Public Health England

Protecting and improving the nation's health

National Infection Service - Reference Colindale

Bacteriology Reference Department user manual

Version 13, October 2020, Q-Pulse BRDW0078

About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. We do this through world-leading science, research, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health and Social Care, and a distinct delivery organisation with operational autonomy. We provide government, local government, the NHS, Parliament, industry and the public with evidence-based professional, scientific and delivery expertise and support.

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Bacteriology reference department (BRD)

Bacteriology Reference Department (BRD) is part of the National Infection Service (NIS) which is Public Health England's (PHE) scientifically led service to protect and reduce the burden of infectious disease to the UK public. It is a national and international reference department for a wide range of bacterial infections and receive clinical samples and bacterial isolates from public health departments, National Health Service (NHS) and commercial laboratories across the UK and internationally for specialist testing, bacterial characterisation and susceptibility testing.

The BRD department is made up of 3 units:

The Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit is the national reference laboratory for investigation of antibiotic resistance in, and characterisation of, healthcare associated and sexually transmitted bacterial pathogens.

AMRHAI seeks to define outbreaks and identify transmission pathways using established and developmental phenotypic and genotypic methods to type isolates, to identify biomarkers associated with virulence, 'fitness', host specificity and transmissibility. The unit determine susceptibility for most species of bacteria, with the notable exception of category 3 organisms, detect new and emerging resistances by interpretive analysis of MIC profiles and molecular investigation, and provide therapeutic guidance.

AMRHAI undertakes laboratory-based surveillance, advises on outbreak investigations and investigation of unusual antibiotic resistance, and on any public health risk. The Unit also provides an identification service for difficult to identify bacteria and from culture-negative clinical samples; information and advice on infection control issues; investigation of healthcare- and community-associated infections, aspects of laboratory safety and other related matters.

AMRHAI is the designated World Health Organization Collaborating Centre (WHO CC) for Reference and Research on Antimicrobial Resistance and Healthcare Associated Infections.

The Gastrointestinal Bacteria Reference Unit (GBRU) works at local, regional, national and international levels to reduce the burden of gastrointestinal infection.

Activities include national microbiological reference services for a range of gastrointestinal pathogens as well as the provision of specialist testing for the microbiological examination of clinical, food, water and environmental samples. The unit also undertakes research into the genetic diversity of pathogens and the development of improved detection and characterisation techniques for food, water and environmentally borne diseases. GBRU is able to offer expert advice, education and training on public health aspects of food microbiology and safety.

The Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) provides national and international reference services for a number of bacteria causing respiratory, systemic and vaccine-preventable bacterial infections. RVPBRU receives bacterial isolates and clinical samples which are analysed by a wide range of methodologies in accordance with customer needs. RVPBRU also performs surveillance and advises on incident/outbreak investigation.

RVPBRU is a designated WHO CC for diphtheria and streptococcal infections and a WHO CC for *Haemophilus influenzae* and Streptococcus pneumoniae.

Disclaimer

Amendment history

Version no.	Date	Sections affected	Pages affected
1	April 2014	All Units combined into one BRD user manual following internal reorganisation.	All
2	October 2014	Update links to PHE website, service table and appendixes *for B. pertussis* PCR, *B. pseudomallei*, *C. botulinum*, Helicobacter, Neiserria, Staphylococcus, Treponema, Trichomonas, STBRU TAT. Add pertussis oral fluid.	11 to 18, 22 to 49
3	January 2015	Update service table (and link request forms), and appendixes for *B. pertussis* PCR and *C. diphtheria.* Remove Trichomonas service.	8, 11 to 18, 28, 41
4	April 2015	Update link to quality standards, service table and appendix for Legionella, Salmonella and *C. botulism* TAT. Bartonella service suspended from 1 July 2015. Add BIDS, Leptospira.	12, 15, 20, 21, 32, 50
5	October 2015	Update service table and appendixes for syphilis IgM. Yersinia serodiagnostic service withdrawn July 2015, Group A Streptococcal (antistreptolysin O and anti-DNase) and *S. aureus* antibodies (antistaphylolysin and anti-nuclease) services withdrawn Oct 2015.	Various
6	November 2016	Update Key personnel, medicolegal, summary of contacts, service table and appendixes for TATs, BIDS, Escherichia, Listeria, Neisseria Shigella, Streptococcus and Cronobacter. **Add** Abiotrophia, Aerococcus, Actinomycetes, Bartonella, Dolosicoccus, Dolosigranulum, Elizabethkingia, Facklamia, Gemella, Globicatella, Granulicatella, Helcococcus Ignavigranum, Lactococcus, Leuconostoc, Norcadia, Pediococcus, Rastonia, Tetragenococcus and Vagococcus.	12 to 27, 4, 5, 8, 31, 49, 62, 65
7	January 2017	Update service table and appendix for Listeria and tests provided by STBRU now offered by AMRHAI and CSU (VRD).	4, 13 to 28
8	April 2017	Update contact list, charging for sharing data or isolates, service table and appendixes for *Listeria, Staphylococcus* (add WGS from Apr 2017), Mycoplasma medium.	10, 23, 26, 45, 56, 63

Version no.	Date	Sections affected	Pages affected
9	April 2018	Update BRD, key personnel list, Caldicott Guardian, Quality (UKAS ISO 15189 accredited), service table and appendixes for *M. genitalium, N. gonorrhoeae,* Resistance Mechanism, *C. botulism* and *perfringens, Listeria, Helicobacter, Salmonella,* pertussis oral fluid, Antimicrobial Susceptibility Testing and Resistance Mechanisms Section *C. diphtheriae and* Haemophilus. Remove *L. monocytogenes and* pseudomonas serodiagnosis, *r*eplace resistance with susceptibility and replace sections in GBRU. Add WGS for *S. pneumoniae,* additional charges in AMRHAI, Leptospira culture MLST and pertussis oral fluid.	7, 8, 13 to 27, 31, 42, 50
10	June 2019	Update contact list, medico-legal, HTA, service table and appendixes for antibiotic testing, Bacillus and *C. perfringens TAT, C. trachomatis, Escherichia* (replace VTEC with STEC), *Helicobacter,* Legionella, *Listeria, M. genitalium. N. gonorrhoea and Vibrio (add Aeromonas).*	8, 10, 13 to 28, 33, 34, 45, 53, 66
11	October 2019	Minor updates to add links and contact list	
12	July 2020	Update service table and appendixes for antibiotic testing*, B. pertussis* PCR, Enterococcus, Escherichia, H. *pylori* TAT, Legionella spp., *M. genitalium*, N. gonorrhoeae, Salmonella). Remove *E. Coli* serodiagnostic, Staphylococcus spa and update WGS. Update amendment history.	
13	September 2020	Update Key personnel and contact details	8, 76

Key personnel and contact details

Name	Designation	Telephone
Prof. Neil Woodford	Deputy Director, NIS Labs	020 8327 6511
Dr Nandini Shetty	Interim Lead, BRD Consultant Medical Microbiologist	020 8327 6033
Steve Harbour	Head of Operations, NIS Labs – Reference	020 8327 6432

The following clinical (listed first) and scientific staff are available for advice on any clinical and scientific queries at VRDQueries@phe.gov.uk, phone 0208 327 7887 or the respective BRD Units

Meera Chand Vicky Chalker, Norman Fry	Respiratory and Vaccine Preventable Bacterial Infections RVPBRUqueries@phe.gov.uk
Gauri Godbole Saheer Gharbia	Gastrointestinal Bacterial infections GBRU@phe.gov.uk
Helen Fifer Michelle Cole	Sexually transmitted bacterial infections AMRHAI@phe.gov.uk
Colin Brown Katie Hopkins	Healthcare associated and multidrug resistant infections. AMRHAI@phe.gov.uk

Full contact list and details are found at the end of the user manual.

If you need to contact a Medical Microbiologist please send an email to: ColindaleMedMicro@phe.gov.uk or telephone 0208 327 6736 issues accessing the microbiologist please email: jacinta.santos@phe.gov.uk.

Department addresses

DX address:	Postal address:
PHE Colindale Bacteriology DX 6530002	Public Health England Bacteriology Reference Department 61 Colindale Avenue London NW9 5EQ View map

BRD general office

Telephone: 020 8327 7887 (staffed 9am to 5.30pm Monday to Friday).

PHE MS Colindale switchboard: 020 8200 4400.

How to obtain services

Hours of service

The Department is open from 9am to 5.30pm, Monday to Friday. Telephone enquiries should be directed to 020 8327 7887 from 9am to 5.30pm, Monday to Friday. No routine services are available outside these hours. The Department is closed on public holidays.

Services to the public

BRD does not offer diagnostic services to members of the public except via a registered medical practitioner. Results can only be issued to the requesting physician or medical unit and will not be given to patients directly under any circumstances. We reserve the right to check the authenticity of callers to protect the confidentiality of patients' personal data.

There are no clinical facilities at PHE Colindale, and we are unable to see patients or give telephone medical advice directly to members of the public.

Establishment of service agreement

Each request accepted by the laboratory for examination is considered to be an agreement for work under PHE terms and conditions of business. These may be found on the PHE web site by searching www.gov.uk for 'PHE terms and conditions'.

Specimen submission guidelines

Specimens

All clinical specimens must be labelled with at least 2 of the following unique identifiers:

- surname/forename or other unique patient identifier and/or
- date of birth
- sender's sample number

All environmental specimens must be labelled with a unique specimen identifier/sender's sample number.

Request forms

Request forms must match and include the above information on the sample, as well as:

- 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 sample
- sex
- relevant clinical information
- date of onset
- vaccination history (if relevant to test requested)
- NHS number
- Appropriate travel history in previous 4 weeks

Please complete the forms in black or blue ink only.

Request form completion guidance document is available from the PHE website:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/581444/QW 0159.04_-Guidelines_for_completing_Request_Forms.pdf

Requests for work on isolates that presumptively 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 relevant Unit by telephone. The turnaround time in this instance will vary.

Please use the current versions of request forms where possible and complete all relevant sections. BRD specific request forms are available from the PHE website:
https://www.gov.uk/government/collections/bacteriology-reference-department-brd
https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Pre-addressed and bar-coded request forms to ensure reports are dispatched to the appropriate address are available on request.

Requests for sharing data or isolates.

Commonly requested strains are submitted to NCTC by PHE. BRD requires a material transfer agreement for shared data or strains. A charge will be levied unless prior collaborative PHE project is agreed and in place. Please contact relevant Unit.

Urgent specimens

If a reference service is required urgently, please contact a senior staff from the relevant Unit to discuss prior to dispatch. Always mark ‘urgent’ clearly on the request form.

Forensic and medico-legal (if appropriate) specimens

The department has capabilities to test medico-legal specimens and certain types of forensic specimens. However, whilst the assays performed are accredited under ISO15189:2012 for diagnostic purposes the department is not accredited for performing these tests for forensic work where the results of the sample will go into the criminal justice system.

Due to the legal requirements pertaining to these types of specimens, they will only be processed if the department has been contacted in advance and if all paper work (including the chain of evidence form) is correctly completed. This will enable the department to ensure continuity of evidence throughout testing.

All requests for forensic tests must be discussed with the relevant units prior to sending the specimen to the laboratory.

Specimen transportation

Arrangements must be made by referring laboratories to ensure that time and temperature requirements (detailed under ‘Key factors affecting tests’, below) for sample transportation are maintained. Failure to achieve this may compromise sample

integrity and the validity of test results. Samples which do not meet the sample acceptance criteria may not be processed.

Samples which are dispatched at ambient temperature (10°C to 25°C) must have a transit time of no more than 72 hours. If the date of receipt is greater than 72 hours from the date of dispatch, the referring laboratory will be informed, and the specimens may not be processed.

Specimens sent by post or by courier must be in a sealed container, surrounded by sufficient absorbent packing material to take up any leakage in the event of damage during transit, sealed in a plastic bag and placed in an approved outer container which meets current postal or other transport regulations.

Any organisation sending out cultures or diagnostic specimens has a legal duty to ensure that such items are sent in a safe manner. Infectious substances which break or leak in transit can result in a major incident, putting those handling them and those in receipt of them at risk of infection. It is therefore vital that the correct transport requirements are followed.

Contact the departmental safety manager (020 8327 6447) or the specimen reception manager (020 8327 6063) for further information.

Guidance on the transport of infectious substances (including links to current European agreements and information from the HSE) may be found at:
http://www.dft.gov.uk/vca/dangerousgoods/useful-links.asp

Specimen quarantine policy

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

Please complete request forms as fully as possible. Failure to do so may result in delays or rejection. Some specimens may be rejected if lack of information could expose staff to ‘high risk’ pathogens at the incorrect containment level. Requests for work on isolates that presumptively fall into ACDP Hazard Group 3 must be clearly marked to show the findings of the sending laboratory. See specimen submission guidelines for more details. If a specimen is submitted to BRD for an investigation that we do not offer we will contact the customer and return, forward or archive the sample and issue a report to the sender explaining the reasons for the sample’s rejection. The sample will be returned if requested (within mainland UK) or discarded after 14 days.

The time taken to perform bacterial identification, typing and susceptibility testing 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 or require repeat submission.

Serology tests

For serological tests, separated serum is preferred. Samples which are highly haemolysed or hyperlipaemic should not be sent as lysed blood or heavily blood stained samples can interfere with serological testing.

Heat-inactivated samples may give rise to erroneous results in several assays and should not be sent – please contact the relevant Unit prior to sending the specimen if no other sample is available.

Services available

The Department undertakes tests as listed on the following pages. Key factors affecting individual tests are noted against the relevant test, including minimum sample volumes where relevant.

Further information is available from:

- https://www.gov.uk/health-protection/services
- https://www.gov.uk/health-protection/infectious-diseases

Turnaround times

Turnaround times (TAT) are from day of receipt to issue of reports in calendar days. The times shown are the typical TATs achieved by the laboratory but may be longer or shorter depending on the availability of staff and the complexity of the investigation. BRD staff are committed to the fastest possible issue of reports, consistent with accuracy, on the specimens they examine. TATs may vary during seasonal outbreaks; testing may be conducted more frequently during epidemic seasons. We seek to process at least 80% of specimens received within the published TATs.

Requests for additional tests: time limits and specimen retention

If additional laboratory testing is required on a sample previously submitted to BRD, please contact the relevant unit in the first instance. Original specimens are normally retained for at least1month (up to several years in the case of certain specimens) but further testing may not be possible due to sample volume constraints, specimen viability or other factors. The Unit will be able to advise on the feasibility of using the original specimen for analysis.

A to Z listing of services available

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Abiotrophia spp.	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Achromobacter spp.	Species identification, molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Acinetobacter spp.	Species identification, molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Actinomycetes (aerobic only)	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
	Identification and confirmation	Pure culture, Agar slope	7 days	M1, H2	AMRHAI
Aerococcus spp.	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Alloiococcus spp.	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Antibiotic testing	The antibiotic susceptibility service includes interpretive analysis of MIC profiles and therapeutic guidance. The main examples are mentioned in this 'Services' table but most species other than Mycobacterium spp. and anaerobes are covered. MIC determination is not undertaken	Pure culture, Agar slope	15 days (non-fastidious spp.)	H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
	on rectal/faecal isolates or environmental isolates submitted for carbapenemase detection. Lack of EUCAST clinical breakpoints is NOT sufficient justification alone for referral. Diagnostic laboratories should familiarise themselves with EUCAST guidance for susceptibility testing of organisms/agents for which there are no EUCAST clinical breakpoints. We use standard antibiotic panels based on EUCAST and CLSI recommendations.				
Bacillus (other than *B. anthracis)*	Identification of *Bacillus* spp. Molecular typing of *B. cereus* for outbreak investigations	Pure culture on agar slopes	10 days	L4	GBRU
	Detection of *B. cereus* emetic toxin gene by PCR	Pure culture on agar slope	10 days	L4	GBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Bacterial Identification Service (BIDS): Isolate identification (unknown, atypical fastidious, emerging bacteria)	Species identification	Pure culture on agar slope	7 days	H2, M1	AMRHAI
	Isolate identification (unknown, atypical, fastidious, emerging bacteria)	Pure culture on agar slope	7 days	H2, M1	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
BIDS: Clinical sample identification (culture negative, unknown)	Bacterial detection and species identification for culture-negative clinical samples from normally sterile sites	Normally sterile site clinical sample: liquid – 200-500 µl, Tissue – 1cm^3, FFPE - up to 3 sections, each with a thickness of up to 10 µm and a surface area of up to 250 mm^2	7 days	M1	AMRHAI
Bartonella spp.	Serology is no longer offered by RVPBRU. AMRHAI can test by 16S rRNA gene PCR and sequencing	Normally sterile site clinical sample except blood/serum (see above BIDS for details)	7 days	M1	AMRHAI
Bordetella spp.	Confirmation of identification, serotyping of *B. pertussis*	Pure culture on a suitable agar slope or growth from a plate on swab in charcoal transport medium	Varies	R3	RVPBRU
	Antimicrobial susceptibility (species other than B. *pertussis*)	Pure culture on agar slope	15 days	H1, H2	AMRHAI
Bordetella pertussis	Serology - anti-PT IgG antibodies (NOT suitable for immune status)	Not less than 400 µL serum in a sterile container (≥2week history of cough)	12 days	R3	RVPBRU
	Oral fluid - anti-PT IgG antibodies (NOT suitable for immune status)	Oral fluid for notified cases 2->17 yrs. - contact HPT for kit. (≥2week history of cough)		Form distributed with kit	
	Further characterisation of *B. pertussis* qPCR positive clinical specimens for surveillance and epidemiological purposes (currently suspended)	Aliquot of original specimen and/or DNA extract	Varies	R3	

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Burkholderia spp.	Species identification, molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Burkholderia pseudomallei	Identification and antimicrobial susceptibility	Pure culture, Agar slope	2-7 days	Contact Lab. H1, H2	AMRHAI
Campylobacter spp.	Identification and MLST by WGS. (Antimicrobial sensitivity by E-test only upon specific request).	Pure culture sent on Amies charcoal swab (preferably) or other suitable medium (for example blood or chocolate agar slope)	14 days	L4	GBRU
Chlamydia (respiratory) Not UKAS accredited	*C. pneumoniae/ C. psittaci/ C. abortus* - PCR assay Only available after discussion and prior agreement with RVPBRU senior staff.	Minimum 200 µL of respiratory sample	Urgent phoned	Contact Lab before sending	RVPBRU
Clostridium botulinum	Detection and identification of C. botulinum from clinical, food or environmental samples by PCR and culture.	Faeces (10g) or rectal washout into anaerobic broth (universal). Faeces (10g) or rectal washout in sterile container. Serum (≥ 2mL) to be collected close to the onset of symptoms (preferably < 3 days) and before antitoxin is given. Note: lysed or EDTA treated blood specimens are not suitable. Food/Drink samples (10g or 10 mL).	9 days	Contact lab before sending specimens. L4 (culture) L5 (specimen)	GBRU
	Detection of botulinum neurotoxins in clinical specimens or food associated with suspected cases.		>=5 days (refer to Appendix 2)	Contact Lab before sending specimens. L5 (specimen) L7 (Food/Env)	GBRU

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Clostridium perfringens	Identification of enterotoxigenic *C. perfringens* by PCR	Pure culture in anaerobic broth or transport swab	5 days	L4	GBRU
	Molecular typing for outbreak investigations	Pure culture in anaerobic broth or transport swab	15 days	L4	GBRU
	Detection of *C. perfringens* enterotoxin in faeces by ELISA	≥1g or 1mL of faeces from cases of diarrhoea collected as close to the onset of symptoms as possible (preferably <3 days)	5 days	L5	GBRU
	C. perfringens Toxin (lethal toxins) typing by PCR	Pure cultures of C. perfringens in anaerobic broth or transport swab	5 days	L4	GBRU
Clostridium tetani	Detection and identification of *C. tetani* by PCR and culture	Pure cultures of *C. tetani* in anaerobic broth. Tissue inoculated into anaerobic broth	5 days	L5	GBRU
	Detection of *C. tetani* neurotoxin in serum (Note: serum will be first tested for tetanus antibody levels by RVPBRU)	Serum (≥ 2mL) to be collected close to the onset of symptoms (< 3 days) and before antitoxin is given. Note: lysed or EDTA treated blood specimens are not suitable	5 days (following antibody results)	L5	GBRU
	Tetanus immunity: serum antibodies	Not less than 200 µL serum in a sterile container	21 days unless urgent	As required R3	RVPBRU
Corynebacterium spp.	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	Contact Lab. H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Corynebacterium diphtheriae/ ulcerans/ pseudotuberculosis	*C. diphtheriae/ ulcerans/ pseudotuberculosis* (that is, potentially toxigenic corynebacteria): Identification and Toxin testing by real-time PCR and Elek test	Pure culture on blood or Loeffler slope (notify RVPBRU prior to submission)	Within 24 hours (PCR) (6day service)	Contact Lab. R3	RVPBRU
	Diphtheria immunity: serum antibodies	Not less than 200 µL serum in a sterile container.	21 days unless urgent	R3	RVPBRU
Corynebacterium jeikeium	*C. jeikeium* Antimicrobial susceptibility	Pure culture	15 days	H1, H2	AMRHAI
Cronobacter spp.	*C. sakazakii* confirmation of identification, Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	Contact Lab. H1, H2	AMRHAI
Cystic Fibrosis (CF) Pathogens	Identification and molecular typing	Pure culture, Agar slope	18 days	H2	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Dolosicoccus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Dolosigranulum spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Elizabethkingia spp.	Identification, molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Enterobacter spp.	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Enterococcus spp.	Species identification and antimicrobial susceptibility (molecular typing currently suspended)	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Escherichia spp.	*E. coli* (ACDP HG 2 only): Whole genome sequencing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
	Identification, serotyping, phage typing and molecular typing by whole genome sequencing. [We can offer fast identification of HG3 *E. coli* (STEC) by PCR upon request]	Pure culture on Dorset's egg or nutrient agar slope	14 days	L4	GBRU
	PCR and Culture detection from faeces for non-O157 STEC	Faecal sample in standard sealed container ≥1 gram	8 days	L5	GBRU
Facklamia spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Gemella spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Globicatella spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Gram-negative bacteria non-fermenter and fastidious organisms	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Gram-positive bacteria (except *C. diphtheriae*)	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Granulicatella spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Haemophilus spp.	*Haemophilus* spp. (excluding *H. ducreyi*): Identification	Pure culture on chocolate agar slope with cap securely screwed down	12 days	R3	RVPBRU
	H. influenzae: Serotyping and capsular genotyping of *H. influenzae*	Pure culture on chocolate agar slope with cap securely screwed down	12 days	R3	RVPBRU
Haemophilus spp. and *Aggregatibacter* spp.	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Helcococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Helicobacter spp.	*H. pylori isolation,* identification and antibiotic susceptibility testing by Etest	Heavy suspension of isolate or Gastric biopsies in sterile saline or a suitable *H. pylori* transport medium	19 days	L4 (culture) L5 (specimen) Please avoid sending samples on Fridays	GBRU

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Ignavigranum spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Klebsiella spp.	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Lactococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Legionella spp.	*L. pneumophila*: 2 commercial EIA assays (confirmation of sending lab testing results only)	Not less than 2mL urine sample (soon after onset), mid-stream, early morning with/without preservatives in a sterile container	8 days unless urgent	R1	RVPBRU
	L. pneumophila PCR (from urinary antigen positive patients only)	Lower respiratory tract samples (sputa, BAL, tracheal aspirate) and other clinical samples in a sterile container	Urgent samples should be notified by phone	R1	RVPBRU
	Legionella spp. (including L. longbeachae) qPCR molecular detection and culture (from urine antigen negative patients admitted with pneumonia,	Lower respiratory tract samples (sputa, BAL, tracheal aspirate) and other clinical samples in a sterile container	Urgent samples should be notified by phone	R1, clearly marked Legionella species	RVPBRU

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
	pneumococci urine/routine respiratory pathogen screen negative patients only). Charged for service				
	Identification and epidemiological typing of clinical or outbreak associated isolates	Pure culture on either BCYE medium or a dense suspension in sterile distilled water or Page's saline	Varies	R1	RVPBRU
Leptospira spp.	BRD offers reference service for specimens that have been referred via Rare and Imported Pathogens Laboratory (RIPL), Public Health England (PHE), Porton Telephone 01980 612 348 only. All specimens should be sent to RIPL in the first instance. They will then be tested and referred to RVPBRU, BRD, PHE Colindale.				RIPL, PHE Email ripl@phe.gov.uk
	Microscopic Agglutination Test (MAT)	1.5mL preferred (500 µL minimum volume), serum, plasma or clotted blood 7-10day post onset and repeat sample 7 days following first	12 working days	DX 6930400 P3	MAT discussion RVPBRU
	Leptospira MLST Specimens from PCR positive patients will be referred to RVPBRU, BRD, PHE Colindale.	CSF: Minimum volume 0.5mL (0.25mL for MLST and 0.25mL for PCR). Serum taken within 7-10 days after the onset of symptoms. Urine taken as soon as possible. Post mortem tissue specimens not fixed, taken ASAP after death.	Varies	P3	MLST discussion RVPBRU

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Leuconostoc spp.	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Listeria spp.	*Listeria* species identification, serotyping and typing of *L. monocytogenes* by whole genome sequencing and SNP analysis	Pure culture on agar slopes	14 days	L4	GBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Lymphogranuloma venereum - LGV	RT-PCR for the detection of LGV	CSU (refer to VRD Use Manual			
Mycoplasma spp.	*M. hominis* and *Ureaplasma* spp.: PCR and/or culture	Minimum volume 200 µL of respiratory, CSF, joint and wound, aspirates in a sterile container	PCR - 5 days Culture – up to 42 days	R1	RVPBRU
	Mycoplasma/Ureaplasma: Characterisation and molecular methods	Pure culture on mycoplasma medium or chocolate/blood agar slope	Varies	R1	RVPBRU
	M. pneumoniae: PCR and determination of mutations associated with macrolide resistance by PCR and sequencing.	Minimum volume 200 µL of respiratory sample (LRT or throat swab) in a sterile container CSF with paired respiratory samples or DNA extract of positive specimen	5 days	R1	RVPBRU

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
	M. genitalium: Molecular detection of the adhesion *MgPa* and *gap* genes and determination of mutations associated with macrolide resistance by PCR and sequencing on all clinical specimens found positive for *M. genitalium*. Molecular detection of fluoroquinolone resistance is only available for patients who have failed quinolone treatment	Residual specimen from unprocessed NAAT swab transport medium (minimum volume =400 µL), fresh dry swab or, urine (minimum volume 3 mL) or extracted DNA (from previously positive specimens)	8 days	B7	AMRHAI
	Other species: Culture, PCR and sequencing when relevant		PCR: 5 days Culture: up to 6 weeks	R1	RVPBRU
Neisseria gonorrhoeae (putative) - for other *Neisseria spp.* please refer to the Bacterial Identification Section).	*N. gonorrhoeae:* Confirmation of identification by MALDI ToF. Further phenotypic and molecular methods will be used if necessary. Medicolegal processing is not available for isolates which have already been confirmed as *N. gonorrhoeae* by 2 different tests	Pure culture on chocolate slope or VCM swab	7 days	B2 – For medicolegal isolates, contact lab before sending	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
	Susceptibility testing: For isolates that exhibit resistance to ceftriaxone, spectinomycin or from suspected treatment failures only	Pure culture on chocolate slope or VCM swab	7 days	B2	AMRHAI
	Programme: Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP)	Isolates submission is based on a pre-agreement between the laboratory and AMRSTI	GRASP Annual Report	Contact Lab before sending isolates	AMRHAI
Nocardia spp.	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
	Identification and confirmation	Pure culture, Agar slope	7 days	M1, H2	AMRHAI
Pandoraea spp.	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Pediococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Pseudomonas spp.	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Resistance Mechanism Detection	Molecular detection of acquired carbapenemase genes and transferable colistin resistance genes in Gram-negatives, and characterisation of linezolid resistance mechanism(s) in staphylococci and enterococci (G2576T mutation, plus plasmid mediated linezolid resistance genes)	Pure culture, Agar slope	14 days	H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
Salmonella spp.	Identification to genus and species level and determination of sequence types and serovar by whole genome sequencing	Pure culture on Dorset's egg or nutrient agar slope	17 days	L4	GBRU
	Analysis of whole genome sequence data to support outbreak investigations	Pure culture on Dorset's egg or nutrient agar slope	By arrangement	L4	GBRU
Serratia spp.	Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Shigella spp.	Identification to genus and species level and molecular typing by whole genome sequencing	Pure culture on Dorset's egg or nutrient agar slope	14 days	L4	GBRU
Staphylococcus spp.	*S. aureus* (multiple isolates from suspected clusters): Molecular typing derived from whole genome sequence data	Pure culture, Agar slope	1 to 14 days	H1, H2	AMRHAI
	S. aureus (single isolates): Molecular typing derived from whole genome sequence data	Pure culture, Agar slope	14 days	H1, H2	AMRHAI
	Staphylococcus coagulase negative: Species identification and Molecular typing	Pure culture, Agar slope	1 to 15 days	H1, H2	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
	Resistance gene detection derived from whole genome sequence data	Pure culture, Agar slope	14 days	H1, H2	AMRHAI

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
	S. aureus: PVL testing only	Pure culture, Agar slope	1-6 days	H1, H2	AMRHAI
	Virulence gene detection (14 genes, incl. PVL) derived from whole genome sequence data	Pure culture, Agar slope	14 days	H1, H2	AMRHAI
	Enterotoxin gene detection (suspected staphylococcal food poisoning) derived from whole genome sequence data	Pure culture, Agar slope	14 days	H1, H2	AMRHAI
Stenotrophomonas	*S. maltophilia*: Molecular typing and antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Streptococcus spp. and related genera	*S. pyogenes* (Lancefield Group A) typing of invasive isolates (and outbreak/cluster associated non-invasive isolates)	Pure culture on blood or chocolate agar slope. Charcoal swabs are not suitable.	6 days	R1	RVPBRU
	S. agalactiae (Lancefield Group B) typing of invasive isolates (and outbreak/cluster associated non-invasive isolates)	Pure culture on blood or chocolate agar slope	6 days	R1	RVPBRU
	Streptococci Lancefield Group C and G typing of invasive isolates (and outbreak/cluster associated non-invasive isolates)	Pure culture on blood or chocolate agar slope	Contact laboratory	R1	RVPBRU
	S. pneumoniae: species confirmation and capsule typing of invasive isolates (by whole genome sequencing)	Pure culture on blood or chocolate agar slope	14 days	R3	RVPBRU

Services	Test type	Sample required	Target TAT*	Request form	Contact unit
	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Tetragenococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Treponema	*T. pallidum* (syphilis): Serological	CSU (refer to VRD User Manual)			
	T. pallidum/*Haemophilus ducreyi* /Herpes Simplex Virus (HSV) complex (Genital ulcer disease) – PCR				
Ureaplasma	Refer to *Mycoplasma*			R1	RVPBRU
Vagococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	R1	RVPBRU
	Antimicrobial susceptibility	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Vibrio (including Aeromonas and Plesiomonas)	Identification to genus and species level and serotyping	Pure culture on Dorset's egg or nutrient agar slope	14 days	L4	GBRU
Yersinia	Identification and antibiotic susceptibility	Pure culture on Dorset's egg or nutrient agar slope	14 days	L4	GBRU

*TAT – Turnaround Times

Reports

Reports will be delivered electronically as PDF documents via E-lab or will be printed and delivered by post if the referring laboratory is not registered to E-lab. Please contact the LIMS Helpdesk (LimsHelpdesk@phe.gov.uk) for details on how to register for E-lab and further information on the system. Reports will only be sent to the requestor named on the request form.

Policy on faxing and emailing reports containing patients' data

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

1. It is PHE Microbiology Services policy that reports containing patients' data should not be sent by fax or email.
2. Emails cannot be relied on to guarantee security of patients' data because they can be intercepted by a third party *en route* (except for those sent within the PHE network, within the NHS.Net network, or by encrypted e- mails)
3. 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 to 'PHE Microbiology Services – recognition of Caldicott recommendations' on page 20 of this manual.
4. 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.
5. Assurance must be sought from the intended recipient of the faxed report, preferably in writing, that the receiving fax machine is a 'safe-haven'.
6. Measures must be taken to minimise the risk of misdialling, either by doublechecking numbers or having frequently used numbers available on the fax machine's memory dial facility.
7. Confirmation must always be sought from the intended recipient that the fax is expected and has been received.
8. Confidential letter submission guidelines - Do not include confidential letters within the bio-bottle or the specimen box which are not related to the specimen. These should be sent separately and be clearly marked/addressed naming the recipient and indicating for the attention of the addressee only.

Quality assurance in BRD and referral site accreditation

BRD is accredited by the United Kingdom Accreditation Service (UKAS) to ISO 15189:2012 and ISO 17025:2017. We receive many requests regarding the accreditation status of BRD and the following information may be of assistance: UKAS ISO 15189:2012 and ISO17025:2017 laboratory accreditation number: **8197**

General information about our accreditation (including copies of certificates): see 'Quality at the laboratories of Public Health England Colindale: National Infection Service – NIS Laboratories', available on the PHE website (https://www.gov.uk/government/publications/quality-standards-microbiology- services-colindale).

List of accredited services: see the schedule of accreditation on the UKAS website for the test repertoire stated on the Schedule of Accreditation (link https://www.ukas.com/search-accredited-organisations) (lab reference 8197).

Participation in EQA and IQA schemes: all BRD laboratories participate in these where available and appropriate for the examination and interpretation of examination results.

Any issues with EQA performance that could affect any of the services provided are communicated directly to service users where relevant. The quality of our systems is also checked by our IQA schemes, which require selection of referred samples for 'blinded' testing at a later date. After processing, the results for IQA samples are unblinded and are assessed against the results originally reported to the sending laboratory. Any discrepancies are fully investigated as to their root cause before any remedial action is implemented. Results of our EQA and IQA performance are discussed at Management Review and Unit meetings as appropriate.

Service updates: Users will be informed in a timely manner of any delays beyond the published turnaround times where these could compromise patient care.
Issue of revised reports: any amendments to original reports will be highlighted to users.

Authorisation of reports: staff authorising reports are competency assessed, and, additionally, medical staff undergo revalidation to meet the professional standards set by the GMC.

Contact BRD Quality Assurance Manager thamayanthy.ramesh@phe.gov.uk for any additional quality-related enquiries, Tel: 020 8327 6642.

Complaints procedure

If there is a problem, or you are not satisfied with the service you have received, contact the appropriate Unit or Section heads or otherwise Deputy Director of

Reference Microbiology, Professor Neil Woodford or Head of Operations, Steve Harbour. We endeavour to be responsive to the changing needs of all users of our services. We welcome comments on how we can improve the provision of these services. Please contact the Department if you have any queries.

PHE microbiology services - recognition of Caldicott recommendations

The recommendations of the Caldicott Report (1997) and the subsequent Information Governance Review (2013) have been adopted by Public Health England and by the National Health Service as a whole. PHE observes Caldicott guidance and has appointed its own Caldicott Guardian. The Caldicott Guardian safeguards and governs the uses of patient information within PHE, actively supports work to enable information sharing and advises on options for lawful and ethical processing of information as required. Caldicott Guardianship is a major component of broader information governance within PHE. All enquiries about the security and use of PID at PHE should be addressed to caldicott@phe.gov.uk.

Compliance with the Human Tissue Act

PHE Microbiology Services Colindale is licensed by the Human Tissue Authority (licence number 12459) to store tissues from deceased people for scheduled purposes. Post mortem samples are submitted 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. Microbiology Services.

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, for example examination of post mortem tissue, following needlestick injury or the coroner has requested that samples are retained for further testing, samples are disposed of within 3 months of the initial test being performed. for example, examination of post mortem tissue following needlestick injury.

When tissue samples from deceased people are received at Microbiology Services Colindale, 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 other clinical samples we receive are disposed of. However, we will adhere to any specific requirements regarding disposal or returning of tissue samples if requested by the sending coroner or pathologist.

Appendix 1: AMRHAI reference unit

For key staff and contact list refer to the last page.

To ensure a pure strain is tested please only send a slope of a single colony pick. Should a second strain/colony variant be identified – this could lead to an extra charge being incurred (contact lab for prices) or the sample being rejected. If you have identified 2 strains which require testing/comparison, please send them as 2 separate slopes of pure cultures.

Bacterial identification Service (BIDS)

BIDS provide specialist identification services for ‘unknowns’, which includes atypical, difficult to isolate or emerging bacterial pathogens detected in culture-negative clinical samples and for isolates with no national reference facility.

Isolate identification

All unknown isolates will first be tested by MALDI-ToF MS, and 16S rRNA gene analysis will only be used where reliable species identification is not achieved. Identification will be supplemented by phenotypic testing for certain groups of organisms where species identification is doubtful or requires additional confirmation.

Atypical and rarely isolated strains

Atypical isolates are those with phenotypic or physiological profiles deviating from the majority of strains belonging to the same genus or species (for example catalase test, oxidase or sugar fermentation), which affects the ability of the laboratory where it has been isolated to confirm its identity accurately.
Laboratories may also seek confirmatory testing, since the tests currently in use target groups of known clinically-significant organisms and may not confirm the identity of emerging and unusual infectious agents.

Bacterial taxa that are difficult to identify to the species level

Such species may have high genetic similarity or paucity in differentiating phenotypic tests resulting in misidentification or may not be well represented in MALDI-ToF MS databases, giving low scores or unreliable identification.

Aerobic actinomycetes

Organisms collectively grouped under the broad category ‘aerobic, Gram-positive with/without branching filaments’ form a very diverse collection of ill-defined genera and species. Often referred to as the ‘non-TB mycobacteria complex’, ‘Nocardia complex’, ‘Rhodococcus – Gordonia – Tsukamurella – Corynebacterium complex’ etc., they may be seen in pathology specimens and are often highlighted by the presence of sulphur granules. The taxonomy of this group has been actively investigated over the last 20 years but is still in a state of flux. Their poor phenotypic identification is often compounded by their slow and poor growth.

NOTE: Some *Mycobacterium* spp. isolates are similar in morphology to aerobic actinomycetes. If the isolate or a clinical sample is suspected to be a member of the *Mycobacterium* TB-complex based on preliminary tests or clinical assessment, then it is not accepted for this service and should be referred to the PHE National Mycobacterium Reference Service in the first instance.

Clinical sample identification

BIDS provide a specialist service for the detection and identification of bacteria in clinical samples from normally sterile samples. Detection is performed by real-time 16S rDNA PCR, which has been shown to improve sensitivity of detection.
Suitable sample types include tissue (for example native heart valves), paraffin-embedded tissue, nonpipettable liquids as pus, and liquids/fluids (for example blood, CSF, blood culture fluid). Unsuitable samples from non-sterile sites include samples in direct contact with skin or mucous membranes and samples in direct connection to the intestine.

Samples should be transported in a small sterile container (for example 1.5ml microfuge tube or universal) without adding any additional water, buffers or preservatives. Sterile water or PBS might be contaminated with DNA from pseudomonas and pseudomonas like bacteria.

The amount of material must be adequate to maintain maximum sensitivity. In general, we recommend 200 µl or more for liquid material (no more than 1ml to be supplied) and a ‘finger nail’-sized piece of tissue for solid samples ~ 1cm^3, selecting the most necrotic diseased area for testing. Please do not submit excess sample material, as this may result in your sample being quarantined or rejected. For blood culture medium, remove 200 µl to 1,000 µl of fluid using aseptic technique to a sterile container. Please ensure the source is clearly indicated by ticking the ‘blood culture fluid’ box on the request form, this helps us to differentiate from other blood samples and for correct protocols to be used for processing.

For paraffin-embedded tissues supply freshly cut sections of FFPE tissue, each with a thickness of up to 10 µm. Up to 3 sections, each with a thickness of up to 10 µm and a surface area of up to 250 mm^2, can be combined in 1 DNA extraction preparation. The histologists should choose tissue sections that show signs of infection or show anything on gram stain and removal of any excess wax from the sections would be most helpful. These sections should be placed in a sterile universal or tube and no buffers or water added.

Outcome of identification tests include:

- identifying atypical isolates or novel pathogens that fail identification by conventional methods
- confirmation of identification of aerobic actinomycetes
- identification of bacteria detected in samples from normally sterile sites

The important factors affecting our ability to provide a timely service include:

- inappropriate or incorrectly completed request forms
- safety question on request form not completed
- slow growing fastidious organisms
- mixed cultures submitted
- insufficient clinical sample submitted
- non-sterile site clinical samples submitted

Opportunistic pathogens section Identification

Identification by MALDI-ToF MS and sequence-based methods is offered for pathogens from patients with cystic fibrosis and for other opportunistic pathogens, especially *Acinetobacter, Burkholderia, Enterobacter, Enterococcus, Klebsiella, Achromobacter, Pandoraea* and *Ralstonia* species, *Stenotrophomonas maltophilia, Burkholderia pseudomallei*, *Cronobacter sakazakii* and medically- important pseudomonads.

Please note: AMRHAI will not perform identification to species level on Pseudomonas spp. (non Pseudomonas aeruginosa strains).

The main factor affecting our ability to offer a timely and clinically relevant service is the lack of clinical information.

Requests for work on presumptive isolates must include:

- full details of sending laboratory's results
- an indication of whether the isolate(s) may be a hazard group 3 organism – failure to provide necessary information on the form can result in an isolate being handled at Containment Level 2 instead of Containment Level 3,

putting staff at risk (in these instances, a report of the incident will be sent to the Health and Safety Executive)
- full clinical details, including clinical and contact history. Failure to provide necessary clinical information on the form can also result in an isolate being tested using inappropriate methods which will delay reporting
- an indication of any recent travel abroad
- reason for typing investigations – that is details of the comparisons sought and of the underlying question posed (we regret that 'ongoing surveillance' and 'vancomycin resistant enterococcus' will not be considered sufficient).

Molecular (DNA-based) typing

For inter-strain comparative purposes, a molecular typing service is available for all the organisms listed above, plus any other species involved in suspected outbreaks of healthcare-associated infection. Techniques used are pulsed-field gel electrophoresis (PFGE), whole genome sequencing (for *Escherichia coli)* or variable number tandem repeat (VNTR) analysis (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Mycobacterium abscessus* complex). However, please note that the enterococcus PFGE molecular typing service was suspended in August 2019; please discuss any such requirements with the Colindale Medical Microbiology Team (ColindaleMedMicro@phe.gov.uk).

In addition, the following are offered:

- further characterisation of isolates of *Acinetobacter baumannii* by detection of *bla*$_{OXA}$ carbapenemase genes, identification of isolates belonging to the major clonal lineages (international clones I, II and III), and determination of repeat numbers at VNTR loci with small repeat units, that can provide discrimination within a PFGE type.
- PCR identification of capsular types K1, K2, K5, K54, and K57 of *Klebsiella* spp., associated with invasive disease, and of 3 putative virulence factors (*rmpA*, *rmpA2* and *wcaG*).

Important factors affecting the performance of the test include:

- poor growers
- isolates where DNA degrades
- autolytic enzymes
- single isolate with no indication of what it should be compared with

Staphylococcus Reference

The 'spa typing' service is no longer available and it is now replaced with Whole Genome Sequencing (WGS) service.

A ‘phage typing service for S. aureus is no longer available. The International Set of *S. aureus* ‘phages, together with their propagating strains, are available from NCTC.

Whole-genome sequencing

The analysis of WGS data to support outbreak investigations was launched in April 2020 Genetic relatedness of isolates linked to a cluster is assessed by Single Nucleotide Polymorphism (SNP) based analyses from WGS data.

Outbreak report includes :

- Lineage (Multi Locus Sequence Type – MLST)
- Cluster Address (derived from SNP analysis)

WGS is available for the characterisation of single isolates of MSSA or MRSA referred for testing. These include isolates referred for reasons such as (i) surveillance - for example, MRSA or MSSA from cases of bacteraemia, (ii) typing, antimicrobial resistance gene and/or toxin gene profiling, (iii) such as the identification of suspected Hospital vs Community vs Livestock-Associated MRSA.

Depending on the nature of the reason for referral, the following will be derived and reported from WGS data:

1. The lineage (MLST)
2. The detection of resistance genes including:
- *mecA* and its homologue *mecC*, which confer resistance to oxacillin (charged)
- *mupA* and *mupB* which confer high-level resistance to mupirocin (charged)
3. Toxin gene profiling (Charged), providing insights into strain virulence, including:
- 9 enterotoxin genes (sea-see and seg-sei)
- 3 exfoliative toxin genes eta,etb and etd
- Toxic shock syndrome toxin gene 1 (tst)
- Panton-Valentine Leukocidin toxin gene (luk-PV)

Key factors affecting the performance of the test include:

- mixed culture
- single isolate with no indication of what it should be compared with
- lack of epidemiological information

Non-enteric disease

When requesting *S. aureus* toxin gene testing, select either PVL-testing only or extended toxin gene profiling (the latter includes all 14 toxin genes listed above). Where the toxin request is NOT diagnostic, we will not charge but the free text field MUST contain the relevant previous referral details of related isolates – for example, MS - Colindale Laboratory reference numbers or details of the outbreak/diagnostic isolates sent previously. If this is not included, we will assume it relates to primary diagnosis and will charge accordingly (please refer to latest price lists).

Enteric disease

Isolates of *S. aureus* from foods and/or cases of suspected food poisoning are screened for 9 enterotoxin genes (A-E and G-J). Where detection of staphylococcal enterotoxins in samples of food or beverages is required, please contact the Gastrointestinal Bacteria Reference unit (GBRU).

PVL testing

Screening of isolates for the presence of the Panton-Valentine Leukocidin toxin genes (*luk-PV*) by real-time PCR is available as a ‘stand alone’ test (Charged).

Identification of coagulase-negative staphylococci (CoNS)

Isolates are identified by MALDI-ToF MS (Charged). Where reliable species identification is not achieved, isolates are analysed by 16S rRNA gene analysis.

Important factors affecting the performance of these tests include:

- slow growers
- organisms with specific growth requirements

Fine strain typing of CoNS

PFGE-based analyses are available for inter-strain comparative purposes, including suspected outbreaks in healthcare or community settings.

Key factors affecting the performance of the test include:

- poor growers
- isolates where DNA degrades
- autolytic enzymes

Antimicrobial Resistance in Sexually Transmitted Infections

The main activities of the AMRSTI section are:

1. Antibiotic susceptibility testing of *N. gonorrhoeae* for national and international surveillance of resistance.Detection and confirmation of alert resistances and investigation of the underlying mechanisms.
2. Characterisation of circulating strain types, including to inform and aid outbreak investigations and suspected treatment failures.
3. Evaluation of new phenotypic and genotypic methods for investigating AMR in gonorrhoea and other STIs.

Referral of putative *N. gonorrhoeae* cultures

A reference is available for isolates that require confirmation of identification because local results were anomalous – all isolates will first be tested by MALDI- ToF MS. Identification will be supplemented by phenotypic testing and where necessary an *N. gonorrhoeae* specific PCR when reliable species identification is not achieved by MALDI-ToF MS.

Antimicrobial susceptibility testing of confirmed isolates of *N. gonorrhoeae* with suspected resistance to ceftriaxone (first line antimicrobial therapy) or spectinomycin or from suspected treatment failures. resistance.

NB: We no longer seek to confirm *N. gonorrhoeae* with azithromycin resistance.

Antimicrobial susceptibility testing of confirmed isolates of *N. gonorrhoeae* that have been related to clinical treatment failure.

Confirmation of identification for medico-legal purposes (please contact lab before sending isolates) – refer to Specimen Submission Guidelines. Please note that medicolegal processing is not available for isolates which have already been confirmed as *N. gonorrhoeae* by 2 different tests.

AMRSTI will accept viable cultures on a chocolate slope or on a VCM swab.

Mycoplasma genitalium

Molecular detection of *M. genitalium* MgPa adhesion and gap genes, and determination of mutations associated with macrolide resistance by PCR and sequencing. This AMR detection will be undertaken automatically on all clinical specimens found positive for *M. genitalium* – there is no opt-out.

Molecular determination of mutations associated with fluoroquinolone resistance is only available for patients who have failed quinolone treatment – this must be made clear on the referral form.

AMRSTI will accept specimens and DNA extracts for:

- *M. genitalium* detection from symptomatic patients, known contact cases or for test of cure
- Specimens accepted include rectal and genital swabs
- Urine
- extracted DNA – ideally only from previously positive specimens as an internal control result may not be available

Please note, charges will be levied for this service.

AMRSTI also performs surveillance of antimicrobial resistance and investigates molecular epidemiology of bacterial STIs through various programmes and projects.

The Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP)

GRASP is a sentinel surveillance scheme monitoring antimicrobial resistance in *N. gonorrhoeae* across England and Wales, annually. Participating laboratories refer all gonococcal isolates identified over a 2 to 3-month period (July – August/September) to AMRSTI for susceptibility testing. Isolates can be referred frozen on Microbank beads (storage in the referring laboratory must be at -80°C). Frozen batches will be collected by courier by arrangement.)

Antimicrobial Resistance and Mechanisms Service

Confirmation of unusual resistances

AMRHAI investigates isolates found by diagnostic laboratories to have unusual resistances, aiming to identify (i) treatment options (ii) emerging resistance of public health importance (iii) underlying resistance mechanisms (iv) clonal spread of resistant strains. We have the capacity to determine the activity of most antibiotics available in the UK against most species (excluding obligate anaerobes, category 3, *Mycobacteria* spp. and enteric pathogens). Please state your requirements clearly on the request form, failure to provide adequate reasons for submission to AMRHAI may result in rejection of your isolate without testing.

Diagnostic laboratories should familiarise themselves with EUCAST guidance for susceptibility testing of organisms/agents for which there are no EUCAST clinical breakpoints. Lack of EUCAST clinical breakpoints is NOT sufficient justification alone for referral to AMRHAI. This includes genera such as *Bacillus* spp., *Nocardia* spp. and organisms formerly classified as nutritionally variant streptococci amongst many others. Isolates should only be referred to AMRHAI if from invasive sites of infections (eg blood, CSF, joint or pleural fluid), or if local testing identifies unusual resistance that requires confirmation. Please contact AMRHAI if you require advice on growth conditions or assistance with interpreting your minimum inhibitory concentration (MIC) values.

While AMRHAI is willing to examine a wide range of resistance phenotypes for customers, diagnostic laboratories should be aware of EUCAST guidelines regarding intrinsic resistance and exceptional resistance phenotypes. Tables 1 and 2 that follow identify combinations of organism/resistance phenotypes that AMRHAI views as exceptional. Any bacterial isolates exhibiting these resistance phenotypes should first be reviewed by diagnostic laboratories in line with EUCAST guidance to ensure accuracy of the results. If resistance is confirmed, we advise referral of the isolates to AMRHAI.

We are happy to examine other unusual combinations of resistance(s) and organism(s), and cases where the sender has obtained conflicting results by different methods (eg where an automated system identifies an isolate as having a particular resistance phenotype but this cannot be confirmed by classical methodology).

Determination of MIC values for referred isolates is undertaken by broth microdilution, agar dilution and occasionally by gradient strip test. Interpretative reading of these antibiograms allows assessment of the likely dominant underlying resistance mechanisms. The antibiotics we report MIC values for are those listed in EUCAST or CLSI guidelines as appropriate for the species. NHS diagnostic laboratories will be charged for MIC determination unless exceptional resistance is confirmed. A charge per additional antibiotic is also applied for determining MICs of antibiotics outside of our standard panels. A charge will also apply for resolution of mixed cultures (susceptibility testing will not be performed on submissions that yield more than 2 colony variants).

The request will be rejected, and a standard charge applied when the bacterial identification obtained by AMRHAI differs significantly from the identification stated on the referral form (eg Gram-negative referred but Gram-positive organism isolated; Enterobacterales referred but Gram- negative non-fermenter isolated).
For the correct interpretation of susceptibilities, use of appropriate breakpoints and interpretation of mechanisms, isolates must be correctly identified to species level. You will be charged if unidentified ‘coliform/gram-negative rod’ isolates are submitted, unless also formally sent for reference identification.

Please note we no longer offer a service for detecting extended-spectrum beta-lactamase (ESBL) or acquired AmpC as there are now many commercial diagnostic

tests available to undertake this work locally. By following EUCAST guidance diagnostic laboratories should be able to identify, and differentiate between, ESBL and AmpC activity without the need for referral to AMRHAI.

We no longer determine MICs for confirmed carbapenemase-producing Enterobacterales (CPE) from rectal/faecal screens (that is, gut colonisation), or for those isolated from environmental samples. Should a patient go on to develop an infection, we will be happy to perform susceptibility testing on the clinically relevant isolate. We will also continue to determine MICs for CPE from other sample types (if required), and for all isolates sent in for carbapenemase testing that are found PCR-negative.

For an up-to-date list of other charges please contact lab for the PHE Price List. If an isolate is submitted for 'confirmation of results', please be aware that we can only comment if the results requiring confirmation are stated.

If you have a query about a report, please telephone the validator, using the contact details listed on our reports.

Key factors affecting the performance of the test include:

- slow growers
- organisms with specific growth requirements
- organisms which have not been identified

Table 1: Exceptional resistance phenotypes in Gram-negative bacteria.

Organism	Antimicrobial resistance phenotype confirmed by diagnostic laboratory	Should this isolate be sent to AMRHAI?	Test(s) to be provided by AMRHAI	Further information
Any Enterobacterales[a]	Meropenem MIC >0.12 mg/L or meropenem (10 µg) disc diffusion zone diameter <28 mm (N.B. if meropenem 25-27 mm only follow up IF piperacillin/tazobactam <17 mm and/or temocillin <11 mm). These isolates should be screened for the 'big 4/5' carbapenemases (KPC, OXA-48-like, NDM, VIM +/- IMP) by PCR or immunochromatographic assay	YES - isolates positive for the 'big 4' carbapenemase families and from invasive infections only should be referred for inclusion in the national strain archive Isolates negative for the 'big 4' carbapenemases should be referred to rule out presence of rarer carbapenemase families	Real-time PCR to screen for an extended panel of carbapenemase genes[b] MICs on request for isolates from clinical sites only	https://www.gov.uk/government/publications/smi-b-60-detection-of-bacteria-with-carbapenem-hydrolysing-lactamases-carbapenemases http://www.eucast.org/resistance_mechanisms/ Do NOT send isolates of *Enterobacter* spp. that have borderline resistance to ertapenem, but remain fully susceptible to other carbapenems Do NOT send isolates of *Serratia*, *Morganella* or *Proteus* spp. that are borderline resistant to imipenem, but susceptible to other carbapenems We are regularly asked to define criteria for referring carbapenem-resistant bacteria for investigation. These are subjective and under regular review
	High-level temocillin resistance (MIC >64 mg/L; zone diameter <11 mm) AND piperacillin/tazobactam resistance (MIC >64	YES - isolates positive for an OXA-48-like carbapenemase and from invasive infections only should be referred for	MICs on request for isolates from clinical sites only	https://www.gov.uk/government/publications/smi-b-60-detection-of-bacteria-with-carbapenem-hydrolysing-lactamases-carbapenemases

	mg/L; zone diameter <17 mm). These isolates should be screened for OXA-48-like carbapenemases by PCR or immunochromatographic assay	inclusion in the national strain archive		http://www.eucast.org/resistance_mechanisms/
	Ceftazidime/avibactam resistance	YES – but only if isolates are confirmed as negative for class B (NDM, VIM or IMP) carbapenemases	Confirmation of resistance; testing of alternative agents if requested	N/A
	Pan-aminoglycoside resistance (ie resistance to all of amikacin, gentamicin and tobramycin)	NO – confirmation by diagnostic laboratory only.	N/A	
	High-level tigecycline resistance (MIC >4mg/L)	NO – confirmation by diagnostic laboratory only.	N/A	
Any Enterobacterales[a] (except *Serratia* spp., *Proteus* spp. *Hafnia* spp. and *Morganella* spp.)	Colistin resistance as determined by broth microdilution (use of automated system, disc diffusion or gradient strip test is NOT appropriate)	YES – but only isolates are found to be resistant via broth microdilution by the diagnostic laboratory	Confirmation of resistance by broth microdilution; testing of alternative agents if requested Screening for transmissible colistin resistance (*mcr*) genes	http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Warnings/Warnings_docs/Warning_-_colistin_AST.pdf
Acinetobacter spp.	Isolates suspected to produce a metallo-carbapenemase: meropenem or imipenem resistance AND exhibit	YES	Real-time PCR to screen for an extended panel of carbapenemase genes[b]	https://www.gov.uk/government/publications/smi-b-60-detection-of-bacteria-with-carbapenem-hydrolysing-lactamases-carbapenemases

	strong imipenem/EDTA synergy		MICs on request for isolates from clinical sites only	http://www.eucast.org/resistance_mechanisms/ Do NOT send isolates of *Acinetobacter* spp. that are resistant to ertapenem, but susceptible to other carbapenems. Ertapenem resistance is inherent in the genus We are regularly asked to define criteria for referring carbapenem-resistant bacteria for investigation. These are subjective and under regular review
	Colistin resistance – see Enterobacterales section	see Enterobacterales section	see Enterobacterales section	see Enterobacterales section
Pseudomonas aeruginosa	Resistance to ALL of imipenem, meropenem, ceftazidime and piperacillin/tazobactam, AND exhibiting strong imipenem/EDTA synergy (irrespective of susceptibility/resistance to aztreonam). These isolates should be screened locally for NDM, VIM and IMP carbapenemases by PCR or immunochromatographic assay	YES - isolates positive for NDM, VIM or IMP carbapenemase families and from invasive infections only should be referred for inclusion in the national strain archive Isolates negative for VIM, NDM or IMP carbapenemases AND exhibiting strong imipenem/EDTA synergy should be referred to rule out presence of rarer carbapenemase families	Interpretive reading of antibiogram derived by AMRHAI to infer underlying resistance mechanism Real-time PCR to screen for resistance mechanisms if appropriate[b]	https://www.gov.uk/government/publications/smi-b-60-detection-of-bacteria-with-carbapenem-hydrolysing-lactamases-carbapenemases http://www.eucast.org/resistance_mechanisms/ Do NOT send isolates of *Pseudomonas* spp. that are resistant to ertapenem, but susceptible to other carbapenems. Ertapenem resistance is inherent in the genus We are regularly asked to define criteria for referring carbapenem-resistant bacteria for investigation. These are subjective and under regular review

	Colistin resistance – see Enterobacterales section	see Enterobacterales section	see Enterobacterales section	see Enterobacterales section
	Ceftolozane/tazobactam resistance (MIC >2 mg/L)	YES	Interpretive reading of antibiogram derived by AMRHAI to infer underlying resistance mechanism Real-time PCR to screen for resistance mechanisms if appropriate[b]	N/A
Other non-fermenters	Co-trimoxazole resistance	YES	Confirmation of resistance; testing of alternative agents if requested	http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/S_maltophilia_EUCAST_guidance_note_20120201.pdf
	Carbapenem resistance in *Stenotrophomonas maltophilia*, *Aeromonas* spp., *Myroides* spp., *Elizabethkingia* spp. and 'chryseobacteria'	NO	N/A	Do NOT send for investigation of carbapenem resistance because metallo-carbapenemase production is an intrinsic characteristic of these bacteria
Haemophilus influenzae	Resistance to any third-generation cephalosporin or carbapenem	YES	Confirmation of resistance; testing of alternative agents if requested	N/A
	Fluoroquinolone resistance	YES – isolates from invasive infections only. Isolates from other sites – resistance to be confirmed by diagnostic laboratory only	Confirmation of resistance; testing of alternative agents if requested	
Moraxella catarrhalis	Resistance to any third-generation	YES	Confirmation of resistance; testing of	N/A

	cephalosporin or fluoroquinolone		alternative agents if requested	
Organisms/antibiotics for which there are no EUCAST clinical breakpoints	N/A	YES – isolates from invasive infections only. Isolates from other sites - refer to for performing susceptibility testing on organisms/agents for which there are no EUCAST breakpoints		Please contact AMRHAI if you require assistance with interpreting your MIC values

[a] *Salmonella* and *Shigella* strains should be referred to the Gastrointestinal Bacteria Reference Unit.

[b] at the time of writing our real-time PCR screens for carbapenemase genes belonging to class A (KPC, IMI, GES, FRI, SME), class B (NDM, VIM, IMP, DIM, GIM, SIM and SPM) and class D (OXA-48-like, OXA-23-like, OXA-40-like, OXA-51-like and OXA-58-like), and ESBL genes that may be associated with ceftolozane/tazobactam resistance in *P. aeruginosa* (GES, PER and VEB).

Table 2: Exceptional resistance phenotypes in Gram-positive bacteria.

Organism	Antimicrobial resistance phenotype confirmed by diagnostic laboratory[a]	Should this isolate be sent to AMRHAI?	Test(s) to be provided by AMRHAI	Further information
Staphylococcus aureus	Any resistance to ceftaroline, ceftobiprole, vancomycin, teicoplanin, telavancin, dalbavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin or tigecycline	YES	Confirmation of resistance Molecular screening for linezolid/tedizolid resistance mechanisms where appropriate	Do NOT send isolates for determination of heteroresistance to glycopeptides (GISA, hVISA, VISA). Such isolates should be referred to the Specialist Antimicrobial Chemotherapy Unit, Cardiff
	Oxacillin MIC 2 – 8 mg/L, cefoxitin MIC >4 mg/L or a discrepancy between oxacillin and cefoxitin	YES	Confirmation of cefoxitin resistance	EUCAST recommends the use of cefoxitin rather than oxacillin for MRSA screening as the

			Molecular screening for *mecA*/*mecC* where appropriate	specificity of oxacillin is lower than that of cefoxitin: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Expert_Rules/2019/Staphylococcus_ExpertRules_V3.2_20190613.pdf
coagulase-negative staphylococci	Any resistance to ceftaroline, ceftobiprole, vancomycin (but NOT to teicoplanin alone), telavancin, dalbavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin or tigecycline	YES	Confirmation of resistance Molecular screening for linezolid/tedizolid resistance mechanisms where appropriate	Resistance to teicoplanin but NOT to vancomycin is not exceptional in coagulase-negative staphylococci and does NOT warrant referral to AMRHAI
enterococci	Ampicillin/penicillin resistance in *E. faecalis*. Any resistance to daptomycin (*E. faecalis*: MIC >2 mg/L; *E. faecium*: MIC >4 mg/L), tigecycline, linezolid or tedizolid	YES	Confirmation of resistance. Molecular screening for linezolid/tedizolid resistance mechanisms where appropriate	Do NOT send suspected VRE for confirmation of glycopeptide resistance – these can be detected by disc or automated methods and do NOT require confirmation by AMRHAI
Streptococcus pneumoniae	Any resistance to penicillin (MIC ≥4 mg/L), cefotaxime/ceftriaxone (MIC >2 mg/L), meropenem, vancomycin, teicoplanin, telavancin, dalbavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin, tigecycline and/or rifampicin	YES	Confirmation of resistance; testing of alternative agents if requested	Do NOT submit pneumococci for penicillin MIC determination unless MIC ≥4 mg/L has been confirmed locally according to EUCAST guidance: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Warnings/Warnings_docs/Warning_gradient_for_benzyl_and_pnc_21nov2019.pdf

	fluoroquinolone resistance in respiratory isolates	NO – confirmation by diagnostic laboratory only.	N/A	N/A
Streptococci (groups A, B, C and G, β-haemolytic)	Resistance to penicillin, cephalosporins, vancomycin, teicoplanin, telavancin, dalbavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin, fluoroquinolones, or tigecycline	YES	Confirmation of resistance. Molecular screening for linezolid/tedizolid resistance mechanisms where appropriate	
Corynebacterium spp.	Resistance to vancomycin, teicoplanin, telavancin, dalbavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin or tigecycline.	NO – confirmed by diagnostic laboratory only. Refer to AMRHAI only for testing of alternative agents	N/A	N/A
Organisms for which there are no EUCAST clinical breakpoints	N/A	YES – isolates from invasive infections only. Isolates from other sites - refer to EUCAST guidelines for performing susceptibility testing on organisms/agents for which there are no EUCAST breakpoints		Please contact AMRHAI if you require assistance with interpreting your MIC values

[a] Not all agents may currently be routinely tested by diagnostic laboratories.

Therapeutic guidance

By determining MICs of appropriate antibiotics for submitted isolates, AMRHAI aims to elucidate the most suitable options for treatment. To evaluate susceptibility, we use published clinical breakpoints or, in their absence, advise on the best evidence for any potential antibiotic treatment on a pharmacological basis and/or published evidence (see EUCAST guidance for susceptibility testing of organisms/agents for which there are no EUCAST clinical breakpoints).

Where multiply-resistant isolates are submitted for therapeutic guidance, susceptibilities already established by the sender should be recorded on the submission form, along with appropriate clinical details. Any significant resistance mechanisms relevant to treatment will be interpreted from MIC profiles and reported. In addition, we also undertake interpretation of hospital laboratory data on the telephone when there is an urgency.

Given the primary role of a reference laboratory is to support national epidemic intelligence and not to provide a confirmatory diagnostic service, colleagues are reminded that the results generated by AMRHAI may not always be generated in a clinically useful time for individual case management.

Endocarditis

AMRHAI determines MICs for endocarditis isolates to provide therapeutic guidance, as some laboratories choose not to maintain MIC testing capacity. For enterococci, we automatically report MICs of streptomycin and daptomycin. Since this work does not entail investigating exceptional resistance, it is chargeable. To maximise the speed of our response, submission forms must be clearly marked 'ENDOCARDITIS'.

The appropriate telephone number for reporting the results must be given if your laboratory is not signed up to receive reports electronically via the eLab system, which allows access to results shortly after validation.

Molecular investigation of resistance

Genes and mutations sought are those that confer resistance to agents of last resort, including carbapenems, colistin and linezolid. Molecular investigation of colistin and linezolid resistance are uncharged reference services to NHS laboratories. However, NHS laboratories will be charged for CPE confirmation unless screening for the ‘big 4’ carbapenemase families (KPC, OXA-48-like, NDM and VIM) has been performed locally prior to referral to AMRHAI; there are now many commercial diagnostic tests available to undertake this work.

We strongly recommend implementation of a molecular or immunochromatographic assay locally, where rapid testing will have maximal impact on individual patient management.

Services currently offered include the detection of:

- 23S rRNA mutations and plasmid-mediated genes responsible for oxazolidinine resistance in enterococci, staphylococci or streptococci
- genes encoding acquired carbapenemases in *Acinetobacter* spp., Enterobacteriaceae or *Pseudomonas* spp.
- plasmid-mediated genes responsible for colistin resistance in Enterobacteriaceae, *Acinetobacter* spp. or *Pseudomonas* spp.

New antibiotics

AMRHAI liaises with pharmaceutical companies to test new antibiotics against representative or unusually resistant referred isolates, possibly revealing new treatment options. This is undertaken as contracted research.

New diagnostics

AMRHAI liaises with diagnostics companies to test new kits and platforms against representative or unusually resistant referred isolates. This is undertaken as contracted research.

Surveys of resistance

Point prevalence surveys of antibiotic resistance are undertaken, giving measures of the extent and nature of critical resistance problems.

Other useful Information relevant to susceptibility testing services can be found at:

- BSAC site (www.bsac.org.uk)
- BSAC survey site (www.bsacsurv.org.uk)

Infection Prevention and Control Advice Section

Information and advice is available on:

- infection control issues
- education and training
- research and audit
- disinfection and sterilisation
- investigation of healthcare- and community-associated infection, aspects of laboratory safety and other related matters

Appendix 2: Gastrointestinal bacteria reference unit (GBRU)

For key staff and contact list refer to the last page.
GBRU FAX: 0208 327 7112

GBRU provides a national reference facility for bacteria causing gastrointestinal infections. The range of services offered includes: identification to the genus and species level, phenotypic and molecular typing, whole genome sequencing, antimicrobial susceptibility testing and epidemiological typing.

GBRU offers a primary diagnostic service for the detection of Shiga toxin-producing *E. coli* (STEC) in faeces from cases where there is a clinical suspicion of STEC infection, including haemolytic uraemic syndrome (HUS). For the diagnosis of STEC-HUS, if STEC PCR is not available at the local or regional hospital diagnostic laboratory, a faecal specimen (or rectal swab if a faecal specimen is not available) should be rapidly referred to GBRU, as early in the care pathway as possible and before administering antibiotics. The unit also provides the national reference facility for the epidemiological typing and toxin testing for a range of Gram-positive bacteria associated with foodborne infection and intoxication. On identification of a presumptive potential pathogen or high level of toxin.

GBRU is required to notify the appropriate Environmental Health Officer, Consultant in Communicable Disease Control and all other relevant people. Notification will be through a designated, competent senior member of staff.

Bacillus, Clostridia and Listeria

Bacillus species

Identification of *Bacillus* species, other than *B. anthracis*, and molecular typing of Bacillus isolates associated with foodborne outbreaks and other healthcare associated incidents.

Samples or specimens to send are pure cultures of Bacillus on agar slopes isolated from:

- vomitus, faeces or foods suspected to be or linked with cases of food poisoning
- isolates from blood cultures, or from sites that are normally sterile, or other sites where invasive or other diseases are confirmed or suspected
- clinical and environmental sources where cross-infection is suspected

- foods or beverages with levels of Bacillus species including *B. cereus* of ≥104 cfu per g or mL clinical and environmental sources where cross-infection is suspected
  As foods may be contaminated simultaneously with several species of Bacillus; a selection of different colonial types should be sent.

Please fill in the correct request form as completely as possible including your address and telephone number; your specimen/sample reference number; specimen/sample details; your presumptive identification of the isolate together with the testing you require. Brief clinical and epidemiological information including patient details should be included with cultures from cases of infection.

Clostridium botulinum

Diagnostic service for botulism includes the detection of botulinum neurotoxin and PCR detection and isolation of *Clostridium botulinum* from clinical specimens, food and environmental samples associated with suspected cases of botulism.
Tests on food and referred isolates are UKAS ISO 17025 accredited (BRD is UKAS accredited testing laboratory No. 1595).

There are 5 routes by which botulism can arise in humans: foodborne, intestinal colonisation, wound, accidental or deliberate. Details on clinical presentation, diagnosis and laboratory tests for *C. botulinum* are available on the PHE website. Antitoxin for treatment is available on request through the Colindale Duty Doctor System (24 hours telephone: 020 8200 4400) for treatment of foodborne and wound botulism. Advice on treatment and prevention of infant botulism can also be obtained through the Duty Doctor System or from the Infant Botulism Treatment and Prevention Programme, California Department of Health:
http:/www.infantbotulism.org/

Suspected cases of all forms of botulism should be discussed with the Botulism service at Colindale (Dr Gauri Godbole 0782 6859642 and the Colindale Duty Doctor out of hours (0208 200 4400). The service will also discuss and ensure that the most appropriate samples are taken and sent under optimal conditions. Specimens should be sent immediately to the reference laboratory and GBRU notified of their arrival so that necessary preparations for testing can be made.

Specimens or samples to send include:

- 10g or 10mL of suspected food and drink samples (refrigerated)
- serum – at least 2mL to be collected as close to the onset of symptoms as possible (within 3 days) and before antitoxin is given – lysed or EDTA treated blood specimens are not suitable

- faeces – 10g faeces or rectal wash out for toxin detection and a pea-sized portion inoculated into cooked meat broth or other anaerobic media for rapid PCR detection and isolation of *C. botulinum*
- vomitus, gastric washings or gut content – at least 10g in a sterile container
- pus or debrided tissue – to be placed as soon as possible into cooked meat broth or other anaerobic culture medium
- post mortem specimens such as heart blood if not haemolysed – specimens of faeces, gut contents or infected wounds may be useful
- all pure cultures suspected of being *C. botulinum* should be sent in a cooked meat medium using as category A transport

Please complete the correct request form in full including your address and telephone number, patient (specimen) details or food (sample) details including your reference number, major patient symptoms, recent travel history, your identification of the isolate and what testing you require. Brief clinical and epidemiological information should be included. If botulism is suspected, by any route, it is essential that the local CCDC is notified immediately. Please also notify the Microbiology Services Colindale, Duty Doctor (020 8200 4400).

Emergency situations:

During working hours contact a senior member of staff for appropriate urgent attention. Outside working hours, contact the Colindale Duty Doctor (020 8200 4400). Urgent transport of samples to GBRU by taxi or courier should be considered if a clinical diagnosis of food botulism is suspected.

Turnaround times are shown in calendar days and reflect the proportion of tests requiring prolonged observation in order to establish a negative result. They are dependent upon receipt of sample and request as described above and may vary dependent upon the clinical or public health urgency. If neurotoxin is detected, a turnaround time of >5 days may be required to establish the toxin type. Rapid PCR detection of *C. botulinum* can be performed within 3 hours if the appropriate sample (specimen in anaerobic medium) is received into the laboratory during the working day. Positive results will be reported immediately.

For further information or for tests requiring urgent attention, please contact the appropriate member of staff.

Clostridium perfringens

Identification of *C. perfringens* toxin genes in cultures, typing of enterotoxigenic *C. perfringens,* and the detection of *C. perfringens* enterotoxin in faeces.

Specimens or samples to send are pure cultures of *C. perfringens* in anaerobic broth isolated from:

- faeces from cases of diarrhoea obtained after alcohol shock treatment or on direct isolation
- faeces, gut contents or gut biopsy in cases of suspected necrotising enterocolitis
- foods
- faeces and food may be contaminated with several types of *C. perfringens* – it is recommended at least 3 colony picks in separate CMB should be sent from faeces and food

Faeces for enterotoxin detection in cases of diarrhoea:

- minimum sample at least 1g or at least 1mL collected within 3 days from onset of symptoms.
- in cases of suspected necrotising enterocolitis: faeces or gut contents

Information to send:

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, your identification of the isolate and what testing you require. Brief clinical and epidemiological information should be included from cases of *C. perfringens* diarrhoea in particular the date of onset of symptoms. Please indicate if a relationship with other cases by common source is suspected and if the cases are suspected to be food-borne or as a result of person to person spread.

Clostridium tetani

Diagnostic of tetanus in humans by the detection of *C. tetani* neurotoxin in serum or/and by the isolation and identification of toxigenic *C. tetani* (toxin gene detection) from tissues/wound/pus.

Samples or specimens to send are:

- pure cultures suspected to be *C. tetani* in an anaerobic broth
- tissue to be placed into an anaerobic broth
- serum at least 2ml collected as close to the onset of symptoms as possible – serum specimen must be collected before antitoxin is given and lysed or EDTA samples are not suitable and will be tested for the presence of tetanus antibodies before toxin detection is performed

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, your identification of the isolate and what testing you require. Brief clinical and epidemiological information should be included with cultures/specimens from cases of infection.

Listeria

Identification of *Listeria* species and typing of *L. monocytogenes* isolates by WGS.

The samples or specimens to send include:

- pure cultures on agar slopes – isolates from all cases of human listeriosis should be sent for typing, with all reports incorporated into a database for national surveillance of listeriosis
- ideally all Isolates of *L. monocytogenes* from foods and the environment should be sent for typing including in the following circumstances – if the isolates form part of a coordinated survey or follow up investigation
- if there is a concern with a specific food product
- if there is an association with a case of listeriosis
  Foods may be contaminated simultaneously by several species of Listeria, or several strains of *L. monocytogenes*, multiple (ideally 3 to 5) subcultures should therefore be examined for each sample

GBRU offers a service for the identification of *Listeria* species and this may be helpful when laboratories are experiencing difficulties in this area. Isolates of *Listeria* species other than *L. monocytogenes* where these are present at high numbers in food should also be sent.

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, your identification of the isolate and what testing you require. Brief clinical and epidemiological information should be included with cultures from cases of human listeriosis. A more detailed surveillance questionnaire for completion will be sent for each case.

Molecular typing by WGS is performed on all isolates of *L. monocytogenes* submitted to the GBRU for surveillance purposes and to assist in outbreak investigations. These results are not reported routinely but are available on request.

Campylobacter and Helicobacter

Campylobacter

The specimens or samples to send include:

- pure culture sent on Amies charcoal swab (preferably) or other suitable media (for example blood or chocolate agar slope)

It is advisable to pick Campylobacter isolates from a non-selective medium to minimise overgrowth by contaminants. If an overnight delay before posting is anticipated then the isolate should be stored at 40C.

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, recent travel history, your identification of the isolate and what testing you require.

Please note we do not provide a serodiagnostic service for Campylobacter. Preston Microbiology Services offer Campylobacter serology testing (telephone: 01772 522100). GBRU will impose a handling charge for dealing with such requests.

Helicobacter pylori (charged service)

The specimens or samples to send include:

- *H. pylori* cultures: should be harvested from a 48 to 72 hour culture – a heavy suspension (visibly cloudy) should be prepared in either a suitable H. pylori transport medium, sterile saline or any rich broth (for example Brain Heart Infusion)
- Alternatively, Amies charcoal swabs may be used. Isolates should be transported as soon as possible after harvesting.
- Gastric biopsies for culture of *H. pylori* should be sent without delay, preferably within 24 hours. Ideally biopsies should be sent in a suitable *H. pylori* transport medium or alternatively, in sterile saline. Additional details below.

Instructions to optimise the growth of *Helicobacter* spp. from gastric biopsy material:

1. Perform multiple gastric biopsies (5-6), at least 2 from the antrum and 2 from the anterior and posterior corpus respectively.
2. Larger volumes increase the yield.
3. All microbiology specimens should be taken with sterile forceps before the histology specimens are taken to reduce risk of contamination.

4. There is a risk with pooling biopsy specimens that the organism may not be isolated if any contaminating microbial flora from one biopsy cross contaminates others.
5. Gastric biopsies for culture of *H. pylori* should be sent without delay, preferably within 24 hours of collection. Ideally biopsies should be sent in a suitable *H. pylori* transport medium. Alternatively, biopsies can be sent in sterile saline. If a biopsy is not posted / couriered on the day of receipt in your laboratory then please store at 4°C (drying and exposure to air/oxygen kills *Helicobacter* spp. easily).
6. Endoscopic biopsies should be performed in the middle of the week (avoid Fridays) and sent via courier or Dx within 24 hours to the Gastrointestinal Bacteria Reference Unit Colindale.
7. Have treatment free interval before biopsy, at least 2 weeks off PPI and 4 weeks off antibiotics.

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, recent travel history, your identification of the isolate and what testing you require.

We do not provide a serodiagnostic service for Helicobacter. GBRU will impose a handling charge for dealing with such requests.

Escherichia coli, Shigella, Vibrio, Yersinia

Escherichia coli

Services offered include:

- species identification of the genus Escherichia
- *E. coli* serotyping
- *E. coli* Shiga toxin-producing (STEC) O157 phage typing
- typing of STEC O157 by whole genome sequencing
- detection of Shiga toxin (stx) genes by PCR
- identification by PCR of virulence genes in STEC and in strains that may belong to other groups of *E. coli* associated with diarrhoeal illness – these enterovirulent E. coli include enteropathogenic (EPEC), enteroaggregative (EAEC), enterotoxigenic (ETEC), enteroinvasive (EIEC).
- testing of faecal samples for STEC and, by arrangement, other enterovirulent E. coli

The specimens or samples to send include:

- pure culture on Dorset's Egg or Nutrient agar slopes
- faecal sample in standard sealed container ≥ 1 gram

When submitting a culture to GBRU please pick from a non-selective medium or check the purity before sending. Submitting a pure culture significantly reduces sample processing time.

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, recent travel history, your identification of the isolate and what testing you require. If you have any reason to suspect that the agent being submitted is an ACDP HG3, please indicate this clearly on form.

Shigella, Vibrio and Yersinia species

Services offered include:

- species identification of the genus Shigella
- serotyping of *Sh. dysenteriae*, *Sh. flexneri* and *Sh. boydii*
- molecular typing of *Sh. sonnei*, *Sh. dysenteriae*, *Sh. flexneri* and *Sh. boydii*
- species identification of the genus Yersinia (including *Yersinia pestis)*
- species identification of the genus Vibrio (including *Aeromonas* spp. and *Plesiomonas shigelloides*)
- *V. cholerae* serotyping

The specimens or samples to send include:

- pure culture on Dorset's Egg or Nutrient agar slopes.

When submitting a culture to GBRU please pick from a non-selective medium or check the purity before sending. Submitting a pure culture significantly reduces sample processing time.

Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, recent travel history, your identification of the isolate and what testing you require.

Salmonella

Services offered include:

- whole genome sequencing of all Salmonella species providing sequence type and inferred serovar
- analysis of whole genome sequencing data to support outbreak investigations
- investigation of the genetic basis of antibiotic resistance in enteric bacteria

The samples to send include:

- suspect Salmonella cultures: should be submitted on nutrient agar or Dorset egg slopes in screw-capped containers
- urgent submissions: Advise the relevant contacts (from Table) by telephone of any urgent specimen that is – being dispatched to GBRU.

Information to send:
Please complete the correct request form including your address and telephone number, patient (specimen) details including your reference number, major patient symptoms, recent travel history, and your identification of the isolate including the hazard group and what testing you require.

Appendix 3: Respiratory and vaccine preventable bacteria reference unit (RVPBRU)

For key staff and contact list refer to the last page.

The unit is happy to discuss and advise upon particular clinical or epidemiological problems and outbreak investigations with the Unit/Section head in the first instance.

Specimen submissions regarded by the sending laboratory as especially important or urgent should be notified to the Unit by telephone to ensure that the appropriate level of priority is accorded to these specimens immediately upon receipt.
Turnaround times will vary depending on the nature of the enquiry and the complexity of the investigation required. Priority will always be given to outbreak associated isolates.

Where services are offered as reference services (that is, 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.

Respiratory and systemic bacteria section (RSBS)

Lancefield Group A Streptococci (GAS), *Streptococcus pyogenes*

Genotypic classification and epidemiological typing of Group A streptococci (GAS), Streptococcus pyogenes. Typing of GAS is useful in the investigation of both community and hospital outbreaks of GAS infection.

Typing of GAS is based upon determination of the M-protein which is inferred by sequencing 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. Results are reported as an *emm* sequence type, which usually correlates with the M protein type, for example: *emm* ST12 = M type 12

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

The specimens or samples to send:

- pure culture on blood or chocolate agar slope – charcoal swabs are not suitable.

RSBS offers a reference service for typing of *S. pyogenes* strains from non-invasive infections if linked to a cluster of infection under investigation. Non-invasive isolates of *S. pyogenes* (that is; isolates from throat swabs) will be charged.

Please indicate details on the referral form regarding cases under investigation for cross-infection/cluster investigation.
If seeking MICs only from non-invasive *S. pyogenes* isolates please refer directly to AMRHAI.

Lancefield Group B Streptococci (GBS), Streptococcus agalactiae

Serological classification and epidemiological typing of Lancefield group B streptococci (GBS).

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 in the UK are serotypes Ia, Ib, II or III. Serotype III is most commonly associated with neonatal infections.

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 national surveillance of invasive disease due to GBS in this age group (0 to 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.

We do not offer a reference service for typing of *S. agalactiae* isolates from non-invasive infections unless linked to a cluster of infection under investigation. An admin charge will be applied for the referral of non-cluster related superficial isolates of *S. agalactiae* (for example isolates from ear/rectal swabs).

Where typing of non-invasive *S. agalactiae* is required in the investigation of clusters/instances of suspected cross-infection in hospitals/other please contact RVPBRU, and details must be included on the referral form.

If seeking MICs only from non-invasive *S. agalactiae* isolates please refer directly to AMRHAI.

Lancefield Group C and Group G Streptococci

For urgent public health investigations and in other relevant clinical circumstances, after discussion and agreement with Unit/Section heads similar typing to GAS can be undertaken.

Group C and G streptococci may cause both nosocomial (for example: burns unit crossinfection 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, for example *S. equi subsp zooepideicus* 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´ terminus 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.

We do not offer a reference service for typing of Group C and G streptococcal strains from non-invasive infections unless linked to a cluster of infection under investigation. Non-invasive Group C and G streptococcal isolates (that is; isolates from ear/rectal swabs) will not normally be tested and an admin charge may be applied in these cases. If testing is required please contact us and a charge will be levied.

Where typing of non-invasive Group C and G streptococcal isolates may be helpful in the investigation of clusters/instances of suspected cross-infection in hospitals/other please indicate details on the referral form.

If seeking MICs only from non-invasive S. agalactiae isolates please refer directly to AMRHAI.

Identification of streptococci and related genera

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 the section head.

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 for example 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.*

Isolates that needs MIC/MBC and that are not streptococci and may be an enterococcus or a Gram-positive rod will be referred to the AMRHAI Unit. The turnaround time in this instance will vary.

Legionella

A range of reference and confirmatory tests useful in the investigation of individual cases and outbreaks of legionella infection.

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 contact Legionella@phe.gov.uk

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 Team 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. We will perform 2 commercial EIA assays to enable the confirmation of the submitting laboratory's findings.

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

Legionella genome detection and culture from clinical material

These services are provided to assist 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, PCR positive or culture positive patients is particularly encouraged as such samples are likely to yield useful epidemiological typing data. Lower respiratory tract specimens from urine antigen test positive patients taken within 2 days of admission are more likely to be positive by isolation molecular detection and yield typing data.

Respiratory specimens will be tested by qPCR and culture; however, these services are not offered for primary diagnosis unless part of an HPT led investigation. In exceptional circumstances L. pneumophila PCR and culture may be requested as a referred (charged) service after discussion and agreement with the laboratory.

The most commonly referred specimens are sputum and bronchoalveolar lavage (BAL), though the laboratory is pleased to receive other clinical specimens for examination from patients with other evidence of legionella infection.

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

Legionella species detection including *L. longbeachae*

Culture and attempted molecular detection (from urinary antigen negative, intensive care, contact with horticultural growth medium in 14 days prior to onset, pneumococci urine/routine respiratory pathogen screen negative patients only). This is a charged for service.

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.

Identification is made by nutritional characteristics and 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.

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

Leptospira

All specimens tested at PHE Colindale are received and referred via RIPL PHE Porton following positive polymerase chain reaction (PCR) result and/ or positive Leptospiral ELISA IgM testing. RVPBRU offers isolation and confirmatory testing, including the serological diagnosis of human leptospirosis by the microscopic agglutination test (MAT) and Multi-Locus Sequence Type (MLST) of Leptospira from clinical material.

The Unit offers a referred (charged for) service for the confirmation of Leptospira. Costs are included in initial specimen processing charges to RIPL, PHE Porton and additional charges will not be made for specimens received via RIPL, PHE Porton.

Leptospira serology

The microscopic agglutination test (MAT) is the gold standard test with high specificity, allowing for the detection of agglutinating antibodies. Antibodies to Leptospira can be detected 5 to 10 days' post onset of disease. To determine alterations in titre and give an indication of the presumptive infecting serovar in acute Leptospiral infection it is necessary to examine more than one specimen, with a second specimen requested 714 days after the initial specimen.

Leptospira MLST

MLST is performed based on a scheme targeting 7 loci (glmU, pntA, sucA,tpiA, pfkB,mreA, caiB) of 7 pathogenic Leptospira species *(L. alexanderi*, *L. borgpetersen*i, *L. interrogans*, *L. kirschneri*, *L. noguchii, L. santarosai*, *L. weilii*). MLST is performed on clinical samples that have tested PCR positive. It is possible to determine Leptospira species directly direct from urine, serum, cerebral spinal fluid and tissue (biopsies and post-mortem).

Mycoplasma pneumoniae

RSBS 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.

Referral of Mycoplasma pneumoniae samples can be found at: https://www.gov.uk/government/publications/mycoplasma-pneumoniae-referral-ofsamples.

Quick view table (further details below):

Target	Test	Turnaround time	Preferred specimen	Minimum sample Vol.
M. pneumoniae	PCR and determination of point mutations associated with macrolide resistance	5 days	Respiratory sample (LRT or throat swab) Positive DNA extract	0.2mL 0.1mL
Neonate screen: *M hominis / Ureaplasma spp.*	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 (respiratory, CSF, joint and wound, aspirates)	0.2mL
Target	Test	Turnaround time	Preferred specimen	Minimum sample Vol.
Isolates	Culture, PCR and sequencing when relevant	Species dependant (see below)	Culture on blood agar or in transport medium*	N/A

* Please use transport medium for respiratory *Chlamydia, mycoplasmas* and *ureaplasmas* (for example VCM) and not viral transport medium (for example VTM) as not suitable for culture.

The detection of *Mycoplasma pneumoniae* DNA in clinical samples

The unit provides a (charged) *Mycoplasma pneumoniae* primary diagnostic service and a (free of charge) service for confirmation of infection on samples tested positive locally and for identification of presumptive mycoplasma isolates. Reference laboratory confirmatory diagnosis and macrolide resistance testing is provided free of charge.

Referral of *Mycoplasma pneumoniae* samples can be found at: https://www.gov.uk/government/publications/mycoplasma-pneumoniae-referral-ofsamples.

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 (LRT) specimen or throat swab. DNA extracts from known positives can also be referred for determination of point mutations associated with macrolide resistance.

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

Mycoplasma / ureaplasma from clinical material

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.

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 except haemoplasmas.

Relevant PCR, sequencing and culture results will be available dependant on the organism in question. Culture results will be available ASAP following successful isolation. Some species such as M. hominis take only a few days while others such as *M. pirum* may take as long as 6 weeks to isolate.

Neonate screen

U. urealyticum, *U. parvum* and *M. hominis*, may be involved in respiratory infection or rarely meningitis/septicaemia 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.

Detection in genital specimens of *U. urealyticum, U. parvum* and *M. hominis* is not undertaken.

The identification of putative isolates of mycoplasmas and ureaplasmas

This reference service is undertaken by molecular methods including 16S rDNA sequencing.

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

Priority will always be given to isolates of current clinical relevance.

Respiratory chlamydiae

The Unit can provide a reference service for chlamydia DNA detection by PCR, which may be useful in the investigation of potential 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.

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. The PCR assay is not UKAS accredited.

Vaccine Preventable Bacteria Section (VPBS)

Bordetella pertussis and *Bordetella* spp.

The VPBS 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, identification and, where appropriate, phenotypic and genotypic characterisation of isolates, including other *Bordetella* spp.

The laboratory works very closely with the Immunisation and Countermeasures Division, NIS and reports all clinically relevant results to them. National surveillance of pertussis is led by Dr Gayatri Amirthalingam and Dr Helen Campbell, who can be contacted via the switchboard (0208 200 4400).
See: https://www.gov.uk/government/publications/pertussis-guidelines-for-public-healthmanagement

Bordetella pertussis serology

The laboratory 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 of cough for any individuals with a history of prolonged cough.

Please note: This service is NOT suitable for assessment of immune status as there are no agreed correlates of protection for anti-PT IgG.

Bordetella pertussis oral fluid antibody testing

The laboratory offers a service testing for anti-pertussis toxin (PT) IgG antibody levels in oral fluid samples using an in-house EIA, for the diagnosis of pertussis and for national surveillance, as an alternative to serology testing.

This service is offered where the following criteria are met: patients must be aged between 2 and 17 years of age and their local Health Protection Team must be notified of the clinical suspicion of pertussis by their GP in order for the patient to be sent an oral fluid testing kit. The patient takes their own oral fluid sample (2 weeks after onset of cough) and posts it (in a prepaid envelope) directly to the Unit.

Further characterisation of *Bordetella pertussis* isolates

The laboratory 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.

Further characterisation of *Bordetella pertussis* PCR positive specimens

The laboratory requests referral of clinical specimens and/or DNA extracts from specimens found to be *B. pertussis* PCR positive for surveillance purposes and further characterisation. Please note this service is currently been suspended. Contact the laboratory before sending any samples.

Streptococcus pneumoniae identification and capsular typing of pneumococci

We request submission of ALL *S. pneumoniae* isolates from blood, CSF and other normally ‘sterile site’ from episodes of invasive disease for confirmation of identity and capsule serotyping as part of the national surveillance function of our laboratory. Results of pneumococcal capsule typing are shared with the Immunisation and Countermeasures Division, Colindale and contribute to National Surveillance.

Presently available and likely future pneumococcal vaccines contain specific, generally 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.

From October 2017, most *Streptococcus pneumoniae* isolates from invasive disease sent to PHE Colindale for confirmation of identity and capsular typing will undergo

routine whole genome sequencing (WGS). Identification and capsular type will be derived from WGS but will be reported in the same format as previously. Identification and capsular typing will still be carried out using phenotypic methods in a small number of cases when required.

The Unit liaise closely with the AMRHAI in studies of antibiotic resistant pneumococci.

Other information:
There are currently at least 92 distinct pneumococcal capsular polysaccharide serotypes defined by the Danish classification scheme (SSI Diagnostica). Some of the 92+ serogroups/serotypes may be divided into specific serotypes or subtypes that is; types carrying the same number, but different letters, for example 6A, 6B, 9A, 9L, 9V.

Subtyping is undertaken on all isolates from normally sterile sites, in particular for any episode of systemic infection associated with possible vaccine failure.

The laboratory together with the Immunisation and Countermeasures Division, 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 4 September 2004. Typing of isolates to assist in the management of clusters of pneumococcal disease, including non-sterile site isolates can be undertaken. Such requests should be made via the local Health Protection Team or contact RVPBRU.

We do not offer a reference service for typing of *Streptococcus pneumoniae* strains from non-invasive infections. Unless part of an investigation as described above, non-invasive isolates of *S. pneumoniae* (that is; isolates from *eye swabs, sputum*, will not normally be tested) or can be tested as a charged test.

Where capsular typing of non-invasive pneumococci may be helpful in the investigation of instances of suspected cross-infection in hospitals, other residential institutions and day care centres (or similar) for children please contact the Health Protection Team and discuss with Senior RVPBRU staff prior to sending isolates.

If seeking MICs from non-invasive pneumococcal isolates please refer directly to AMRHAI.

We do NOT carry out tests for *S. pneumoniae* antibodies. *S. pneumoniae* serology is performed by PHE Vaccine Evaluation Unit, Manchester Medical Microbiology Partnership, Clinical Sciences Building 2, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL Please contact Professor Ray Borrow (0161 276 6793).

Identification and toxigenicity testing of *Corynebacterium diphtheriae* and other potentially toxigenic corynebacteria

Identification/confirmation and toxigenicity testing of potentially toxigenic Corynebacteria (*C. ulcerans* and *C. pseudotuberculosis*) is performed initially by real-time PCR (qPCR) on the submitted isolate. Isolates which are qPCR positive for the toxin gene (*tox*) will also be tested by the Elek test for toxin expression. Although *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* toxin gene PCR positive results will be confirmed by the Elek test, a toxin gene PCR positive result should be acted upon without waiting for the Elek result. A toxin gene PCR result of 'not detected' (that is, tox PCR negative) is final and no further toxigenicity testing will be reported on these isolates.

Infections with 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 contact with farm animals and/or companion animals and/or raw milk.

UK microbiological laboratories are encouraged to submit all clinical isolates of *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* to RVPBRU for toxigenicity testing and surveillance purposes. The Unit is a designated WHO Collaborating Centre for diphtheria.

Urgent isolates received by midday will usually be processed on the same day (Monday to Saturday). 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.

Notify RVPBRU (telephone 0208 327 7887) before sending an isolate for toxigenicity testing within working hours on a weekday. Outside these hours, please notify the Colindale duty doctor on 0208 200 4400. If possible, always use the RVPBRU Request Form (R3) and always ensure full contact telephone numbers are provided on the form.

Contact the PHE Immunisation and Countermeasures Colindale or duty doctor out-of-hours if considering the use of diphtheria antitoxin (0208 200 4400). They will advise on details of current stock and dosing as suppliers change and dosing is product specific.

Details in immunoglobulin handbook:
https://www.gov.uk/government/publications/immunoglobulin-when-to-use

Information on immunisation against infectious diseases can be found in the Green Book: https://www.gov.uk/government/collections/immunisation-against-infectiousdisease-the-green-book and advice is available from the PHE Immunisation Lead (Immunisation.Lead@phe.gov.uk)

Please refer to: Public health control and management of diphtheria (in England and Wales) 2015 Guidelines – Diphtheria Guidelines Working Group for further details https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/416108/D iphtheria_Guidelines_Final.pdf

Haemophilus influenzae

The laboratory requests submission of ALL *H. influenzae* isolates from invasive disease (that is; blood, CSF and other normally sterile sites) for confirmation of their identification and capsular serotyping.

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

The laboratory requests submission of ALL *H. 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 for detecting Hib vaccine failures in children. This surveillance is being conducted in collaboration with the Immunisation and Countermeasures Division, NIS Colindale. Requests for antimicrobial sensitivity testing can be included with submissions.

The laboratory is happy to discuss and advise upon clinical or epidemiological problems.

Other information:
Identification of *H. influenzae* is based upon X and V factor requirement and a species-specific PCR directed at the *ompP2* gene. Serotyping is performed using a combination of slide agglutination using antisera and PCR-based typing. There are 6 capsular serotypes of *H. influenzae* (a-f) based on the capsular polysaccharide of the organisms. Before the introduction of a conjugate vaccine against serotype b (Hib), this serotype caused the majority of serious human infections in the UK. However, other capsular serotypes, notably types e and f, and non-capsulated strains can also cause serious infections

The Unit will refer requests for antimicrobial susceptibility testing to AMRHAI.

VPBS does not offer a routine service for typing or susceptibility testing of *H. influenzae* strains from non-invasive infections. Non-invasive isolates of *H. influenzae* (that is; isolates from eye swabs, sputum) 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. Requests for antimicrobial susceptibility testing of non-invasive isolates should be made to AMRHAI directly.

We also do NOT carry out tests for Hib antibodies. Hib serology is performed by Professor Ray Borrow, PHE Vaccine Evaluation Unit, Manchester Medical Microbiology Partnership, Clinical Sciences Building 2, Manchester Royal Infirmary, Oxford Road, Manchester, M139WL. Please contact Professor Ray Borrow (0161 276 6793).

Identification of Haemophilus species (excluding *Haemophilus ducreyi*)

RVPBRU will confirm the identity of strains of other *Haemophilus* species isolated from cases of invasive disease. For isolates not confirmed as *Haemophilus* spp. a preliminary report will be issued and the isolate forwarded to AMRHAI (BIDS) for full identification, who will issue a report in due course.

Diphtheria immunity/vaccination studies

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 6 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 IU/mL

Tests are batched every 3 weeks, unless a sample is deemed to be urgent. Please supply details of vaccination history (if known) with all requests plus relevant clinical details.

Tetanus immunity

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 supporting a clinical diagnosis of tetanus. Absence of detectable antibody or levels below or close to the minimum protective level lends support to the clinical diagnosis while higher levels do not.

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

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

According to demand tests are normally batched every 3 weeks. If a sample is deemed to be urgent, please contact RVPBRU before sending to discuss.

List of contacts

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)			
Prof. Neil Woodford	Unit head	neil.woodford@phe.gov.uk	020 8327 6511
Susceptibility testing, resistance mechanism, interpreting antibiogram, commercial opportunities			
Dr Katie Hopkins	Section head	katie.hopkins@phe.gov.uk	020 8327 7061
Infection prevention and control, site visits			
Mr Peter Hoffman	Section head	peter.hoffman@phe.gov.uk	020 8327 7274
Staphylococci ID and typing, PVL / other toxins			
Dr Bruno Pichon	Section head	bruno.pichon@phe.gov.uk	020 8327 7227
Opportunistic pathogens typing			
Dr Jane Turton	Section head	jane.turton@phe.gov.uk	020 8327 7224
Bacterial identification, culture negative clinical specimens (16S rDNA sequencing)			
Dr Julie Logan	Section head	julie.logan@phe.gov.uk	020 8327 6059
Antibiotic resistance in sexually transmitted bacteria			
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Gastrointestinal Bacteria Reference Unit (GBRU)			
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