UK. The diagnostic and reference samples received are analysed by a wide range of methodologies in accordance with customer needs. The laboratory includes the HPA Reference Units for streptococci, diphtheria, haemophili, mycoplasma, legionella, respiratory chlamydiae and bordetella. The first three of these units are WHO Collaborating Centres and as such provide laboratory and advisory support for national (and other) centres worldwide. The laboratory and its staff work closely with other colleagues in the HPA and beyond on the diagnosis and surveillance of infections within its remit.

The Respiratory and Systemic Infection Laboratory is resourced to provide a range of free of charge reference services to NHS and HPA laboratories in England and Wales. A number of referred services associated with primary diagnostic activity are also provided to NHS and HPA laboratories in England and Wales, and these are subject to a charge. Tests provided to laboratories in England and Wales, for which a charge is levied, are indicated as such in the relevant sections of the User Manual.

All submissions for both reference and referred testing from laboratories outside England and Wales are subject to charging. Please note this includes all work undertaken for Scotland, Northern Ireland, the Channel Islands and the Republic of Ireland. For enquiries regarding the level of charging for individual tests, please contact the laboratory director and/or Unit Heads for details.

Postal Address:

HPA - Respiratory and Systemic Infection Laboratory
Colindale
61 Colindale Avenue,
London NW9 5HT

DX Address:

HPA Colindale
DX6530011

Section 2.0 HOW TO OBTAIN SERVICES

2.1 HOURS OF SERVICE

The Laboratory is open from 9a.m. to 5p.m. Monday to Friday.
No routine services are available outside these hours.
The Laboratory is closed on public holidays.

A 24 hour on-call service is available for Identification and toxigenicity testing of *Corynebacterium diphtheriae* (Refer to 3.6).

Out of hours and emergency contact via the Colindale Duty Doctor System,
Tel: 020 8200 4400

2.2 SPECIMEN LABELLING, PACKAGING AND TRANSPORTATION

Transportation:

To ensure that specimens are processed as rapidly as possible, the receiving Unit (SDRU, APU, and HRU) and or the Laboratory name (RSIL) should be clearly identified on the address label. If in doubt, please use the telephone contacts directory listed in 2.7 to check on the appropriate identification.

A small but significant proportion of samples received by Colindale are poorly or inappropriately packaged. This often leads to samples leaking or being damaged during transport, therefore posing a serious risk to HPA staff handling them. HPA hopes to eliminate this risk by helping laboratories to understand basic packaging requirements.

The following guidelines are intended to cover the transport of clinical samples from humans, or cultures of micro-organisms isolated from such samples to another laboratory for diagnostic or other clinical testing within the U.K. where the micro-organisms suspected of causing the disease are all either Hazard groups 2, 3 or 4.

The terms Category A and Category B are limited to classifying samples / microbial cultures being transported to another laboratory.

Sample Description

Category A samples are known or suspected to contain a microbial agent with the following definition "an infectious substance which is transported in a form that if exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease to humans or animals" .The majority are Hazard Group 3 or 4

For practical reasons to allow referral / reference services to continue a limited number of Category A agents have exempted from being transported as Category A. These are Vero-cytotoxin

Packaging Requirement

Assign to UN2814
(Humans) Packaging
Instructions PI620
Supporting documentation
as per ADR
Transport as category A
ADR licensed courier

Assign UN3373 Packaging
instruction PI650
Send by courier, Royal

producing *Escherichia coli* (VTEC),
Mycobacterium tuberculosis and *Shigella*
dysenteriae

mail will NOT accept

Category B samples are those that do not meet
the definitions of Category A

Assign UN3373 Packaging
instruction P1650

Post or courier, Royal mail
WILL accept

These guidelines are not intended as a substitute for reading the advice given by
Department for Transport and Department of Health

Department of Health guidance on the transport of infectious substances may be
found at:

http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_075439

Labelling and Packaging:

All specimens/cultures sent to the RSIL must be packed in accordance with IATA
regulations 650/602.

Label the specimen/culture bottle with the name of the patient (or unique
identifier) and the laboratory number.

The top of the specimen/culture bottle must be fixed on firmly so that there is no
chance of leakage. It may be necessary to use parafilm to ensure that the top
remains on tight during transport. This will also prevent desiccation of the
specimen/culture in transit which will compromise successful culture. Wrap the
bottle in absorbent material and seal inside a bag. We will endeavour to process
the material if leakage occurs but this is likely to compromise the chance of
successful culture (if a primary specimen) and we will request the user to send us
an additional specimen)

Place the specimen/culture inside a leak proof plastic container with
enough absorbent material to be able to absorb all the contents of the bottle in
case of leakage.

Place the plastic container inside a fibreboard box.

Place the form between the plastic container and the outer cardboard box. DO
NOT PLACE IT INSIDE THE PLASTIC CONTAINER. In the event of
leakage/breakage the whole shipment may be destroyed without opening.

Specimens may be sent by Royal Mail or Courier. We recommend that to
minimise delays, specimens are sent by routine courier e.g DX or other
specialised courier.

Reporting incidents during transportation that may affect the safety of personnel

RSIL will report to users any leaking containers and improperly packaged parcels.
Repeated offences will be referred to the HPA Safety Committee for further
actions.

2.3 RSIL SPECIMEN SUBMISSION GUIDELINES

Specimens

All Clinical Specimens **MUST** be labelled with at least two of the following:-

- Surname/Forename or other Unique Patient Identifier
- Date of Birth
- Sender's Sample Number
- Date of Collection of Specimen

All Environmental specimens **MUST** be labelled with the following:-

- Unique Specimen Identifier/Sender's Sample Number
- Date of Collection of Specimen

Request forms

Request Forms * **MUST** match and include the above information on the sample

Plus Name and contact information of requester (vital for urgent requests)

- Tests required
- Specimen type and site
- Hazard group, if known, or suspected to be Category 3
- Sender's Sample Number
- Consultant or GP name (if applicable)

Request Forms should also have:-

- Date of dispatch
- Sex
- Relevant clinical information
- Date of onset
- Vaccination history (if relevant to test requested)
- NHS number

Please complete the forms in BLACK or BLUE pens (NOT red or any other colour).

Failure to comply with our specimen submission guidelines may lead to specimen rejection and/or delay of reports.

Requests for work on presumptive isolates that fall into ACDP Hazard Group 3 MUST be clearly marked to show the findings of the sending laboratory.

If an additional test is required, please discuss with the Unit Head or Laboratory by telephone before the sample is dispatched. The turnaround time in this instance will vary.

Full clinical information on the accompanying request forms will ensure that all required tests and interpretations are provided (see above). Please use the current versions of SDRU, APU and HRU request forms where possible and complete all relevant sections. All RSIL request forms may be downloaded from the HPAI website: www.hpa.org.uk/SRMTTests

*Laminated request forms suitable for repeated photocopying have been sent to many of our customers. To help ensure accurate data entry onto our Laboratory

information management system the forms are pre-addressed and bar-coded. This also helps us to ensure reports are dispatched to the appropriate address.

If you have not received or require additional laminated request forms, please contact Tony McNiff on 020 8327 6533 or e-mail: tony.mcniff@hpa.org.uk

2.4 URGENT SPECIMENS

If a specimen result is required urgently, prior telephone contact with the receiving unit or Laboratory Director or Laboratory Manager will ensure priority. Always clearly mark 'URGENT' on the request form.

2.5 KEY FACTORS AFFECTING SPECIMEN PERFORMANCE

The time taken to perform bacterial identification and typing tests is dependent on the receipt of pure cultures. Cultures that require purification or that cannot be retrieved because they are no longer viable may increase turnaround time significantly.

If a specimen is received in RSIL which is unsuitable for examination, we will endeavour to contact the sender to discuss the problem.

If a specimen is submitted to RSIL for an investigation that we do not offer, we will return the sample to the sender (if they are in the UK) together with a report explaining the reasons for the sample's rejection.

TURNAROUND TIMES

Turnaround times are shown in calendar days and are based on average monthly figures for processing 75% of specimens. They are dependant upon receipt of an appropriate sample and fully completed specimen request form as described above and may vary dependent upon the clinical or public health urgency.

2.6 CPA ACCREDITATION & EQA SCHEMES

Our CPA Accreditation Certificate can be found at the following website address: http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947329857

There are no NEQAS schemes for the specialist assays performed within RSIL. We do undertake an extensive IQA programme and, whenever possible, participate in formal and informal international EQAs with similar laboratories overseas. Overall performance is satisfactory. We can provide more specific information on EQA on separate request to the individual Unit Heads.

2.7 **LIST OF RSIL CONTACT DETAILS**

Colindale Main Switchboard: 020 8200 4400
RSIL Fax No: 020 8205 6528

Acting Director: Tim Harrison PhD, SRCS
020 8327 7222
Email: tim.harrison@hpa.org.uk

Director's PA: Mrs Joanna Tambouridou/Fatima Faki
020 8327 7219

Security of Information Officer: Mrs. Joanna Tambouridou
Email: joanna.tambouridou@hpa.org.uk

General Office:
020 8327 7330

Laboratory Manager: Mrs Nita Doshi BTech FIBMS CSci
020 8327 7235
Email: nita.doshi@hpa.org.uk

IF THERE IS A PROBLEM, OR YOU ARE NOT SATISFIED WITH THE SERVICE YOU HAVE RECEIVED:

In the first instance contact appropriate Unit Head and subsequently contact Director or Laboratory Manager.

STREPTOCOCCUS/DIPHThERIA REFERENCE UNIT: 020 8327 7288/89

Head: Dr Androulla Efstratiou PhD, SRCS
020 8327 7270
Email: androulla.efstratiou@hpa.org.uk

ATYPICAL PNEUMONIA UNIT: 020 8327 7327/31

Head: Dr Tim Harrison PhD, SRCS
020 8327 6906
Email: tim.harrison@hpa.org.uk

HAEMOPHILUS REFERENCE UNIT: 020 8327 7331

Head: Dr Mary Slack MA MB BChir FRCPath.
020 8327 6091
Email: mary.slack@hpa.org.uk

Our endeavour is to be responsive to the changing needs of all users of both our routine and reference microbiology services. We welcome comments on how we can improve the provision of these services. Please contact any of the above.

Section 3.0 **STREPTOCOCCUS & DIPHTHERIA REFERENCE UNIT**

The **Streptococcus and Diphtheria Reference Unit (SDRU)** comprises the HPA Reference Units for streptococci (including pneumococci) and diphtheria. The reference services offered are listed below.

3.1 **LANCEFIELD GROUP A STREPTOCOCCI (*STREPTOCOCCUS PYOGENES*)**

DETAILS OF SERVICES OFFERED

The Streptococcus and Diphtheria Reference Unit (SDRU) offers genetic and serological classification and epidemiological typing of Group A streptococci (GAS), *Streptococcus pyogenes*.

The laboratory requests submission of ALL GAS isolated from blood culture or other normally sterile sites as part of the surveillance of invasive disease due to GAS.

Typing of GAS is useful in the investigation of both community and hospital outbreaks of GAS infection. Scarlet and rheumatic fevers and post-streptococcal nephritis are associated with particular M/*emm* types; typing of isolates from such cases may assist in confirming these clinical diagnoses.

The laboratory is pleased to discuss and advise upon particular clinical or epidemiological problems and outbreak investigations; ask for Dr Androulla Efstratiou (020 8327 7270) in the first instance.

TURNAROUND TIME FOR *EMM* GENE TYPING/OFT-TYPING:

The turnaround time is 12 days (see 2.5)

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request forms (section 2.3) and supply a pure culture on blood or chocolate agar slopes.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to SDRU by telephone (020 8327 7270/7289) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

OTHER INFORMATION

- Typing of GAS is based upon the detection of T protein antigens, opacity factor, and determination of the M-protein by sequencing the *emm* gene.
- The T protein is an epidemiological marker and is used in the 'first stage' of GAS typing. Opacity factor is closely related to the M protein and is present amongst some GAS carrying specific M-protein antigens. The M protein is the major virulence factor of GAS and is encoded by the *emm* gene.
- *Emm* genotyping (genotypic detection of the *emm* gene, which encodes M protein) is performed by sequencing the 5' hypervariable region of the *emm* gene. More than 130 *emm* sequence types, ST(s) have been identified and information on these types can be found at: <http://www.cdc.gov/ncidod/biotech/strep/emmtypes.htm>. Results are reported as an *emm*

sequence type, which usually correlates with the M protein type, for example: *emm* ST12 = M type 12.

- There are over 100 well-defined M-protein antigens; the most common serotypes currently associated with clinical disease within the UK are MT1, MT3, MT12, MT28 and MT89. Serological GAS typing is only performed in exceptional circumstances, where atypical correlations between the T pattern, opacity factor and *emm* sequence type are obtained.

Virulence /toxin genotyping (identification of the streptococcal pyrogenic exotoxin and other virulence genes for group A streptococci using PCR is available but **only after discussion and agreement** with Dr Androulla Efstratiou (020 8327 7270).

REFERENCES

Further information on group A streptococcal infection is available from the HPA website:
<http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1202487089439>

Group A streptococcus typing:

Efstratiou A, Sriskandan S, Lamagni T, Whatmore A. β -Haemolytic Streptococci. Chapter 1. In: Principles and Practice of Clinical Bacteriology. Editors: Stephen H Gillespie, Peter M Hawkey. John Wiley and Sons Ltd pubs, England 2006

Johnson DR, Kaplan EL, Sramek J, Bicova R, Havlicek J, Havlickova H, Motlova J, Kriz P. Laboratory diagnosis of group A streptococcal infections. World Health Organisation, Geneva, 1996. ISBN 92 4 254495 7

Beall, B., R. Facklam, and T. Thompson. 1996. Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci, *J.Clin.Microbiol.*, 1996; **34**:953-958.

Johnson DR, Kaplan EL, VanGheem A, Facklam RR, Beall B. Characterization of group A streptococci (*Streptococcus pyogenes*): correlation of M-protein and *emm*-gene type with T-protein agglutination pattern and serum opacity factor. *J Med Microbiol*, 2006;55:157-164

Facklam R, Beall B, Efstratiou A and 13 other authors. *emm* typing and validation of provisional M types for group A streptococci. *Emerg Infect Dis*, 1999;5:247-253

Igwe EI, Shewmaker PL, Facklam RR, Farley MM, van Beneden C, Beall B. Identification of superantigen genes *speM*, *ssa*, and *smeZ* in invasive strains of beta-hemolytic group C and G streptococci recovered from humans. *FEMS Microbiol Lett.*, 2003, 229:259-64

Luca-Harari B, Darenberg J, Neal S, Siljander T, Strakova L, Tanna A, Creti R, M, Tassios PT, van der Linden M, Straut M, Vuopio-Varkila J, Bouvet A, Efstratiou A, Schalén C, Henriques-Normark B; the Strep-EURO study group, Jasir A. Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol.* 2009 Apr;47 (4):1155-65.

Lamagni TL, Neal S, Keshishian C, Alhaddad N, George R, Duckworth G, Vuopio-Varkila J, Efstratiou A. Severe *Streptococcus pyogenes* infections, United Kingdom, 2003-2004. *Emerging Infectious Diseases*, 2008,14:202-209.

3.2 **LANCEFIELD GROUP B STREPTOCOCCI (GBS), STREPTOCOCCUS AGALACTIAE**

DETAILS OF SERVICES OFFERED

SDRU offers serological classification and epidemiological typing of Lancefield group B streptococci (GBS).

GBS are a relatively common cause of puerperal and neonatal infections, which may be nosocomially acquired. Epidemiological typing may assist in the investigation of apparent clusters or outbreaks of GBS sepsis in all age groups.

The laboratory requests submission of ALL group B streptococci isolated from blood culture or other normally sterile sites of neonates as part of the surveillance of invasive disease due to GBS in this age group (0-90 days).

GBS may also cause systemic infection in adults (non-pregnancy related). We are pleased to receive blood culture or other "sterile site" isolates for typing and surveillance purposes.

The laboratory is happy to discuss and advise upon particular clinical or epidemiological problems and outbreak investigations, ask for Dr Androulla Efstratiou (020 8327 7270) in the first instance.

TURNAROUND TIME

The turnaround time is 10 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply a pure culture on blood or chocolate agar slopes.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to SDRU by telephone (020 8327 7270/7222/7289) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

OTHER INFORMATION

- The serological classification of GBS is based upon the identification of polysaccharide and protein antigens. There are currently ten polysaccharide antigens designated, Ia, Ib, II, III, IV, V, VI, VII, VIII, IX.
- The most common polysaccharide antigens are serotypes Ia, Ib, II or III. Serotype III is most commonly associated with neonatal infections.
- Molecular typing of GBS is available upon written request and discussion with the Reference Laboratory.

REFERENCES

Further information on group B streptococcal Infection is available from the HPA website:
<http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1202487089475>

Group B streptococcus typing:

Efstratiou A, Sriskandan S, Lamagni T, Whatmore A. β -Haemolytic Streptococci. Chapter

1. In: Principles and Practice of Clinical Bacteriology. Editors: Stephen H Gillespie, Peter M Hawkey. John Wiley and Sons Ltd pubs, England 2006

Colman G. Typing of *Streptococcus agalactiae* (Lancefield group B). European: J Clin Microb & Infect Dis, 1988;7:226-231

3.3 LANCEFIELD GROUP C AND GROUP G STREPTOCOCCI

DETAILS OF SERVICE OFFERED

We are no longer able to offer the above reference service on a “routine basis”.

However, the service will continue to be available for urgent public health including outbreak, investigations and in other relevant clinical circumstances but **only after discussion and agreement** with Dr Androulla Efstratiou (020 8327 7270).

Group C and G streptococci may cause both nosocomial (e.g.: burns unit cross-infection episodes) or institutional outbreaks.

Group C and G streptococci may also cause systemic infections in adults and in particular the taxonomy of group C streptococci may have clinical implications, as (with the exception of the human species *S. dysgalactiae subsp equisimilis*) they are all primarily animal species.

Group C streptococci of animal origin e.g.: *S. equi subsp zooepidemicus* may cause severe systemic infections in humans. Such infections may occur in clusters and have been associated with the consumption of raw milk.

The current typing methodology for these streptococci is based upon the detection and sequence of the *emm* gene, which encodes the major virulence factor, the M protein. The human group C and group G streptococci carry M protein antigens that are both serologically and genotypically distinct from those carried by the Lancefield group A streptococcus and are useful epidemiological markers.

Emm sequencing is based upon the heterogeneity of the 5' end of the *emm* gene which gives rise to the different sequence types. More than 40 *emm* types of group C and group G have been identified and information on these types can be found at: http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm

TURNAROUND TIME

This is not a routine service and turnaround times will therefore vary depending on the nature of the enquiry and the complexity of the investigation required (see 2.5).

HOW TO OBTAIN SERVICE

After discussion with Dr Androulla Efstratiou (020 8327 7270), use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply a pure culture on blood or chocolate agar slopes.

REFERENCES

Further information on group C/G streptococcal Infection is available from the HPA website: <http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1202487089506>

Group C and G typing:

Efstratiou A, Sriskandan S, Lamagni T, Whatmore A. β -Haemolytic Streptococci. Chapter 1. In: Principles and Practice of Clinical Bacteriology. Editors: Stephen H Gillespie, Peter M Hawkey. John Wiley and Sons Ltd pubs, England 2006

3.4 **PNEUMOCOCCI (STREPTOCOCCUS PNEUMONIAE)**

DETAILS OF SERVICES OFFERED

SDRU offers serological classification and epidemiological typing of pneumococci.

There are currently over 90 different pneumococcal capsular polysaccharide serotypes based upon the Danish classification scheme.

We request submission of ALL blood, CSF and other "sterile site" isolates from episodes of invasive disease for this national surveillance function of our laboratory. Results of serotyping of these isolates are shared with the Immunisation, Hepatitis and Blood Safety Department of Health Protection Services: Colindale and contribute to National Surveillance.

Presently available and likely future pneumococcal vaccines contain specific, common, capsular polysaccharide antigens. For this reason it is important to monitor the capsular type distribution of isolates from invasive disease in both adults and children.

Capsular typing of pneumococci may also be helpful in the investigation of instances of suspected cross-infection in hospitals, other residential institutions and day care centres (or similar) for children.

The laboratory is happy to discuss and advise upon particular clinical or epidemiological problems and outbreak investigations, ask for Dr Androulla Efstratiou (020 8327 7270) or Dr Mary Slack (020 8327 6091) in the first instance.

SDRU liaise closely with the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) in studies of antibiotic resistant pneumococci.

TURNAROUND TIME:

The turnaround time is 10 days (see 2.5). In period of very heavy workload priority will be given to isolates referred from children (see below).

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply a pure culture on blood or chocolate agar slopes.

Specimen submissions regarded by the sending laboratory as urgent should be notified to SDRU by telephone (020 8327 7270//7289) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

OTHER INFORMATION

- Some of the 90 serogroups/serotypes may be divided into specific serotypes or subtypes i.e.; types carrying the same number but different letters, e.g. 6A, 6B, 9A, 9L, 9V. Subtyping is undertaken on all sterile site isolates, in particular for any episode of systemic infection associated with possible vaccine failure.
- The more common serotypes are currently (October 2011), in order of prevalence, 7F, 3, 19A, 8, 22F, 1, 6C and 15A. RSIL, together with the

Immunisation, Hepatitis and Blood Safety Department of Health Protection Services: Colindale, are actively following up all cases of invasive pneumococcal disease in the childhood age groups targeted for vaccination in order to ascertain immunisation history and determine vaccine effectiveness. This applies to anyone born after 4th September 2004.

REFERENCES

Further information is available from the HPA website:

<http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1203008863939>

Streptococcus pneumoniae serotyping:

Lund E, Henrichsen J. Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*. Methods in microbiology, vol. 12. Academic Press, London, New York and San Francisco 1978

3.5 IDENTIFICATION OF STREPTOCOCCI AND RELATED GENERA

DETAILS OF SERVICES OFFERED

SDRU offers a referred (charged for) taxonomic identification service for streptococci and other related gram positive, catalase negative genera from systemic and other significant infections.

However, a free-of-charge reference service will continue to be available for urgent public health investigations, outbreaks and incident management, either nosocomial or community based. This should be discussed and agreed with Dr. Androulla Efstratiou (020 8327 7270).

The laboratory is also happy to discuss and advise upon particular clinical or epidemiological problems, ask for Dr Androulla Efstratiou (020 8327 7270) in the first instance.

SDRU liaise closely with the Antibiotic Resistance and Monitoring Reference Laboratory (ARMRL) in studies of the antibiotic susceptibility of referred isolates, if asked to do so by the requesting laboratory.

TURNAROUND TIME

The turnaround time is 12 days (see 2.5).

Isolates that needs MIC/MBC will be referred to the Antibiotic Resistance Monitoring Reference Laboratory (ARMRL) Isolates that are not streptococci and may be an enterococcus or a Gram positive rod are referred to the Laboratory of HealthCare Associated Infections (LHCAI). The turnaround time in this instance will vary.

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply a pure culture on blood or chocolate agar slopes.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to SDRU by telephone (020 8327 7270/7289) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

OTHER INFORMATION

- An identification scheme incorporating updated taxonomic methodologies is used.
- Updated nomenclature based upon both the UK and USA classification schemes is used to subdivide streptococci into many species e.g. the 'sanguinis group' is subdivided into *S.sanguinis*, *S.parasanguinis*, *S.gordonii* and *S.cristatus*; *S.australis*; the 'anginosus group' is subdivided into *S.anginosus*, *S.constellatus subsp constellatus*, *S.intermedius* and *S.constellatus subsp pharyngis*.

REFERENCES

Further information is available from the HPA website:

<http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1202487089434>

3.6 **IDENTIFICATION AND TOXIGENICITY TESTING OF CORYNEBACTERIUM DIPHtherIAE AND OTHER POTENTIALLY TOXIGENIC CORYNEBACTERIA (C. ULcerANS AND C. PSEUDOTUBERCULOSIS)**

DETAILS OF SERVICES OFFERED

This service is available 24 hours a day, seven days a week. For advice on laboratory diagnosis of diphtheria and/or submission of samples to Colindale during normal working hours contact Dr Androulla Efstratiou on 020 8327 7270 or Miss Gina Mann on 020 8327 7289.

Out of hours and weekends, advice and service is available via the HPA Colindale main switchboard operator/Vodafone answering service (Tel. 020 8200 4400) who will access the on-call staff by mobile telephone.

Cultures should be submitted to the laboratory by courier (if deemed urgent), but only after establishing contact with SDRU (as described above) to inform them that a culture is on its way.

Advice on immunisation against diphtheria, provision of vaccine and provision of diphtheria antitoxin for **therapeutic** use is available from the Immunisation, Hepatitis and Blood Safety Department of HPA-Colindale on 020 8200 6868 (Dr. M. Ramsay, 020 8200 7085 or Joanne White 020 8327 7446) during normal hours. Out of hours via HPA-Colindale Duty Doctors on 020 8200 4400.

Toxigenic *C.diphtheriae* is very uncommon within the UK and is almost always imported. A travel and immunisation history should **always** be obtained from suspected cases of diphtheria and, if feasible, their close contacts.

Some strains of *C.ulcerans* (and very rarely *C.pseudotuberculosis*) may produce diphtheria toxin and the illness caused may present as clinical diphtheria. Such infections should be treated as diphtheria with the important proviso that person-to-person transmission is extremely rare. Infection is usually acquired from raw milk and/or contact with farms and farm animals and also perhaps close contact with companion animals.

UK microbiological laboratories are encouraged to submit all isolates of *C.diphtheriae* and other potentially toxigenic corynebacteria to SDRU for surveillance and monitoring purposes. The unit is a designated WHO Collaborating Centre for reference and research on diphtheria.

TURNAROUND TIME

Turnaround times vary according to the degree of clinical and public health urgency. If the situation warrants it, a rapid result for the detection of diphtheria toxin can be issued within 24 hours of receipt of a pure culture. All results are communicated by telephone. Under normal circumstances, a final written report is issued within 5 days of receipt and all interim results are given by telephone usually within 24 hours (see 2.5).

Isolates that are cystinase negative may be referred to the Laboratory of HealthCare Associated Infections (LHCAI) for full identification. The turnaround time in this instance will vary.

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply a pure culture on blood or Loeffler slopes.

NB. Do not delay submission if you do not have the requisite forms or slopes.

Specimen submissions regarded by the sending laboratory as especially important or urgent should always be notified to SDRU by telephone as described above.

OTHER INFORMATION

- PCR for detection of the diphtheria toxin gene is not undertaken routinely. The gold standard test for detection of toxigenicity is the phenotypic Elek test which is the key test used by the reference unit. PCR can be undertaken but only in urgent circumstances and only after discussion with the Unit Head (Dr Efstratiou). The definitive and final result is always based upon the Elek phenotypic test.

REFERENCES

Further information is available from the HPA website:

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1191942152928?p=1191942152928>

Corynebacterium diphtheriae/diphtheria:

Efstratiou A, Maple PAC. Manual for the Laboratory Diagnosis of Diphtheria. 1994. World Health Organisation Regional Office for Europe. ICP/EPI 038 (C)

Efstratiou A, Engler KH, Mazurova IK, Glushkevich T, Vuopio-Varkila J, Popovic T. Current approaches to the laboratory diagnosis of diphtheria. J Infect Dis, 2000,181(Suppl 1):S138-S145

3.7 **DIPHTHERIA IMMUNITY/VACCINATION STUDIES**

DETAILS OF SERVICE OFFERED

SDRU offers a referred (charged for) service for the determination of serum antibodies to diphtheria toxin.

Diphtheria immunity status is determined by a tissue culture toxin neutralisation assay of serum antibodies specific for diphtheria toxin. Test plates are incubated for up to six days before a final report is issued. This assay is more reliable than ELISA, particularly for detecting susceptible individuals.

Results are reported in International Units/ml and classified as:

- Individual is susceptible: <0.016 IU/ml;
- Levels conferring some protection: 0.016 – 0.09 IU/ml;
- Protective levels: 0.1 – 0.9 IU/ml;
- Levels conferring long-term protection: ≥ 1.0 IU/ml.

TURNAROUND TIME

Tests are batched every three weeks, unless a sample is deemed to be urgent. The turnaround time is 21 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply not less than 200 μ l of serum in a sterile container.

Please supply details of vaccination history (if known) with all requests plus relevant clinical details.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to SDRU by telephone (020 8327 7270/7289) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

REFERENCES

Further information is available from the HPA website:

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1191942152928?p=1191942152928>

Diphtheria serology:

Efstratiou A, Maple PAC. Manual for the Laboratory Diagnosis of Diphtheria. 1994. World Health Organisation Regional Office for Europe. ICP/EPI 038 (C)

3.8 **TETANUS IMMUNITY**

DETAILS OF SERVICE OFFERED

SDRU offers a referred (charged for) service for the determination of serum antibodies to tetanus toxin.

Tetanus immunity status is determined by an ELISA for serum antibodies specific for tetanus toxin.

Provided serum is collected prior to therapeutic administration of antitoxin, determination of tetanus immunity status can be useful in confirming a clinical diagnosis of tetanus. Absence of detectable antibody or levels below or close to the minimum protective level lend support to the clinical diagnosis whilst higher levels do not.

Results are reported in International Units/ml. Minimum protective level is presently defined as 0.1 IU/ml.

TURNAROUND TIME

According to demand tests are normally batched every three weeks, on occasion less frequently. If a sample is deemed to be urgent, a same day result can be produced.

The turnaround time is 21 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Streptococcus and Diphtheria Reference Unit request form (See 2.3) and supply not less than 200 µl of serum in a sterile container.

Please supply details of vaccination history (if known) with all requests plus relevant clinical details.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to SDRU by telephone (020 8327 7270/7289) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

REFERENCES

Further information is available from the HPA website:

http://www.hpa.org.uk/infections/topics_az/tetanus/menu.htm

Tetanus serology:

Budd R, Budd C, Alcock F, George R, Broughton K, Bradwell A. An enzyme immunoassay for measuring IgG antibodies to tetanus toxoid. 14th European Congress of Clinical Microbiology and Infectious Diseases, Prague, Czech Republic, 1-4 May 2004. Clin Microbiol & Infect, 2004, Supplement 3; 1632

Section 4.0 ATYPICAL PNEUMONIA UNIT

The Atypical Pneumonia Unit (APU) offers a range of reference, confirmatory and referred (charged) tests for the following organisms: legionella, bordetella mycoplasma/ureaplasma, respiratory chlamydia, and bartonella.

Where services are offered as reference services (i.e. free of charge) for customers in England and Wales, they are offered on the assumption that the primary diagnostic work has been undertaken already. Evidence of such primary testing should be noted on the specimen request forms or a charge will be levied.

Note: Turnaround times and other information given below apply to 'routine' referrals only. All urgent and/or outbreak investigation specimens will be processed on receipt after prior telephone discussion with the Unit.

4.1 LEGIONELLA

DETAILS OF SERVICES OFFERED

The APU offers a range of reference and confirmatory tests useful in the investigation of individual cases and outbreaks of legionella infection. These are urinary antigen detection, antigen or genome detection and/or culture from clinical material, identification and where appropriate epidemiological typing of referred isolates.

The laboratory is happy to discuss and advise upon particular diagnostic and clinical problems and laboratory aspects of epidemiological investigations, ask for Dr Tim Harrison (020 8327 6906) or Dr Norman Fry (020 8327 6776) in the first instance.

The laboratory works very closely with the colleagues responsible for national surveillance and reports all clinically relevant results to them. National surveillance of Legionnaires' disease is undertaken by Dr Nick Phin who may be contacted on 020 8327 6989.

If samples are submitted as part of an outbreak or incident investigation please ensure this is made clear on the request form and the relevant Health Protection Unit is identified.

LEGIONELLA PNEUMOPHILA SGP 1 URINARY ANTIGEN DETECTION

The laboratory encourages and requests the submission of **all** urine specimens for reference and confirmatory testing which have been found to be positive, equivocal or unexpectedly negative using commercially available *L. pneumophila* urinary antigen kits. Due to the specificity of the RSIL "in-house" assay we use, not only does this allow us to confirm the submitting laboratory's finding, but also allows us to determine if the infecting strain was *L. pneumophila* serogroup 1 (mAb2+ve)¹ or not. Samples submitted as positive but found to be negative in our in-house assay will be re-examined using commercial kits to determine if they are genuinely positive (but the infecting strain is either *L. pneumophila* serogroup 1 mAb2-ve or non-serogroup 1) or are falsely positive.

¹ Most human disease is caused by a subset of *L. pneumophila* serogroup 1 strains that have a virulence associated epitope detected by a monoclonal antibody (designated mAb2). This mAb is used in the RSIL assay.

- Specimens should be collected as soon as possible after onset of symptoms. Antigen excretion typically continues for 7 - 14 days after onset but may continue for longer in severe cases.
- Almost any urine specimen is suitable for examination, but clean-catch, mid-stream, early morning samples, with or without preservative, are most suitable for examination. Grossly contaminated samples may not be suitable.

TURNAROUND TIME

Under normal circumstances these tests are processed weekly. The turnaround time is 8 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply not less than 2 ml urine in a sterile container

Please supply details of the assay used and results obtained from primary testing otherwise you will be charged for these tests

Specimen submissions regarded by the sending laboratory as urgent should be notified to APU by telephone (020 8327 7331/6906/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

LEGIONELLA GENOME DETECTION AND CULTURE FROM CLINICAL MATERIAL

These services are provided to assist diagnostic laboratories in the investigation of outbreaks of legionella infection and other incidents of potential Public Health significance. Submission of any lower respiratory tract samples from **all** *L. pneumophila* urinary antigen positive patients is particularly encouraged as such samples are likely to yield useful epidemiological typing data.

These services are not usually offered for primary diagnosis although *L. pneumophila* PCR may be requested as a referred (charged) service after discussion with the laboratory.

The most commonly referred specimens are sputum and bronchoalveolar lavage (BAL), though the laboratory is pleased to receive any clinical specimens for examination from patients with **other evidence of legionella infection**. Where appropriate samples will also be examined by direct immunofluorescence for the presence of *L. pneumophila*.

Where appropriate (see above) samples may also be tested by PCR for evidence of *L. pneumophila* DNA.

TURNAROUND TIME

As these services are primarily offered to support outbreak investigation, specimens will be accorded a high level of priority. Telephone discussion with the Laboratory in advance of submission will ensure this is the case (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply lower

respiratory tract samples (sputa, BAL, tracheal aspirates etc.) and other clinical samples in a sterile container – as much volume as possible.

Please supply details of the assay used and results obtained from primary testing.

Specimen submissions regarded by the sending laboratory as urgent should be notified to APU by telephone (020 8327 7331/6906/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

IDENTIFICATION AND EPIDEMIOLOGICAL TYPING OF LEGIONELLA ISOLATES

The laboratory encourages submission of **all** legionellae isolated from clinical material for confirmation and national surveillance purposes. We are also happy to receive any putative legionella isolate from clinical and other sources which is of public health significance.

Currently, there are 54 named species of legionellae comprising more than 60 serogroups. *L. pneumophila*, the most frequently encountered species, comprises 16 serogroups.

Identification is initially made by nutritional characteristics and serological reactivity. For difficult species, the above methods are augmented by genotypic methods.

Specialised typing methodologies including monoclonal antibody subgrouping and DNA-sequence based typing are available as part of epidemiological investigations or, when appropriate, **after discussion** with the laboratory.

TURNAROUND TIME

Turnaround times will vary depending on the nature of the enquiry and the complexity of the investigation required. Priority will **always** be given to clinical and outbreak associated isolates (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply pure cultures on either BCYE medium or a dense suspension in sterile distilled water or Page's saline.

Please supply details of the assay used and results obtained from primary testing.

Specimen submissions regarded by the sending laboratory as urgent should be notified to APU by telephone (020 8327 7331/6906/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

LEGIONELLA PNEUMOPHILA SEROLOGY

The APU will undertake *L.pneumophila* serogroup 1 serology at the request of HPUs in support of outbreak or incident investigations. This service is not offered for primary diagnosis or for the confirmation of results obtained using commercial assays.

OTHER INFORMATION

The APU is able to provide extensive laboratory support for any outbreak investigations after prior discussion with the Unit.

REFERENCES

Birtles RJ, Harrison TG, Samuel D, Taylor AG. Evaluation of urinary antigen ELISA for diagnosing *Legionella pneumophila* serogroup 1 infection. J Clin Pathol 1990;43:685-90

Helbig JH, Uldum SA, Luck PC, Harrison TG. Detection of *Legionella pneumophila* antigen in urine samples using the Binax NOW immunochromatographic assay and comparison with both Binax *Legionella* Urinary antigen enzyme immunoassay (EIA) and Biotest *Legionella* Urin antigen (EIA). J Med Microbiol 2001;50:1-8

Harrison TG, Doshi N. Evaluation of the Bartels Legionella Urinary Antigen Enzyme Immunoassay Eur J Clin Microbiol Infect Dis 2001;20:738-40

Harrison TG, Uldum S, Alexiou-Daniel S, Bangsberg J, Bernander S, Drasar V, Etienne J, Helbig J, Lindsay D, Lochman I, Marques T, de Ory F, Tartakovskii I, Wewalka G, Fehrenbach F. A multicenter evaluation of the Biotest legionella urinary antigen EIA. Clin Microbiol Infect 1998;4:359-64

4.2 BORDETELLA PERTUSSIS AND OTHER BORDETELLA

DETAILS OF SERVICES OFFERED

The APU offers a range of reference, enhanced surveillance, and referred tests useful in the investigation of individual cases and outbreaks of pertussis infection. These are serology, genome detection by PCR (for *B.pertussis*), identification and, where appropriate, phenotypic and genotypic characterisation of isolates, including other *Bordetella* spp.

The laboratory is happy to discuss and advise upon particular diagnostic and clinical problems and laboratory aspects of epidemiological investigations, ask for Dr. Norman Fry (020 8327 6776) or Dr Tim Harrison (020 8327 6906) in the first instance.

The laboratory works very closely with the Immunisation, Hepatitis and Blood Safety Department of Health Protection Services: Colindale and reports all clinically relevant results to them. National surveillance of pertussis is undertaken by Dr Gayatri Amirthalingam who can be contacted on 02083276407.

BORDETELLA PERTUSSIS SEROLOGY

The APU offers a referred (charged for) serological service for the diagnosis of pertussis. Anti-pertussis toxin (PT) IgG antibody levels are determined using an in-house EIA.

This service is offered where the following criteria are met: single samples taken >2 weeks after onset for any individuals with a history of prolonged cough.

Please note: This service is **NOT** suitable for assessment of immune status.

TURNAROUND TIME

Under normal circumstances sera are processed weekly and sera found to be positive on screening are re-assayed to determine their endpoint. The turnaround time is 10 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply not less than 400 µl serum in a sterile container

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6906/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

BORDETELLA PERTUSSIS GENOME DETECTION

Currently two PCRs are undertaken on each sample. One is directed against the *B. pertussis* pertussis toxin (PT) promoter and the second is directed against the insertion element IS481, which occurs in *B. pertussis*, *B. holmesii* and some strains of *B. bronchiseptica* and *B. parapertussis*.

This service is offered free of charge in England and Wales where the following criteria are met: Pernasal swabs or Nasopharyngeal aspirates from an acutely ill child age ≤ 12 months admitted to PICU or paediatric ward with respiratory illness

compatible with pertussis. It is offered as a referred (charged) service for colleagues in Scotland and Northern Ireland.

Correct specimen types for this test are pernasal swab (with flexible wire shaft and rayon/Dacron/nylon bud) or nasopharyngeal aspirate. Please do not submit nose, nasal or throat swabs.

Do not submit samples that have been collected more than 72 hours previously without first discussing this with the laboratory.

TURNAROUND TIME

We try to process samples daily and therefore results will be available the same day for specimens received by 10am. **An accurate contact telephone number for receipt of results must be provided.**

Written confirmation of telephone reports will be provided, usually within 6 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply Pernasal swabs (with flexible wire shaft and rayon/Dacron/nylon bud) or NPA (nasopharyngeal aspirate, not less than 200 µL) in a sterile container.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6906/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

THE IDENTIFICATION AND CHARACTERISATION OF *BORDETELLA PERTUSSIS* ISOLATES

The laboratory encourages submission of all *Bordetella pertussis* isolates for confirmation and national surveillance purposes.

TURNAROUND TIME

The turnaround time is 10 days

IDENTIFICATION AND CHARACTERISATION OF *BORDETELLA* SPECIES

The APU is pleased to receive putative isolates of *Bordetella* spp. from any human source. These will be fully characterised by a range of phenotypic and genotypic methods.

TURNAROUND TIME

Turnaround times will vary depending on the nature of the enquiry and the complexity of the investigations required (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply pure cultures on a suitable agar slope or growth from a plate in charcoal transport medium

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6906/7222) to ensure that the appropriate level of priority is accorded to

these specimens immediately upon receipt.

OTHER INFORMATION

- In conjunction with the Immunisation, Hepatitis and Blood Safety Department of Health Protection Services: Colindale, the APU will provide laboratory support for any investigations into pertussis outbreaks. Contact the laboratory before sending any samples.

REFERENCES

Further information is available from the HPA website:

http://www.hpa.org.uk/infections/topics_az/whoopingcough/menu.htm

Fry NK, Duncan J, Wagner K, Tzivra O, Doshi N, Litt D, Crowcroft N, Miller E, George RC, Harrison TG. Role of PCR in the diagnosis of pertussis infection in infants: 5 years experience of provision of a same-day real-time PCR service in England and Wales from 2002 to 2007. *J Med Microbiol* 2009;58:1023-1029.

4.3 MYCOPLASMA

DETAILS OF SERVICES OFFERED

The APU offers confirmatory and referred services useful in the investigation of individual cases and outbreaks of mycoplasma and ureaplasma infection. These are genome detection and/or culture from clinical material and identification of referred isolates.

The laboratory is happy to discuss and advise upon particular diagnostic and clinical problems and laboratory aspects of epidemiological investigations, ask for Dr Vicki Chalker (020 8327 6776) or Dr Tim Harrison (020 8327 6906/7331) in the first instance.

QUICK VIEW TABLE (further details below)

Target	Test	Turnaround time	Preferred specimen	Minimum specimen Volume
<i>M. pneumoniae</i>	PCR	5 days	Respiratory sample (LRT or throat swab)	0.2mL
Neonate screen: <i>M hominis</i> <i>Ureaplasma spp.</i>	PCR with culture on PCR positives	5 days	ETS, NPA	0.2mL
Other species	Culture, PCR and sequencing when relevant	Species dependant (see below)	Case dependant (e.g. respiratory, CSF, joint and wound, aspirates)	0.2mL
Isolates	Culture, PCR and sequencing when relevant	Species dependant (see below)	Culture on blood agar or in VTM	N/A
<i>M. genitalium</i>	See STBRL (telephone)020 8327 6464)			

THE DETECTION OF MYCOPLASMA PNEUMONIAE DNA IN CLINICAL SAMPLES

This referred (charged) service is available where *M. pneumoniae* infection is of increased likelihood or would be of major clinical significance.

The presence of *M.pneumoniae* DNA in clinical material taken from an acutely ill patient is determined by using a PCR directed against the P1 adhesin gene. Any respiratory specimen is suitable for this test, preferably a lower respiratory tract specimen or throat swab.

CSF samples are rarely, if ever, positive for *M.pneumoniae* and are therefore not routinely tested for *M.pneumoniae* DNA.

TURNAROUND TIME

Under normal circumstances, these tests are processed twice weekly.
The turnaround time is 5 days.

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply respiratory samples (sputa, BALs, NPAs, etc.) in a sterile container – as much volume as possible **but not less than 0.2mL**.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6776/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

THE DETECTION OF MYCOPLASMA / UREAPLASMA FROM CLINICAL MATERIAL

Detection and culture of mycoplasma is laborious and expensive. This referred (charged) service is not intended for the routine investigation of respiratory illness, but is available where mycoplasma infection is of increased likelihood or would be of major clinical significance.

NEONATE SCREEN

U. urealyticum, *U. parvum* and *M.hominis*, may be involved in respiratory infection or rarely meningitis/septacemia in neonates, especially low birth weight infants. The presence of *U. urealyticum*, *U. parvum* and *M. hominis* DNA in clinical material is determined using PCR amplifying the urease gene in ureaplasmas with species-specific probes (Yi *et al.*, 2005) and the glyceraldehyde-3-phosphate dehydrogenase (gap) gene in *M.hominis* (adaption of Baczynska *et al.*, 2004 with an house probe design). Culture will be attempted on all PCR positive specimens.

OTHER SPECIMENS

Mycoplasma and ureaplasmas may cause respiratory and other infections in the immunocompromised. Respiratory specimens from such patients are suitable for investigation. Mycoplasmas have occasionally been isolated from other extra-pulmonary sites including CSF, blood cultures, wound and joint aspirates. The presence of mycoplasmas will be determined using PCR, sequencing and culture when relevant for all human and zoonotic mollicute species excepting haemoplasmas.

TURNAROUND TIME

Neonate screen: PCR will be run at least twice a week.
The turnaround time is 5 days

Culture results will be available ASAP following successful isolation
The turnaround time is 5 days following PCR positive results.

Other specimens: Relevant PCR, sequencing and culture results will be available dependant on the organism in question. Some species such as *M. hominis* take only a few days whilst others such as *M.pirum* may take as long as 6 weeks to isolate (see 2.5).

THE IDENTIFICATION OF PUTATIVE ISOLATES OF MYCOPLASMAS AND UREAPLASMAS

This reference service is undertaken by biochemical characterisation, growth inhibition studies, and molecular methods including 16S rDNA sequencing.

The laboratory is happy to receive any putative isolates from **clinical material**. The most frequently referred species include *M. hominis*, *U. urealyticum*, *U. parvum* and *M. pneumoniae*.

TURNAROUND TIME

Turnaround times will vary depending on the nature of the enquiry and the complexity of the investigation required including length of time required to culture species under study. Priority will **always** be given to isolates of current clinical relevance (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply pure culture on mycoplasma medium or chocolate/blood agar

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6776/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

MYCOPLASMA GENITALIUM

All enquiries relating to *M.genitalium* should be referred to STBRL (telephone 020 8327 6464).

REFERENCES

Further information is available from the HPA website:

http://www.hpa.org.uk/infections/topics_az/mycoplasma/menu.htm

Baczynska A, Svenstrup HF, Fedder J, Birkelund S, Christiansen G. Development of real-time PCR for detection of *Mycoplasma hominis*. BMC Microbiol. 2004 6;4:35.

Pitcher D, Chalker VJ, Sheppard CL, George RC, Harrison TG. Real-time detection of *Mycoplasma pneumoniae* in respiratory samples with an internal processing control. J Med Microbiol 2006;55:149-55

Yi J, Yoon BH, Kim EC. Detection and biovar discrimination of *Ureaplasma urealyticum* by real-time PCR. Mol Cell Probes. 2005 19(4):255-60.

4.4 RESPIRATORY CHLAMYDIAE

DETAILS OF SERVICES OFFERED

The APU offers a reference service for chlamydia DNA detection by PCR, which may be useful in the investigation of individual cases and outbreaks of respiratory chlamydia infections. This service is **only offered where there is a clear public health need** to establish the diagnosis. **Please contact the laboratory to discuss before sending any samples.**

The laboratory is happy to discuss particular diagnostic and clinical problems, ask for Dr Tim Harrison (020 8327 6776/7331) in the first instance.

All enquiries relating to *C.trachomatis* should be referred to STBRL laboratory (telephone 020 8327 6464).

TURNAROUND TIME

This is not a routine service and turnaround times will therefore vary depending on the nature of the enquiry and the complexity of the investigation required (see 2.5).

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6776/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

REFERENCES

Further information is available from the HPA website:

http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1195733811051

4.5 **BARTONELLA**

DETAILS OF SERVICES OFFERED

The APU offers a referred (charged for) serological service for the diagnosis of infections such as Cat Scratch Disease, and endocarditis which may be caused by *Bartonella* spp.

The laboratory is happy to discuss and advise upon particular diagnostic and clinical problems, ask for Dr Tim Harrison (020 8327 7331) in the first instance.

BARTONELLA HENSELAE AND B.QUINTANA SEROLOGY

The assay used determines antibody levels against *B.henselae* and *B.quintana*: IgM and IgG are estimated separately.

TURNAROUND TIME

The turnaround time is 10 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Atypical Pneumonia Unit request form (See 2.3) and supply not less than 400 µl serum in a sterile container

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to APU by telephone (020 8327 7331/6776/7222) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

REFERENCES

Further information is available from the HPA website:

http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733782342?p=1200577814562

Harrison TG, Doshi N. Serological evidence of *Bartonella* spp. infection in the UK. *Epidemiol Infect* 1999;123:233-40

Section 5.0 THE HAEMOPHILUS REFERENCE UNIT

The Haemophilus Reference Unit (HRU) is the HPA-Colindale Reference Unit for *Haemophilus influenzae* and other *Haemophilus species* (excluding *H.ducreyi*). The reference services offered are listed below.

5.1 HAEMOPHILUS INFLUENZAE

DETAILS OF SERVICES OFFERED

HRU offers identification, serological typing and capsular genotyping of strains of *Haemophilus influenzae* isolated from cases of invasive disease.

Conjugate *H.influenzae* type b vaccine is routinely offered to all infants in the UK. Typing of strains of *Haemophilus influenzae* is invaluable in determining whether the strain is *H.influenzae* type b that is a vaccine preventable serotype, a non-type b serotype or non-capsulated strain.

HRU requests submission of **ALL** *Haemophilus influenzae* isolated from blood culture or other normally sterile sites in patients of **ALL** ages as part of the surveillance of invasive disease due to *H.influenzae* and in children aged 0 – 16 years as part of the surveillance of invasive disease due to *H. influenzae* and Hib vaccine failures in children. This surveillance is being conducted in collaboration with the Immunisation, Hepatitis and Blood Safety Department, HPA Health Protection Services: Colindale

The laboratory is happy to discuss and advise upon particular clinical or epidemiological problems. Please contact Dr Mary Slack (020 8327 6091) .

TURNAROUND TIME

The turnaround time is 10 days (see 2.5).

HOW TO OBTAIN SERVICE

Use the Haemophilus Reference Unit request form (See 2.3) and supply a pure culture on chocolate agar slope. Please ensure cap is securely screwed down to optimise survival of organisms.

Specimen submission regarded by the sending laboratory as especially important or urgent should be notified to HRU by telephone (020 8327 7330/7331) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

OTHER INFORMATION

- Identification of *Haemophilus influenzae* is based upon X and V factor requirement and lack of haemolytic activity on blood agar.
- There are 6 capsular serotypes of *H.influenzae* (a-f) based on the capsular polysaccharide of the organisms. The majority of serious human infections are caused by *H.influenzae* type b, for which a conjugate vaccine is now available.
- Other capsular serotypes, notably types e and f and non-capsulated strains can cause serious infections.

- HRU liaises with the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) and will refer requests for antimicrobial susceptibility testing to ARMRL.
- HRU does not offer a routine service for typing or susceptibility testing *H.influenzae* strains from non-invasive infections. Non invasive isolates of *H.influenzae* (i.e. isolates from eye swabs, sputum, etc.) will only be examined if there are sound clinical or epidemiological reasons for the investigations. The laboratory is happy to discuss any clinical problem that may warrant further investigation. Please contact Dr Mary Slack (020 8327 6091) .
- The HRU does **NOT** carry out tests for Hib antibodies. Hib serology is performed by Professor Ray Borrow, Meningococcal Reference Unit, HPA Microbiology Services, Manchester Medical Microbiology Partnership, Clinical Sciences Building 2, Manchester Royal Infirmary, Oxford Road, Manchester, M139WL Please contact Professor Ray Borrow (0161 276 6793) or Dr Mary Slack (020 8327 6091) for further advice on Hib serology.

IDENTIFICATION OF HAEMOPHILUS SPECIES (EXCLUDING HAEMOPHILUS DUCREYI)

DETAILS OF SERVICES OFFERED

HRU offers an identification service for strains of *Haemophilus* species isolated from cases of invasive disease.

The laboratory is happy to discuss and advise upon particular clinical or epidemiological problems. Please contact Dr Mary Slack (020 8327 6091)

TURNAROUND TIME

The turnaround time is 10 days (see 2.5).

For isolates not confirmed as *Haemophilus spp* a preliminary report will be issued by HRU and the isolate forwarded to LHCAI/MISU for full identification, who will issue a report in due course.

HOW TO OBTAIN SERVICE

Use the Haemophilus Reference Unit request form (See 2.3) and supply a pure culture on chocolate agar slope. Please ensure cap is securely screwed down to optimise survival of organisms.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to HRU by telephone (020 8327 7330/7331) to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.

REFERENCES

Further information is available from the HPA website:

http://www.hpa.org.uk/infections/topics_az/Haemophilus_influenzae/menu.htm

Haemophilus influenzae and species

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McVernon J, Andrews N, Slack MPE, Ramsay ME. Risk of vaccine failure after *Haemophilus influenzae* type b (Hib) combination vaccines with acellular pertussis. *Lancet.* 1003. 361: 1521-3

Trotter CL., Ramsay ME., Slack MPE. Rising incidence of *Haemophilus influenzae* type b disease in England and Wales indicates a need for a second catch-up vaccination campaign. *Commun Dis Public Health* 2003;**6**:55-58

McVernon J, Trotter CL, Slack Mary PE, Ramsay ME. Trends in *Haemophilus influenzae* type b infections in adults in England and Wales: surveillance study *BMJ* 2004; **329**: 655-8

McVernon J, Howard AJ, Slack MPE, Ramsay ME. Long-term impact of vaccination on *Haemophilus influenzae* type b (Hib) carriage in the United Kingdom. *Epidemiol Infect* 2004; 132: 765-7

McVernon J, Slack MP, Ramsay ME. Changes in the epidemiology of epiglottitis following introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines in England: a comparison of two data sources *Epidemiol Infect* 2005;134;570-572

HPA-COLINDALE RECOGNITION OF CALDICOTT RECOMMENDATIONS

The recommendations of the Caldicott Report (1997) have been adopted by the Health Protection Agency as by the National Health Service as a whole. These recommendations relate to the security of patient identifying data (PID) and the uses to which they are put. The HPA-Colindale observes Caldicott guidance in handling PID and has appointed its own Caldicott Guardian for the Centre for Infections. He advises the Director, HPA-Colindale, on confidentiality issues and is responsible for monitoring the physical security of PID in all parts of Colindale. This also applies to the transfer of results of investigations to and from Colindale whether by mail services, telephone or fax. The value of 'safe haven' arrangements or other means of the sender and receiver of information identifying themselves to each other before data is transferred is emphasised. (see attached Colindale Policy on faxing and e-mailing reports containing patients' data).

Colindale is anxious to audit the security of its PID in collaboration with its customers. Customers are invited to review our arrangements in conjunction with individual laboratory directors and/or the Colindale Caldicott Guardian. Customers are also asked to draw to the Colindale Caldicott Guardian's attention any instances where PID security has been threatened or has broken down. Uses that PID are put to outside clinical diagnostic services generally allow patient identifiers to have been removed before hand, and when PID is used for research purposes the proposals are considered first by the HPA Research Ethics Committee. All enquiries about the security and use of PID at Colindale should be addressed to the Caldicott Guardian, Dr Fortune Ncube (fortune.ncube@hpa.org.uk)

Each of the laboratories at Colindale also has their own named Security of Information Officer; for RSIL this is Mrs Joanna Tambouridou (020 8327 7219, joanna.tambouridou@hpa.org.uk)

COMPLIANCE WITH THE HUMAN TISSUE ACT

Submitting tissue samples from deceased people

The Centre for Infections is licensed by the Human Tissue Authority (HTA) (Licence number 12459) to store tissues from deceased people for scheduled purposes. Post mortem samples are submitted to Colindale by coroners or pathologists for examination to help them determine the cause of death.

Obtaining consent to remove, store and use human tissues for a scheduled purpose is one of the underlying principles of the Human Tissue Act. Colindale receives post-mortem samples from Coroners' post-mortems or from NHS establishments across the UK and therefore we are performing the examination under the authority of the coroner. Unless consent has been obtained or the coroner has requested that samples are retained for further testing, samples are disposed of within three months of the initial test being performed.

When tissue samples from deceased people are received at the Centre for Infections they are retained securely and confidentiality is maintained in compliance with [Caldicott principles](#) as are all samples received at this centre. It is normal practice for tissue samples from the deceased to be disposed of in the same way that all others clinical samples we receive at Colindale are disposed off. However, we will adhere to any specific requirements regarding disposal or returning tissue samples if requested by the sending coroner or pathologist.

COLINDALE POLICY ON FAXING AND EMAILING REPORTS CONTAINING PATIENTS' DATA

The following guidelines are prepared having taken into account the Code of Practice on reporting patients' results by fax prepared by the Department of Health and Caldicott recommendations..

It is HPA-Colindale policy that reports containing patients' data should **not** be sent by fax or email (other than via the e-Lab reporting system).

Emails cannot be relied on to guarantee security of patients' data because they can be intercepted by a third party en route.

In **exceptional** circumstances it may be necessary to send a result by fax but not by email. In this case, the following conditions must be adhered to after telephone discussion with the Laboratory. Refer also the document "Colindale recognition of Caldicott recommendations".

- The report must be sent to a "safe-haven" fax machine. This means that, if the location is in general use, consideration must be given to ensuring that unauthorised personnel are unable to read reports, accidentally or otherwise. Also, the room housing the fax machine must be kept in a secure location which is locked if it is likely to be unattended at the time the fax is sent.
- Assurance must be sought from the intended recipient of the faxed report, preferably in writing, that the receiving fax machine is a "safe-haven".
- Measures must be taken to minimise the risk of mis-dialling, either by double-checking numbers or having frequently used numbers available on the fax machine's memory dial facility.
- Confirmation must always be sought from the intended recipient that the fax is expected and has been received.