UK Health Security Agency

UK Health Security Agency
Public Health Microbiology Division

Bacteriology Reference Department
User Manual

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Foreword

The UK Health Security Agency (UKHSA), Bacteriology Reference Department (BRD) is a national and international reference department for a wide range of bacterial infections. BRD has 3 designated World Health Organization Collaborating Centre (WHO CC), WHO Collaborating Centre for Reference and Research on Antimicrobial Resistance and Healthcare Associated Infections, WHO Collaborating Centre for Haemophilus influenzae and Streptococcus pneumoniae and WHO Collaborating Centre for Diphtheria and Streptococcal Infections. BRD is made up of following 4 units.

The Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) reference unit

This is the national reference laboratory for investigation of antibiotic resistance in, and characterisation of, healthcare associated pathogens. The unit 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 and determine susceptibility for most species of bacteria, with the notable exception of category 3 organisms, detect new and emerging resistances by interpretive analysis of minimum inhibitory concentration (MIC) profiles and molecular investigation, and provide therapeutic guidance. The unit also undertakes laboratory-based surveillance, advises on outbreak investigations and investigation of unusual antibiotic resistance, and on any public health risk and provides an identification service for difficult-to-identify bacteria and from culture-negative clinical samples. It gives information and advice on infection control issues, the investigation of healthcare- and community-associated infections, and aspects of laboratory safety and other related matters.

The Gastrointestinal Bacteria Reference Unit (GBRU)

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)

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 and outbreak investigation.

The Sexually Transmitted Infections Reference Laboratory (STIRL)

The Sexually Transmitted Infections Reference Laboratory (STIRL) provides specialist and reference services to detect, investigate and characterise clinically important STI pathogens. The laboratory uses established and developmental phenotypic and genotypic assays to define antimicrobial susceptibility of bacterial STIs to relevant antimicrobials for individual case management, as well as for surveillance purposes. In addition, we seek to define and investigate outbreaks and diagnostic escape mutants, as well as offering diagnostic, scientific and clinical advice on STI infections.

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Amendment history

Version number	Date	Sections affected	Pages affected
12	July 2020	Update service table and appendixes. Remove *E. Coli* serodiagnostic, update Staphylococcus spa with WGS.	13
13	September 2020	Update Key personnel and contact details	8, 76
14	November 2021	Updates to all sections following transition from PHE to UKHSA.	All
15	September 2022	Remove Lepto MLST, PFGE, update BRD phone no., email addresses to ukhsa, clinical staff, Antimicrobial resistance and mechanism service	All
16	August 2023	Add The Sexually Transmitted Infections Reference Lab (STIRL), update A-Z listing and staff list, remove *C.tetani* in serum, Resp Chlamydia UKAS accredited.	All

Key personnel and contact details

Name	Designation	Email	Telephone
Neil Woodford	Deputy Director, Public Health Microbiology Division	neil.woodford@ukhsa.gov.uk	020 8327 6511
Hemanti Patel	Head of Laboratory Scientific Services	hemanti.patel@ukhsa.gov.uk	020 8327 7705
Sunita Gurung	Head of Business and Commercial Services (interim)	sunita.gurung@ukhsa.gov.uk	020 8327 6758

Scientific staff are available for the respective BRD Units below for advice in the first instance on any scientific queries and if required arrangements will be made for a Clinical Medical Microbiologist to assist on any medical advice. Full contact details for BRD staff members are found at the end of this manual on page 64.

BRD Units	Email
Antimicrobial Resistance and Healthcare Associated Infections	amrhai@ukhsa.gov.uk
Gastrointestinal Bacterial Infections	gbru@ukhsa.gov.uk
Respiratory and Vaccine Preventable Bacterial Infections	rvpbruqueries@ukhsa.gov.uk
Sexually Transmitted Infections	stilab@ukhsa.gov.uk

UKHSA Public Health Microbiology switchboard: 020 8200 4400

BRD General Office: 020 83277887 staffed 9am to 5pm Monday to Friday
email: vrdqueries@ukhsa.gov.uk

DX address
UKHSA Colindale
Bacteriology
DX 6530002

Postal address
UK Health Security Agency
Bacteriology Reference Department
61 Colindale Avenue
London NW9 5EQ

How to obtain services

Hours of service

The Department is open from 9am to 5pm, Monday to Friday. Telephone enquiries via the BRD general office are available from 9am to 5pm, 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 UKHSA 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 UKHSA terms and conditions of business. Specific requests for service level agreements or contracts should be made to business@ukhsa.gov.uk.

Specimen submission guidelines

Specimens

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

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

All clinical and environmental specimens submitted from the same patient or source must be labelled with a unique specimen identifier or sender's sample number.

Request forms

Please use the current versions of request forms where possible and complete all relevant sections. BRD specific request forms are available on the UKHSA website.

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

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.

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.

Services available

The department undertakes tests as listed on the following pages. The main factors affecting individual tests are noted against the relevant test, including minimum sample volumes where relevant. Further information is available from:

- Services: detailed information
- Infectious diseases: detailed information

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.

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.

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.

Requests for additional tests

If additional laboratory testing is required on a sample previously submitted to BRD, please contact the relevant unit by telephone in the first instance. Original specimens are normally retained for at least one month 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. The turnaround time in this instance will vary.

Requests for sharing data or isolates

Commonly requested strains are submitted to the National Collection of Type Cultures (NCTC) by UKHSA. BRD requires a material transfer agreement for shared data or strains. A charge will be levied unless prior collaborative UKHSA project is agreed and in place. Please contact the 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 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 paperwork (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

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. Contact the departmental safety manager (Marlette Vigille on 020 8327 6447) or the Specimen Reception manager (Fiona Clode on 020 8327 7129 or 020 8327 6063) for further information. Any organisation sending out cultures or diagnostic specimens has a legal duty to ensure that such items are sent in a safe manner.

UKHSA follows the Guidance on regulations for the transport of infectious substances 2021 to 2022, published by WHO. Specimens sent to BRD laboratories must meet the criteria in these guidelines. Samples which are not packaged appropriately may not be processed.

Arrangements must be made by referring laboratories to ensure that time and temperature requirements (detailed under ‘Main 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.

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.

A to Z listing of services available

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
Abiotrophia spp.	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Alloiococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Antibiotic testing (susceptibility service)	Interpretive analysis of MIC profiles and therapeutic guidance. The main examples are in this 'Services' table but most species other than *Mycobacterium* spp. and anaerobes are covered. MIC determination not undertaken on rectal or 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 or agents for which there are no EUCAST clinical breakpoints. We use standard antibiotic panels based on EUCAST and CLSI recommendations.	Pure culture, Agar slope	15 days non-fastidious spp.)	H1, H2	AMRHAI
Bacillus (other than *B. anthracis)*	Identification of *Bacillus* spp. by MALDI-TOF	Pure culture on agar slopes	10 days	L4	GBRU
	Molecular typing of *B. cereus* for outbreak investigations	Pure culture on agar slopes	15 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,	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 turnaround times (TATs)	Request form BRD specific request forms	Contact unit
emerging bacteria)					
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 to 500µl), Tissue (1cm^3), FFPE (up to 3 sections, each thickness of up to 10µm and a surface area of up to 250mm^2)	7 days	M1	AMRHAI
Bartonella spp.	16S rRNA gene PCR and sequencing	Normally sterile site clinical sample **except blood or 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 to >17 years. Contact HPT for kit (≥2week history of cough)		Form distributed with kit	
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 to 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)	*C. pneumoniae/ C. psittaci/C. abortus* - PCR assay	Minimum 200 µL of respiratory sample (sputum, bronchoalveolar lavage, lung biopsy or throat swab). Other sample type contact lab (see page 52).	5 days	R1	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) or in sterile container.	Clinical specimens: 9 days Food: Varies – see page 41)	Contact Lab before sending specimens. L4 (culture)	GBRU

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
		Wound, pus, debrided tissue, preferably inoculated in anaerobic broth (universal) – NO SWABS. Food or drink samples (10g or 10 ml). Pure Culture suspected to be of *C. botulinum* into anaerobic broth (universal) - NO SWABS.		L5 (specimen) L7 (Food/Env)	
	Detection of botulinum neurotoxins in clinical specimens or food associated with suspected cases.	Faeces (10g) or rectal washout in sterile container. Serum (≥ 5ml;) collected close to the onset of symptoms (preferably < 3 days) and before antitoxin is given (lysed or EDTA treated blood specimens are not suitable). Food or drink samples (10g or 10 ml).	Varies – see page 41)	Contact Lab before sending specimens. L5 (specimen) L7 (Food/Env)	GBRU
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 less than 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	9 days	L5 (specimen) L4 (culture)	GBRU
	Tetanus immunity: serum antibodies	Not less than 200 µL serum in a sterile container	21 days unless urgent	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 turnaround times (TATs)	Request form BRD specific request forms	Contact unit
Corynebacterium diphtheriae/ulcerans/ pseudotuberculosis	*C. diphtheriae/ ulcerans/ pseudotuberculosis* (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) 6-day 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 a*ntimicrobial 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	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Dolosigranulum spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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
Enterococcus spp.	Species identification and antimicrobial susceptibility (molecular typing for clinical clusters only)	Pure culture, Agar slope	15 days	H1, H2	AMRHAI
Escherichia spp.	*E. coli* (ACDP HG 2 only): Whole genome sequencing (WGS) 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	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Gemella spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
	Antimicrobial susceptibility	Pure culture, Agar slope			
Globicatella spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Helicobacter spp.	*H. pylori* isolation, identification and antibiotic susceptibility testing by Etest. PCR detection (from biopsy specimens only) when a pure culture cannot be isolated.	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
Ignavigranum spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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	15 days	H3, H4	AMRHAI

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
		agar slope			
	Antimicrobial susceptibility	Pure culture, Agar slope			
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 or 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 - >2mL (sputa, BAL, tracheal aspirate) and other clinical samples in a sterile container	Urgent samples - notify by phone	R1	RVPBRU
	Legionella spp. qPCR molecular detection and culture: from urine antigen negative patients admitted with pneumonia (including *L. longbeachae)* and pneumococci urine or routine respiratory pathogen screen negative patients only. Charged for service (free of charge for registered legionella COVID-19 pneumonia and ECMO surveillance sites)	Lower respiratory tract samples - >2mL (sputa, BAL, tracheal aspirate) and other clinical samples in a sterile container	Urgent samples - notify by phone	R1 clearly marked 'Legionella species'	RVPBRU
	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
Leuconostoc spp.	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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
Mycobacterium abscessus complex	Molecular typing	Pure culture, Agar slope/Liquid culture	15 days	H1, H2	AMRHAI
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 or 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	Minimum volume 200 µL of	5 days	R1	RVPBRU

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
	macrolide resistance by PCR and sequencing.	respiratory sample (LRT or throat swab) in a sterile container. CSF with paired respiratory samples or DNA extract of positive specimen			
	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 available for patients who have failed quinolone and macrolide treatment, and where clinically indicated, i.e, pregnancy.	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). Samples must be received within 2 weeks of collection to maintain clinical relevance. *N.B*. as per current guidance, specimens will be inactivated prior to DNA extraction in case of mpox infection.	8 days	B2	STIRL
	Other species: Culture, PCR and sequencing when relevant.		PCR: 5 days Culture: up to 42 days	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 where 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	STIRL
	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	STIRL
	Programme: Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP)	Isolates submission is based on a pre-agreement between the laboratory and STIRL	GRASP Annual Report	Contact Lab before sending isolates	STIRL
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	15 days	H3, H4	AMRHAI

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
		agar slope			
	Antimicrobial susceptibility.	Pure culture, Agar slope			
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
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 sp.	*S. aureus* (multiple isolates from suspected clusters): molecular typing derived from whole genome sequence data.	Pure culture, Agar slope	14 days	H3, H4	AMRHAI
	S. aureus (single isolates): molecular typing derived from whole genome sequence data.	Pure culture, Agar slope	14 days	H3, H4	AMRHAI
	Staphylococcus coagulase negative: species identification and molecular typing.	Pure culture, Agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility.	Pure culture, Agar slope	15 days	H3, H4	AMRHAI
	Resistance gene detection derived from whole genome sequence data.	Pure culture, Agar slope	14 days	H3, H4	AMRHAI
	S. aureus: PVL testing only.	Pure culture, Agar slope	6 days	H3, H4	AMRHAI
	Virulence gene detection (14 genes, incl. PVL) derived from whole genome sequence data.	Pure culture, Agar slope	14 days	H3, H4	AMRHAI
	Enterotoxin gene detection (suspected staphylococcal food poisoning) derived from whole genome sequence data.	Pure culture, Agar slope	14 days	H3, H4	AMRHAI
Stenotrophomonas	*S. maltophilia*: molecular typing and antimicrobial susceptibility.	Pure culture, Agar slope	15 days	H3, H4	AMRHAI
Streptococcus spp. and related genera *Streptococcus* spp. and	*S. pyogenes* (Lancefield Group A): Typing of invasive and outbreak/cluster associated non-invasive isolates.	Pure culture on blood or chocolate agar slope (charcoal swabs not suitable).	8 days	H3, H4	AMRHAI

Services	Test type	Sample required	Target turnaround times (TATs)	Request form BRD specific request forms	Contact unit
related genera	*S. agalactiae* (Lancefield Group B: Typing of invasive and outbreak/cluster associated non-invasive isolates.	Pure culture on blood or chocolate agar slope	8 days	H3, H4	AMRHAI
	Streptococci (Lancefield Group C and G): Typing of invasive and outbreak/cluster associated non-invasive isolates.	Pure culture on blood or chocolate agar slope	8 days	H3, H4	AMRHAI
	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
	Streptococcus spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Tetragenococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
Ureaplasma	Refer to *Mycoplasma*			R1	RVPBRU
Vagococcus spp.	*Streptococcus* spp. and related genera identification	Pure culture on blood or chocolate agar slope	15 days	H3, H4	AMRHAI
	Antimicrobial susceptibility	Pure culture, Agar slope			
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 to genus and species level	Pure culture on Dorset's egg or nutrient agar slope	14 days	L4	GBRU

Reports

Reports will be delivered electronically via E-lab or will be printed and delivered by post if the referring laboratory is not registered to E-lab. For details on how to register for E-lab and further information, please email LimsHelpdesk@ukhsa.gov.uk.

Please note reports will only be sent to the requestor named on the request form.

Policy on emailing reports

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

1. It is Reference Laboratories Colindale policy that reports containing patients' data should **not** be sent by email.

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

3. Reports cannot be sent by fax: there are no fax facilities at UKHSA Colindale.

Quality assurance in BRD

Referral site accreditation information

We receive many requests regarding the accreditation status of BRD. The department is accredited to ISO 15189:2012 and 17025:2017.

BRD is a UK Accreditation Service (UKAS) accredited medical laboratory No. 8197.

General information about our accreditation (including copies of certificates) and ISO 17025:2017. See Quality at the laboratories of the UK Health Security Agency, Colindale.

List of accredited services: see the schedule of accreditation on the United Kingdom Accreditation Service (UKAS) website (lab reference 8197).

External Quality Assurance and Proficiency Testing: 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.

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.

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.

Key contact for general quality-related enquiries:
Quality Assurance Manager: Nazim Chowdhury, Tel: 020 8327 6642,
nazim.chowdhury@ukhsa.gov.uk

Complaints

If there is a problem, or you are not satisfied with the service you have received, in the first instance contact the appropriate unitor section head. Contact details at the end of the user manual. Otherwise contact:

Quality Implementation and Compliance Manager:
Ifeoma Ekwueme (20 8327 7552)
ifeoma.ekwueme@ukhsa.gov.uk
or

Deputy Director, Public Health Microbiology Division:
Neil Woodford (020 8327 6511)
neil.woodford@ukhsa.gov.uk

Complaints will be responded to within 5 working days of receipt and if resolution cannot be achieved within 20 days, the complainant will be notified.

Our endeavour is 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.

UKHSA Colindale: recognition of Caldicott recommendations

The recommendations of the Caldicott Report (1997) and the subsequent Information Governance Review (2013) have been adopted by UKHSA and by the National Health Service as a whole. These recommendations relate to the security of patient identifying data (PID) and the uses to which they are put. UKHSA observes Caldicott guidance in handling PID and has appointed its own Caldicott Guardian.

All enquiries about the security and use of PID at Public Health Microbiology Colindale should be addressed to the Caldicott Guardian at caldicott@ukhsa.gov.uk.

Compliance with the Human Tissue Act

UKHSA 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.

Please note that consent is mandatory for all scheduled purposes. Samples taken from deceased persons that are sent to UKHSA Colindale for testing, where such testing is not related to determining the cause of death as directed by the coroner, will require appropriate consent from the deceased person or their relatives. For example, testing of post mortem material for infectious agents following a needlestick injury sustained during the post mortem will require consent. It is the obligation of the requesting clinician or pathologist to ensure that appropriate consent has been obtained.

Obtaining consent to remove, store and use human tissues for a scheduled purpose is one of the underlying principles of the Human Tissue Act. Public Health Microbiology Laboratories Colindale receives post-mortem samples from coroners' post-mortems or from NHS establishments across the UK and therefore we are performing the examination under the authority of the coroner. Unless consent has been obtained or the coroner has requested that samples are retained for further testing, samples are disposed of within 3 months of the initial test being performed.

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

The Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) reference unit

The staff contact list is in the final section of this document.

To ensure a pure strain is tested please only send a slope of a single colony pick. Should a second strain or colony variant be identified, this could lead to an extra charge being incurred or the sample being rejected. Please contact lab for prices. 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 or 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’ and so on, 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 UKHSA 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 or 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 200µl or more for liquid material (no more than 1ml to be supplied) and a ‘fingernail’-sized piece of tissue for solid samples appromiately 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 10µm. Up to 3 sections, each with a thickness of up to 10µm and a surface area of up to 250 mm^2, can be combined in one 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
- low growing fastidious organisms
- mixed cultures submitted
- insufficient clinical sample submitted
- non-sterile site clinical samples submitted

Opportunistic pathogens section

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. *Burkholderia pseudomallei* is identified by species specific PCR.

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

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 whole genome sequencing (WGS) (for *Escherichia coli*) or variable number tandem repeat (VNTR) analysis (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Mycobacterium abscessus* complex).

Previously, other bacterial opportunistic pathogens (for example, *Acinetobacter spp.*, *Enterobacter cloacae* complex*, Enterococcus spp*., *Burkholderia spp*., *Serratia spp*., *Stenotrophomonas maltophilia*) were typed by pulsed-field gel electrophoresis. This service is no longer supported, although we may still use it on occasion to compare some isolates. We are developing WGS protocols for these organisms; please contact jane.turton@ukhsa.gov.uk if you require comparison of any of these for cross-infection and outbreak investigation purposes. Please note that we no longer provide typing of screening isolates of *Enterococcus spp*.

In addition, the following are offered:

- 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*)

Requests for typing must include.

1. The reason for the request – 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).
2. Please include an accurate identification; wrong identifications can result in organisms going through inappropriate typing methods and irrelevant comparisons.
3. Please do not send single isolates for typing with no indication of any relevant comparison. Any relevant details (for example, ward information) that you can give that link potentially related organisms are much appreciated.
4. Please use the H1 multiple isolates form (available at Healthcare pathogens request form: multiple isolates) for typing investigations, which should detail the comparison required.

Staphylococcus and Streptococcus Reference Section (SSRS)

Staphylococcus species

The ‘spa typing’ service is no longer available, and it is now replaced with 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:

- surveillance - for example, MRSA or MSSA from cases of bacteraemia
- typing, antimicrobial resistance gene and/or toxin gene profiling
- such as the identification of suspected hospital versus community versus 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:
    - *mec*A and its homologue *mec*C, which confer resistance to oxacillin (charged)
    - *mup*A and *mup*B 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)

Main 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 or 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 to E and G to J). Where detection of staphylococcal enterotoxins in samples of food or beverages is required, please contact the London FW&E (FWEM@ukhsa.gov.uk).

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.

Main factors affecting the performance of the test include:

- poor growers
- isolates where DNA degrades
- autolytic enzymes

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 characterisation 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

SSRS 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) referred without a notification that is linked to cluster investigation will be charged.

Please indicate details on the referral form regarding cases under investigation for cross-infection or cluster investigation.

If seeking MICs only from non-invasive *S. pyogenes* isolates, please refer directly to the Antimicrobial Resistance and Mechanisms Service (ARMS).

Lancefield Group B Streptococci (GBS), Streptococcus agalactiae

Serological classification and epidemiological typing of Lancefield group B streptococci.
The serological classification of GBS is based upon the identification of polysaccharide and protein antigens. There are currently 10 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 or rectal swabs).

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

If seeking MICs only from non-invasive *S. agalactiae* isolates, please refer directly to Antimicrobial Resistance and Mechanisms Service (ARMS).

Lancefield group C and group G streptococci

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

Group C and G streptococci may cause both nosocomial (for example, burns unit cross-infection episodes) or institutional outbreaks.

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

Group C streptococci of animal origin, 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 typing is based on sequence analysis of the hypervariable portion of the *emm* gene that dictates the M serotype. Further information available on the Centers for Disease Control and Prevention: Streptococcus Laboratory webpage.

The laboratory requests submission of **ALL** group C and G streptococci isolated from blood culture or other normally sterile sites as part of the national surveillance of invasive disease due to group C and G streptococci.

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 or rectal swabs) without notification that is part of cluster investigation will be charged.

If seeking MICs only from non-invasive group C and G streptococcus isolates, please refer directly to Antimicrobial Resistance and Mechanisms Service (ARMS).

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 and that are not streptococci and may be an enterococcus or a Gram-positive rod will be referred to the ARMS below. The turnaround time in this instance will vary.

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 will result in rejection of your isolate without testing.

Diagnostic laboratories should familiarise themselves with EUCAST guidance for susceptibility testing of organisms or agents for which there are no EUCAST breakpoints. Lack of EUCAST clinical breakpoints is **not** sufficient justification alone for referral to AMRHAI. This includes genera such as *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 (for example 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 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 expert rules and expected phenotypes. Table 1 and Table 2 identify combinations of organism or 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 (for example, 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 (Table 3 lists bacterial genera and antibiotics for which MICs are determined by broth microdiluton), gradient strip test (used to determine MICs for all other bacteria and/or antibiotics not listed in Table 3), agar dilution (fosfomycin only) and disc diffusion (cefiderocol only). 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 and a further charge per additional antibiotic is 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 (for example, 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’ or ‘Gram-negative rod’ isolates are submitted, unless also formally sent for reference identification.

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 isolates from rectal or faecal screens (that is isolates representing patientcolonisation), 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 carbapenemase-producing Enterobacterales from other sample types (if required).

Main factors affecting the performance of the test include:

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

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.

Table 1. Exceptional resistance phenotypes in Gram-negative bacteria

Organism	Antimicrobial resistance phenotype confirmed by diagnostic laboratory	Should this isolate be sent to AMRHAI [note 1]	Test(s) to be provided by AMRHAI [notes 2, 3]	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 to 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 [note 2]. MICs on request for isolates from clinical sites only.	UK SMI B 60: detection of bacteria with carbapenem hydrolysing β lactamases (carbapenemases). EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. 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 mg/L; zone diameter <17 mm). These isolates should be screened for OXA-48-like carbapenemases by PCR or immunochromatographic assay.	Yes: isolates positive for an OXA-48-like carbapenemase and from invasive infections only should be referred for inclusion in the national strain archive.	MICs on request for isolates from clinical sites only.	UK SMI B 60: detection of bacteria with carbapenem hydrolysing β lactamases (carbapenemases). EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance.
	Ceftazidime/avibactam, meropenem/vaborbactam or imipenem/relebactam 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 (that is, resistance to all of amikacin, gentamicin and tobramycin).	No: confirmation by diagnostic laboratory only.	N/A	
	High-level tigecycline resistance (MIC >4mg/L) (except *Proteus* spp., *Providencia* spp. and *Morganella* spp.)	No: may be reviewed in the future.	N/A	
Any Enterobacterales (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 if 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.	Antimicrobial susceptibility testing of colistin - problems detected with several commercially available products

Organism	Antimicrobial resistance phenotype confirmed by diagnostic laboratory	Should this isolate be sent to AMRHAI [note 1]	Test(s) to be provided by AMRHAI [notes 2, 3]	Further information
Acinetobacter spp.	Isolates suspected to produce a metallo-carbapenemase: meropenem or imipenem resistance **and** exhibit strong imipenem/EDTA synergy.	Yes	Real-time PCR to screen for an extended panel of carbapenemase gene [note 2]. MICs on request for isolates from clinical sites only.	UK SMI B 60: detection of bacteria with carbapenem hydrolysing β lactamases (carbapenemases) EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance 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 or 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 or 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[c]	UK SMI B 60: detection of bacteria with carbapenem hydrolysing β lactamases (carbapenemases) EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance 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 [note 2].	N/A

Organism	Antimicrobial resistance phenotype confirmed by diagnostic laboratory	Should this isolate be sent to AMRHAI [note 1]	Test(s) to be provided by AMRHAI [notes 2, 3]	Further information
Other non- fermenters	Co-trimoxazole resistance	Yes	Confirmation of resistance; testing of alternative agents if requested.	Stenotrophomonas maltophilia
	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 cephalosporin or fluoroquinolone.	Yes	Confirmation of resistance; testing of alternative agents if requested.	N/A
Organisms or antibiotics 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 or agents for which there are no EUCAST breakpoints.		Please contact AMRHAI if you require assistance with interpreting your MIC values

Note 1: Confirmation of resistance will be performed on isolates from clinical sites only.
Note 2: 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 [note 1]	Should this isolate be sent to AMRHAI?	Test(s) to be provided by AMRHAI [note 2]	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 or 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 to 8 mg/L, cefoxitin MIC >4 mg/L or a discrepancy between oxacillin and cefoxitin.	Yes	Confirmation of cefoxitin resistance. Molecular screening for *mecA*/*mecC* where appropriate.	EUCAST recommends the use of cefoxitin rather than oxacillin for MRSA screening as the specificity of oxacillin is lower than that of cefoxitin.
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 or 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 or 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.
	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 or 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 or agents for which there are no EUCAST breakpoints.		Please contact AMRHAI if you require assistance with interpreting your MIC values.

Note 1: Not all agents may currently be routinely tested by diagnostic laboratories.
Note 2: Confirmation of resistance will be performed on isolates from clinical sites only.

Table 3. Antibiotics tested by broth microdilution

Bacterial genus	Antibiotics tested by broth microdilution
Enterobacterales	ampicillin amoxicillin/clavulanate temocillin aztreonam cefotaxime ceftazidime cefepime piperacillin/tazobactam ceftazidime/avibactam ceftolozane/tazobactam ertapenem meropenem imipenem amikacin gentamicin ciprofloxacin tigecycline colistin
Acinetobacter spp.	amikacin gentamicin imipenem meropenem colistin ciprofloxacin tigecycline
Pseudomonas spp.	amikacin gentamicin aztreonam ceftazidime cefepime imipenem meropenem piperacillin/tazobactam ceftolozane/tazobactam ceftazidime/avibactam colistin ciprofloxacin
staphylococci	gentamicin cefoxitin teicoplanin vancomycin

Bacterial genus	Antibiotics tested by broth microdilution
	clindamycin erythromycin linezolid levofloxacin daptomycin fusidic acid rifampicin
enterococci	gentamicin ampicillin teicoplanin vancomycin linezolid daptomycin

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 breakpoints).

Where multi-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. 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. UKHSA publishes a guidance to provide the evidence base to support diagnostic laboratories in making an informed choice on the implementation of commercially-available carbapenemase detection assays when considering local business needs.

Services currently offered include the detection of:

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

Table 4 summarises carbapenemase gene families that are targeted using our reference service PCR. Where an 'exceptional' carbapenemase and species combination result (cells without a ¥ symbol in Table 4) has been identified, or where an unusual organism has been identified with an acquired carbapenemase (that is, any bacterial genera other than a member of the Enterobacterales, *Pseudomonas* spp. or *Acinetobacter* spp.), isolates should be sent to the AMRHAI Reference Unit for confirmation.

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.

Table 4: Distribution of carbapenemase genes covered by AMRHAI Reference Unit PCR (based on AMRHAI data) [note 1].

Carbapenemase gene family	Associated with common 'host' organism		
	Enterobacterales	*Pseudomonas* spp.	*Acinetobacter* spp.
KPC	¥	<10[D]	<10[D]
OXA-48-like	¥	<10[D]	0
NDM	¥	¥	¥
VIM	¥	¥	<10[D]
IMP	¥	¥	¥
IMI/NMC-A	¥[B]	0	0
GES	¥	¥	<10[D]
FRI	<10	0	0
SME	<10[C,D]¥	0	0
DIM	0	<10[D]	0
GIM	<10[D]	<10[D]	0
SIM	0	<10[D]	0
SPM	0	<10[D]	0
OXA-23-like	<10[D]	0	¥
OXA-40-like	0	0	¥
OXA-51-like[A]	0	0	¥
OXA-58-like	0	0	¥

Note 1: Table 4 uses the following symbols:
¥ = combinations of mechanism and species would not be considered as exceptional.
A = intrinsic to *A. baumannii* and only expressed when associated with an insertion element.
B = almost exclusively reported in *Enterobacter* spp. with less than a handful of reports in other genera.
C = reported only in *Serratia marcescens*.
D = fewer than 10 in total ever identified by the AMRHAI Reference Unit.

Gastrointestinal Bacteria Reference Unit (GBRU)

The staff contact list is in the final section of this document.

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. 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 by MALDI-TOF, 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
- food environment sources

- 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 in full including:

- your address
- your telephone number
- your specimen or sample reference number
- specimen or 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.

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 UKHSA 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.

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 5mL to be collected as close to the onset of symptoms as possible (within 2 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, 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 time

The turnaround times for the detection and identification of *C. botulinum* are shown in calendar days and reflect the proportion of tests requiring prolonged observation in order to establish a negative result.

The variable turnaround time for food testing reflects, prior to testing, the microbiological confirmation of botulism in patient(s) associated with the food and the emergency of the situation. The variability in the turnaround time also reflects the lengthier procedure for food associated with confirmed cases of infant botulism.

The turnaround time for the detection of neurotoxin will vary depending on decision for testing: the use of the bioassay is restricted by the Home Office and depends on the level of clinical suspicion of botulism in the patient, the adequacy of the specimens and the availability, prior testing of other specimens by culture and PCR. Therefore, it is recommended to contact GBRU with a complete clinical history before submitting samples to make sure that the most appropriate samples are sent in order to maximise the likelihood of detecting the toxin if present. When performed, the bioassay test takes 5 days to complete. If neurotoxin is detected, more than 5 days is required to confirm and establish the toxin type.
Depending on the emergency of the situation, intermediate and final results are communicated to the clinician prior release of the final reports.

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

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

Please complete the correct request form including your address, telephone number, patient (specimen) details including your reference number, ILOG or HPZ 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 is by the detection of neurotoxigenic C. tetani by cultures and PCR from pure culture or from debrided tissues or 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

Please complete the correct request form including your address, telephone number, patient (specimen) details and your specimen 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 to send are pure cultures on agar slope isolated from:

- all cases of human listeriosis should be sent for typing, with all reports incorporated into a database for national surveillance of listeriosis
- from foods and the environment. These cultures 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, telephone number, patient (specimen) details and your specimen 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.

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 40°C

Please complete the correct request form including your address, telephone number, patient (specimen) details and your specimen 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:

1. ***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 or sterile saline and should be sent to our laboratory for testing as soon as possible after harvesting.
2. **Gastric biopsy specimens** for isolation of *H. pylori*: should be sent to our laboratory for testing without delay, preferably within 24 hours. Ideally, biopsies should be sent in a suitable *H. pylori* transport medium or alternatively, in sterile saline. If a biopsy is not posted or couriered on the day of receipt in your laboratory, then please store at 4°C. Drying and exposure to air or oxygen easily kills *Helicobacter* spp.

Note: Do not put biopsies into a histological casette as they cannot be safely opened in our laboratory. Any biopsies that are received in a histological cassette will be reported as 'not examined'.

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

1. Perform multiple gastric biopsies (5 to 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. 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.
6. 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, telephone number, patient (specimen) details and your specimen 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 – this enterovirulent *E. coli* includes 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 an appropriate standard container with an inner seal, and should be more than 1 gram but not overfilled

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 and your specimen 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 (excluding *Yersinia pestis*) as Porton Down is the reference lab for *Yersinia pestis,* not Colindale
- 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, telephone number, patient (specimen) details, your specimen reference number, major patient symptoms, recent travel history, your identification of the isolate and what testing you require.

Salmonella

Services offered include:

- WGS of all Salmonella species providing sequence type and inferred serovar
- analysis of WGS 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 by telephone of any urgent specimen that is being dispatched to GBRU

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

Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU)

The staff contact list is in the final section of this document.

The unit is happy to discuss and advise on any clinical or epidemiological problems and outbreak investigations. Contact the 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.

Zoonotic and Acute Respiratory Section (ZARS)

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. For national surveillance of Legionnaires' disease contact legionella@ukhsa.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 or routine respiratory pathogen screen negative patients only). This is a charged for service. For registered laboratories this service is free of charge for pneumonia or ECMO patients as part of Legionella COVID-19 surveillance.

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.

Mycoplasma and ureaplasma

ZARS 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.

Table 3. Quick list of services

Target	Test	Turnaround time	Preferred specimen	Minimum sample volume
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* or *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
Isolates	Culture, PCR and sequencing when relevant.	Species dependant (see below)	Culture on blood agar or in transport medium [note 1].	N/A

Note 1: 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.

See guidance on Mycoplasma pneumoniae: referral of samples.

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 or 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 and others, 2005) and the glyceraldehyde-3-phosphate dehydrogenase (gap) gene in *M. hominis* (adaption of Baczynska and others, 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. For the occasional non-respiratory sample (for example, eye swab, blood/serum, csf when a respiratory sample is not available) we will report it as 'this is not an optimal sample type, as it has not been validated against this PCR assay'.

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, Colindale 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).

Pertussis: guidelines for public health management provide guidance on the public health management of pertussis (whooping cough).

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 criterion is met:

- single samples taken more than 2 weeks after onset of cough for any individuals with a history of prolonged cough

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 being 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 UKHSA Colindale for confirmation of identity and capsular typing will undergo routine 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-plus serogroups and 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 or 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 in serum. *S. pneumoniae* serology is performed by:

UKHSA Vaccine Evaluation Unit
Manchester Medical Microbiology Partnership
Clinical Sciences Building 2
Manchester Royal Infirmary
Oxford Road
Manchester, M13 9WL

Please contact Professor Ray Borrow on 0161 276 6793.

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

Identification or 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 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 in a timely manner 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 UKHSA 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 can be found in the immunoglobulin handbook.

Information on immunisation against infectious diseases can be found in the Green Book and advice is available from the UKHSA Immunisation Lead at immunisation.lead@ukhsa.gov.uk.

Please refer to public health control and management of diphtheria (in England and Wales) 2015 guidelines by the Diphtheria Guidelines Working Group for further details.

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, Colindale. Requests for antimicrobial sensitivity testing can be included with submissions.

The laboratory works closely with colleagues in the Immunisation and Countermeasures Division and are happy to discuss any queries regarding appropriate investigations and actions.

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 to 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 in serum. Hib serology is performed by:

UKHSA Vaccine Evaluation Unit at Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL, Tel 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 and 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 to 0.09 IU/mL
- protective levels: 0.1 to 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 may 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.

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

Sexually Transmitted Infections Reference Laboratory (STIRL)

The staff contact list is in the final section of this document.

The main activities of STIRL are:

- identification and antibiotic susceptibility testing of *Neisseria gonorrhoeae* and *Mycoplasma genitalium* for patient management and national surveillance
- evaluation of new phenotypic and genotypic methods for investigating AMR in bacterial STIs
- molecular characterisation of strains to inform surveillance, aid outbreak investigations, identify suspect treatment failures and define diagnostic escape variants
- provision of diagnostic, scientific and clinical guidance and advice

Other new activities that are currently outside the scope of ISO 1518 include:

- development of a partnership between Colindale and UKHSA South-west regional laboratory to co-deliver some STI services
- development of antimicrobial susceptibility testing for *Trichomonas vaginalis* – please contact us if you can help us collect samples
- repatriation of other bacterial STI diagnostics such as LGV and *Treponema pallidum* PCR – please refer to the VRD user manual for more information about these tests

Referral of putative *N. gonorrhoeae* cultures

A reference service is available for isolates that require confirmation of identification because local results were anomalous: all isolates will first be tested by MALDI-ToF MS using the extraction method. If a good identification to species level cannot be achieved by MALDI-ToF MS (score <2.3), then identification will be supplemented by phenotypic testing and where necessary an *N. gonorrhoeae* specific PCR.

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 is available. Upon special request, we also offer a PCR for the molecular detection of ceftriaxone resistance. Please contact us if you have any molecular specimens that may be from individuals, or their contacts, with suspected treatment failure or harbour a gonococcal isolate with suspected/confirmed ceftriaxone resistance.

Note: We no longer seek to confirm *N. gonorrhoeae* with azithromycin resistance. Any isolates received will not be tested and an administration fee charged.

Confirmation of identification for medico-legal purposes: Testing will only commence if STIRL has been contacted in advance and if all paperwork (including the chain of evidence form) is correctly completed - refer to the specimen submission guidelines for more details. Please note that medicolegal processing is not available for isolates which have already been confirmed locally as *N. gonorrhoeae* by 2 different tests. Typing/sequencing is not available for medico-legal isolates.

STIRL will accept viable *N. gonorrhoeae* 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 where it is clinically indicated (that is, pregnancy) and for those who have failed macrolide and fluoroquinolone treatment – this must be made clear on the referral form. In addition, STIRL may infer possible treatment failure if different samples are received within 2 months,and will request fluoroquinolone resistance testing on the referrer's behalf.

STIRL will accept specimens and DNA extracts for:

- *M. genitalium* detection from symptomatic patients, known contacts of cases or for test-of-cure
- Specimens accepted include:
    - rectal and genital swabs in unprocessed NAAT swab transport medium (minimum volume 400 µL) or as dry swabs. Pharyngeal swabs will be tested but they are an unvalidated sample type and therefore outside of ISO 15189 scope.
    - urine (minimum volume 3 mL)
    - extracted DNA – ideally only from previously positive specimens as an internal control result may not be available

As per current national guidance in response to the mpox outbreak, all specimens received will be subjected to an inactivation step prior to processing, for further information please contact the STIRL.

Note: Charges will be levied for some services. Please refer to the latest UKHSA price lists for more details.

STIRL 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 to August/September) to the STIRL for susceptibility testing. Isolates can be referred frozen on Microbank beads (storage in the referring laboratory must be at minus 80°C). Frozen batches will be collected by courier by arrangement).

The *Mycoplasma genitalium* Antimicrobial Resistance Surveillance (MARS) was initially run as a pilot in 2019 and 2020, and is being developed into an annual surveillance programme from 2023. Participating laboratories refer positive *M. genitalium* specimens over a 4 month period (March to June) to STIRL for molecular susceptibility testing. This study is currently outside of ISO 15189 scope, but please do contact the lab if you are interested in participating or require more information.

New antibiotics and diagnostics

STIRL liaises with pharmaceutical and diagnostics companies to test new antibiotics and new assays, respectively. We have a large collection of representative and unusually resistant isolates that can be used for evalutions. All work is undertaken as contracted research.

List of contacts

Antimicrobial resistance and healthcare associated infections reference Unit (AMRHAI)			
Neil Woodford	Unit Head	neil.woodford@ukhsa.gov.uk	020 8327 6511
Caroline Motamed	Technical Manager	caroline.motamed@ukhsa.gov.uk	
Antimicrobial Resistance and Mechanisms Service			
Katie Hopkins	Section Head	katie.hopkins@ukhsa.gov.uk	020 8327 7061
Staphylococcus and Streptococcus Reference Section			
Juliana Coelho	Section Head	juliana.coelho@ukhsa.gov.uk	020 8327 6979
Opportunistic pathogens typing			
Jane Turton	Section Head	jane.turton@ukhsa.gov.uk	020 8327 7224
Bacterial identification, culture negative clinical specimens (16S)			
Julie Logan	Section Head	julie.logan@ukhsa.gov.uk	020 8327 6059

Gastrointestinal bacteria reference unit (GBRU)			
Francesco Tripodo	Technical Manager	francesco.tripodo@ukhsa.gov.uk	
E. coli, shigella, vibrio, yersinia			
Claire Jenkins	Pathogen Lead	claire.jenkins@ukhsa.gov.uk	020 8327 6035
Salmonella			
Marie Chattaway	Pathogen Lead	marie.chattaway@ukhsa.gov.uk	020 8327 6171
Bacillus, clostridia (C. perfringens, C. botulinum, C. tetani), listeria			
Corinne Amar	Pathogen Lead	corinne.amar@ukhsa.gov.uk	020 8327 7341
Campylobacter, helicobacter			
Craig Swif	Pathogen Lead	craig.swift@ukhsa.gov.uk	020 8327 6597

Respiratory and vaccine preventable bacteria reference unit (RVPBRU)			
Nita Doshi	Technical Manager	nita.doshi@ukhsa.gov.uk	
Bordetella, diphtheria, haemophilus, pneumococci			
David Litt	Section Head	david.litt@ukhsa.gov.uk	020 8327 7476
Legionella, mycoplasma, ureaplasmas, respiratory Chlamydia			
Baharak Afshar	Section Head	baharak.afshar@ukhsa.gov.uk	020 8327 6495

Sexually transmitted infections reference laboratory (STIRL)			
Paola Barbero	Technical Manager	paola.barbero@ukhsa.gov.uk	020 8327 7674
Neisseria gonorrhoeae, Mycoplasma genitalium			
Michelle Cole	Unit Head (interim)	michelle.cole@ukhsa.gov.uk	020 8327 6465

Quality (general enquires, compliance and complaints)			
Ifeoma Ekwueme	Quality Compliance	ifeome.ekwueme@ukhsa.gov.uk	020 8327 7552
Nazim Chowdhury	Quality Manager	nazim.chowdhury@ukhsa.gov.uk	020 8327 6642

About the UK Health Security Agency

UKHSA is responsible for protecting every member of every community from the impact of infectious diseases, chemical, biological, radiological and nuclear incidents and other health threats. We provide intellectual, scientific and operational leadership at national and local level, as well as on the global stage, to make the nation heath secure.

UKHSA is an executive agency, sponsored by the Department of Health and Social Care.

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