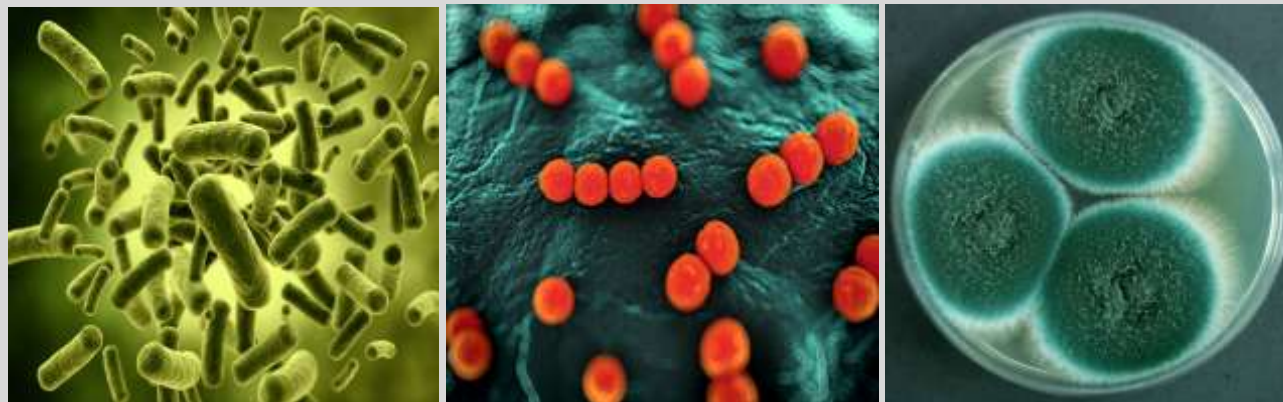


Microbiology

User Manual



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About us

The department of Microbiology, Virology and Infection Control provides a comprehensive, rapid and high-quality service for the diagnosis, management and prevention of infectious disease in patients at Great Ormond Street Hospital.

The Laboratory provides a wide range of both routine and specialised investigations in Bacteriology, Virology, Mycology and Parasitology. We provide environmental monitoring for Pharmacy, Cellular Therapy in addition to that required for prevention of infection, such as environmental cleanliness, air and water quality and for outbreak investigations.

Our expert team is on hand to provide expert clinical advice 24 hours a day all year round. In addition, our infection control team provide full service for the prevention, investigation and control of infection in patient and staff.

The department is highly active in research and development, specialising in molecular diagnostics, including cutting edge high throughput sequencing for diagnostics, epidemiological studies and novel pathogen detection methods.

Disclaimer

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Location

Department of Microbiology, Virology and Infection Control
Level 4
Camelia Botnar Laboratories
Great Ormond Street Hospital
Great Ormond Street
London
WC1N 3JH



Camelia Botnar Laboratories	Level	Room number
Main Microbiology Laboratory and Specimen Reception	4	P4.042
Virology Laboratory and Specimen Reception	4	P4.040
Virology Laboratory	4	P4.036

Contacts

Microbiology and Virology Telephone Numbers		Telephone	Bleep / direct line
Laboratories			
Microbiology Laboratory		5280/ 8661	Bleep 0670/ direct line 0207 829 8661
Virology Laboratory		8506/42401	Direct line 0207 813 8506
Microbiology out of hours service: 20:00 - 08:00 Monday to Friday, all weekend and bank holidays			
Microbiology Laboratory		5280/ 8661	Bleep 0670/ direct line 0207 829 8661
Virology out of hours service 08:00 – 12:00 Saturdays and bank holidays			
Virology Laboratory		8506/42401	Direct line 0207 813 8506
Senior laboratory staff			
Lead Laboratory Manager	Christine Morris	8664	Direct line 0207 829 8664
Laboratory Manager	Tanja Rockenbach	8507	Direct line 0207 813 8507
Deputy Laboratory Manager	Francis Yongblah	5280/8661	Direct line 0207 829 8661
Senior Clinical Scientist (Molecular)	Dr. Kathryn Harris	0437	Direct line 0207 829 0437
Medical staff			
Microbiology and Virology Specialist Registrars		5282	
Microbiology Consultants	Dr. Garth Dixon	8594	Direct line 0207 829 8594
	Dr. John Hartley	7930	Direct line 0207 829 7930
	Dr. James Soothill	5237	Direct line 0207 829 5237
	Dr James Hatcher	4583	
	Prof. J Breuer		
Infection Control	Helen Dunn Elaine Cloutman-Green Helen Saraqi Barbara Brekle	5284/8443	Direct line 0207 813 8443

Note that any of the above staff can be contacted via email, using forname.surname@gosh.nhs.uk

Laboratory Service

Routine Working Hours	
Microbiology	
08:00 – 16:30	Monday to Friday
08:00 – 14:00	Saturday
Clinical advice	
The laboratory specialist registrars and consultants are contactable for clinical advice from 09:00 – 17:30 Monday to Friday. See table above for contact numbers. At all other times a Specialist Registrar or Consultant are on call and contactable via the switchboard.	
Virology	
08:00 – 17:30	Monday to Friday
08:00 – 14:00	Saturday
Clinical advice	
The laboratory specialist registrars and consultants are contactable for clinical advice from 09:00 – 17:30 Monday to Friday. See table above for contact numbers. At all other times a Specialist Registrar or Consultant are on call and contactable via the switchboard.	

Out of Hours	
Microbiology	
17:30 – 08:00	Monday to Friday, plus all weekends and bank holidays
14:00 – 08:00	Saturday
All day	Sunday and bank holidays

Tests Available Out of Hours	
Routine tests	
Microbiology	Virology – By arrangement with On-Call Microbiology staff
Antibiotic assays (Amikacin, Gentamicin, Tobramycin, Vancomycin).	Needlestick injury testing of donor (HIV antibody and Hepatitis B surface antigen)
Blood cultures.	
Bronchoalveolar lavages – Microscopy, culture, mycobacterial microscopy.	
CSF – Microscopy and culture.	
Sterile body fluids and tissues - Microscopy and culture.	
Rapid antigen screening.	
Theatre samples.	
Urine microscopy (until 22:00).	
Other tests available by discussion with BMS on Bleep 0670 or by discussion with On Call Medical Microbiology cover (via switchboard)	

Laboratory Advisory Services

Clinical advice

The laboratory Specialist Registrars and Consultants are contactable for clinical advice including;

- clinical indications and choice of appropriate tests
- advice on individual clinical cases
- professional judgement on the interpretation of the results of examinations

Please refer to the above table for contact details.

Scientific and Technical advice

Biomedical Scientists in the laboratory are available for scientific and technical advice. Please refer to the above table for contact details.

Please also refer to:

- Sample requirements,
 - type
 - volume
 - handing requirements
 - specific contraindications for each assay
- limitations of examination procedures
- frequency of requesting the examination

- Policy on Accepting Unlabelled or Mislabeled Samples (GOS web).

- Samples: requesting, labelling and sampling requirements (GOS web).

Sample Labelling

All samples must be taken and labelled in accordance with the Clinical Procedure Guidelines, which are available on the hospital intranet (GOS web).

- Patient Identification Policy
- Samples: requesting, labelling and sampling requirements

Sample bottles for blood – order of draw			
Order	Colour	Description / anticoagulant	Notes
1	White	Serum	<ul style="list-style-type: none"> • Not for use in Microbiology and Virology
2	Brown	Serum gel	Serum
3	Orange	Lithium heparin	Plasma
4	Green	Sodium citrate	<ul style="list-style-type: none"> • Not for use in Microbiology and Virology
5	Yellow	Sodium fluoride	<ul style="list-style-type: none"> • Not for use in Microbiology and Virology
6	Blue	EDTA for blood transfusion	<ul style="list-style-type: none"> • Not for use in Microbiology and Virology
7	Red	EDTA	Whole blood for Molecular Virology/Bacteriology

Samples must be clearly labelled at the bedside by the person taking the sample, using information from the patient's wristband with surname, forename, hospital number, date of birth, location and date and time of collection.

PIMS generated sticky labels can be used for all samples. Please place the label on the bottle so that it does not obscure the view of the blood.

In instances where a sample fails to meet laboratory acceptance criteria, the requesting ward or doctor will be contacted and a statement to that effect documented in the report. Please refer to the policy: Accepting unlabelled and mislabelled samples (GOS web).

Request Forms

Use PIMS to print request forms wherever possible.

Ensure that the correct patient is identified before proceeding with the request, and that the hospital number is correct. Microbiology and Virology requests for private patients must have a GOS hospital number on both the request form and sample.

If tests are being undertaken on a relative (for example mother, father, sibling) they should be registered on PIMS and allocated a hospital number. In exceptional circumstances, where a relative is not registered on PIMS, then a PIMS downtime form must be used. Full name and date of birth must be used on forms (not 'mother of'). It is never acceptable to use patient forms / stickers with the name changed.

- Identify the consultant and the requester.
- Complete the bleep / telephone number.
- Select the location (ward). Please do not free text inappropriate or temporary locations.
- Select the type of sample and the site (where appropriate).
- Select the investigations required.
- Add any relevant clinical information - this will help to ensure that the correct investigations are targeted by the laboratory.
- Select priority status - urgent, soon or routine.

PIMS will automatically fill in any alerts. If a PIMS form is unavailable, write this information on the downtime form.

If it is not possible to use PIMS request forms, please ensure that the PIMS downtime form contains the above information. This can be downloaded from the GOS web.

After printing the form

Write the date and time of collection in the box provided.

Add any additional specimen details that could not be found on the computer list.

Add any additional or alternate locations where the report, or a copy of the report, should be sent.

Sample Collection and Transport to the Laboratory

The pneumatic chute system should be the primary mode of transport for the delivery of urgent pathology samples. In addition, the Site Services department provides a routine specimen transport service. The pneumatic chute system may be used out of hours for the transport of routine samples if there is a lack of available porter staff.

Department	Chute station
Microbiology and Virology	041

If the chute is unavailable

A porter from Site Services can be booked to deliver a specimen using the CARPS system (GOS Web).

Ward staff may bring specimens to the laboratories, which are located on level 4 Camelia Botnar Laboratories.

Samples for Microbiology and Virology should be placed in the sample reception box in the Microbiology main laboratory, including those which are urgent. Telephone the appropriate laboratory if the sample needs to be processed as a matter of urgency.

Please ensure that samples are sent to the laboratory as soon as they are taken. Please do not store a large batch and dispatch them together, as this causes delay to sample processing.

Computer access to results and turnaround times

Results are accessible on ward computers.

Click on Novell applications and select the 'Pathology Results' icon. Alternatively, use the GOS web/ useful links /pathology results

Enter user ID and password.

Follow prompts for hospital number (or family name and given name) and department.

Grossly abnormal or positive results requiring immediate action are automatically telephoned to the requesting ward or doctor.

Please refer to the tables on the following pages for turnaround times for each test.

In case of computer access problems phone 5066 for the computer help desk.

Requesting additional investigations

If additional investigations are required after the specimen has been dispatched or processed by the laboratory, please telephone as soon as possible, contact details above. You may be asked to supply a fresh request form with the new request, particularly if the specimen is to be forwarded to a different laboratory.

There is a practical time limit for requesting additional investigations: the laboratory stores specimens for a variable time period (depending upon sample type) before disposal. Also note that some specimens deteriorate in storage or may be completely consumed during processing rendering them unsuitable for further investigation.

Please note that any specimen requiring culture becomes less viable as time progresses and so additional testing must be requested at the earliest possible opportunity. If too much time has elapsed the specimen may give a false negative result.

Sample Retention Times

Tissues and biopsies	1 month (minimum)
Fluids excluding Urine	2 weeks (minimum)
Urine	7 days
Faeces and rectal swabs	7 days
MRSA, wound, skin and other swabs	7 days
Blood samples (antibiotic assays)	7days
Blood cultures	2 days post completion of processing
Serum samples (serology)	6 months except for those referred

Quality Assurance and Accreditation

The department operates a robust quality management system and maintains accreditation by UKAS to ISO 15189:2012. The schedule of accreditation can be found on the UKAS website [here](#).

The laboratory currently subscribes to external quality assurance panels provided by UK National External Quality Assessment Scheme (UKNEQAS), Quality Control for Molecular Diagnostics (QCMD) and Instand. Certification to confirm participation is available upon request.

The laboratory also carries out internal quality assurance in the form of anonymous resubmission of previously tested samples.

Laboratory Complaints Procedure

The medical and senior management staff in the Department of Paediatric Laboratory Medicine work very closely with users both within the Hospital Trust and with external referring clinicians. In order to provide the best service to its users, the department encourages both positive and negative feedback. Users can interact with Paediatric Laboratory Medicine staff at clinical presentations and throughout day to day communication.

Details of the laboratory complaints procedure can be found in the following document;

User satisfaction and complaints procedure AQU 013, available on Q-pulse, the Trust's Quality Management System

The Trust also has a general complaints policy, which can be located on the GOS web

Laboratory Policy on Protection of Personal Information

The laboratory adheres to the Trust's Policy on Information Governance to ensure compliance with the key principles of Information Governance. The Trust wishes to ensure all patients and service users to have confidence that their records will be maintained securely and will not be disclosed or shared inappropriately.

Details of the Trust's Information Governance Policy can be located on the GOS web

Special Considerations for Microbiology Investigations

Antibiotic Assays (Amikacin, Gentamicin, Tobramycin, Vancomycin) from Blood or CSF.

Timing of Levels:

Trough Levels – any regimen: should be taken immediately before a dose is given.

Trough and hold levels should be clearly labelled on the form as such so that priority may be given.

Peak levels – should be taken 60 minutes after administration of a dose has finished. Where extra fluid infusion is given to flush the last traces of a dose the dose administration should be considered to have finished before the flush is started.

Please note: BLOOD FOR ANTIBIOTIC ASSAY MUST NEVER BE TAKEN FROM A LINE WHICH HAS BEEN USED TO GIVE THAT ANTIBIOTIC AT ANY TIME.
Samples taken in this way have been shown to give unreliable results.

Antibiotic Regime - please state the dose, patient's weight, the frequency and timing of the dose and sampling on the request form.

Renal Function - please state whether this is normal or not; if impaired give the urea and creatinine.

Results

Antibiotic assay results are available on the [Pathology Results Browser](#) once they are validated.

Ward staff will be notified of levels above the normal range, advice is available from a medical microbiologist or ID consultant regarding modification of dose regime and timing of further assays. Please discuss any results you are not familiar with interpreting, especially CSF levels.

Antibiotic Policy

Antibiotic regimens and normal ranges can be found in the [GOSH Antibiotic Policy](#) on GOS Web, which has been produced under the auspices of the antibiotic subcommittee of the Drugs and Therapeutics Committee after discussion with users.

Amikacin Once-Per-Day policy is [here](#)

Gentamicin Once-Per-day policy is [here](#)

Antibiotic Recommended Normal Ranges are [here](#)

Screening Policy

Antibiotic resistance is an increasing problem. To limit the spread of antibiotic-resistant bacteria at GOSH we aim to screen all patients for carriage of MRSA (nose and throat swabs) and for antibiotic-resistant *Enterobacteriaceae* (faeces)

To facilitate this, computers on each ward automatically provide up to date information on which patients require screening and also information on which require isolation. Click [here](#) for link.

Antibiotic sensitivity test results are issued on MRSA and resistant *Enterobacteriaceae*. However, when these bacteria are isolated on screening, antibiotic therapy is generally not required: the sensitivity results are supplied for infection control purposes only.

The full admission screening policy can be read [here](#).

Blood culture technique

Method A continuous monitoring automated blood culture system is used in the department of Microbiology. The system detects the presence of aerobic and anaerobic bacteria, and fungi by measurement of CO₂ generated in a specially formulated culture medium.

Blood culture sets consist of two bottles - a paediatric aerobic bottle (pink label and silver cap) and an anaerobic bottle (purple label and purple cap) supplied by the Department of Microbiology.

Blood cultures are incubated for 5 days (21 days where endocarditis is suspected) all positives are notified to ward clinicians as soon as possible.

Samples Volumes Up to 5ml of blood should be placed in the aerobic (pink label and silver cap) bottle and up to 10 ml in the anaerobic (purple label and cap) bottle.

Number of Sets In acute bacterial sepsis – at least one set of cultures should be taken prior to starting antibiotic therapy.

In the investigation of Endocarditis three sets should be taken before starting antibiotics.

In patients with central venous and arterial lines, cultures should be taken from each lumen of each line and from a peripheral site if possible.

Labelling Bottles must be clearly labelled and the date, time and site of blood sampling must also be indicated.

Request forms must also be clearly labelled. The unit and consultant must be provided. Appropriate clinical details should be provided and site of sampling also stated.

Unlabelled samples will usually not be accepted. The nurse in charge of the unit or medical personnel involved will be telephoned, a repeat sample requested and the bottles discarded. Documentation of the incident is recorded.

Procedure See - [Blood tests, requesting, sampling & labelling requirements](#)

Microscopy, Culture and Sensitivity

Faeces There are three reasons for sending faecal samples to microbiology: (1) to screen for the presence of antibiotic resistant bacteria, (2) for investigation of gastrointestinal disease (in most cases diarrhoea). **It is vital that if faeces are sent for investigation of disease that this is stated** and that detail are given. Otherwise (unless the specimen is liquid) the sample may be processed as a screening specimen only. (3) **Weekly** screening of stool samples from neutropenic patients as part of their monitoring process. Repeat samples received in the same week without significant clinical details will be discarded.

Respiratory samples Nose and Throat swabs: Please give clinical details as they are part of the routine admission screen and may not get processed for pharyngeal pathogens unless the patient's clinical condition is indicated.

Mouth swabs and Tongue swabs: Mainly for investigation of upper airway specimens and *Candida* sp

Sputum samples : For investigation of lower respiratory infections.

Where NPAs are sent for both Bacteriology and Virology please indicate this or send two specimens.

Per nasal swabs should be sent for cases of suspected *B. pertussis*. This sample may also be sent for *Bordetella pertussis* PCR if an NPA is not received. These swabs should be sent as soon as possible and **not** be put into a charcoal swab container.

Urine samples Because of the high frequency of immunosuppression at GOSH, empirical antimicrobial therapy and the difficulty of collecting specimens from children, urine samples are followed up in more detail than in many other laboratories. Please repeat specimens when clinically indicated and remember that the provision of sensitivity data does not always imply that treatment is necessary.

Skin swabs Please remember to indicate if these are for the investigation of infection or for screening for MRSA.

Soft tissue infections and abscesses For microbiological investigations of such infections tissue or pus are preferred to swabs. Tissue often also requires histopathological investigation and may be sent fresh (the histologists will then place it in formalin) or may be put in formalin by the clinician sending the sample. Formalin kills bacteria and thus makes the samples useless for bacteriological investigation by culture. Before you send a sample to Histopathology and especially **before you put a sample in formalin, consider whether infection is part of the differential diagnosis.**

If TB is a possible diagnosis this should be stated as it requires special culture techniques.

Slow-growing organisms NOTE: results may be generated as 'Additional Reports' added to the previously finalised result.

Bordetella pertussis (Whooping Cough) – cultures are maintained for 5 days

Burkholderia spp – may be slow growing and special plates are incubated for 5 days (used for all cystic fibrosis respiratory specimens; positive results may be in the form of an additional report.

Legionella spp – All Bronchoalveolar lavages are cultured for *Legionella* spp, plates are incubated for 5 days.

Fungi – selective plates are maintained for extended incubation

Laboratory Investigations – Bacteriology - Cultures

Test	Collection requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	External referrals
<u>Microscopy:</u> - Gram stain - AAFB stain - Wet film - Cell count and differential	As per sample requirements for culture.	Same day		Yes, if urgent only.	
<u>Bacterial Culture and Sensitivity:</u> -Blood cultures -Body fluids (other than urine) -Eye swabs -Faeces and Rectal swabs	Aerobic bottle (pink label with silver cap) requires up to 5ml blood. Anaerobic bottle (purple label and cap) requires up to 10ml blood. Sterile plastic universal Charcoal swab Sterile plastic universal or charcoal swab	5 days 2- 5 days 2- 5 days 2- 5 days C.Difficile culture approx. 7	Please note on request form if endocarditis or brucellosis is suspected. Endocarditis requires extended incubation – 21 days. Brucellosis requires extended incubation – 7 days. Please state the type of body fluid. Please label for “Left” or “Right” eye. Please state if requiring investigation for intestinal pathogens.	Not required Yes, if urgent only. Not required Not required	C.difficile isolates referred to: Clinical Microbiology & Public Health laboratory (CMPHL), Level 6, Addenbrookes Hospital,

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		days.			Cambridge, CB2 0QW
-Legionella culture	Sterile plastic universal	5 days		Not required	
-MRSA Screen	Charcoal swab	2- 5 days		Not required	
-Respiratory swabs	Charcoal swab	2- 5 days		Not required	
-Skin and site swabs	Charcoal swab	2- 5 days		Not required	
-Sputum, respiratory secretions, washings or aspirates	Sterile plastic universal	2- 5 days	<i>Burkholderia spp.</i> culture for CF patients completed after 5 days.	Not required	
-Tips	Sterile plastic universal	2- 5 days		Not required	
-Tissue, Biopsy, Pus	Sterile plastic universal	2- 5 days	Primary culture and subculture will be completed in 3 days if negative. All specimens will be given extended incubation which will be completed in 10 days.	Yes, if urgent only.	
-Urine	Sterile plastic universal	3 days	Minimum volume of 0.5ml	Not required	
-Wound and umbilical swabs	Charcoal swab	2- 5 days		Not required	

<u>Fungal Culture and Sensitivity:</u>					
- Skin scrapings (for Dermatophytes)	Scraping kit	21 days		Not required	
- Hair, nails.	Scraping kit or sterile plastic universal	21 days		Not required	
- Other specimen types.	As per specimen type for Bacterial culture and sensitivity	21 days		Not required	
<u>Mycobacterial Culture and Sensitivity:</u>					
-Sputum, bronchoalveolar lavage, body fluids, gastric aspirates, urine	Sterile plastic universal	8 weeks	AAFB microscopy will be performed and reported on day of receipt. AAFB microscopy is not performed on gastric aspirates and urine samples. Isolates requiring sensitivity testing are referred to PHE National Mycobacterial Reference Laboratory. Results may take up to 8 weeks to be completed.	Not required	Positive isolates referred to: National Mycobacterium Reference Unit Colindale London NW9 5HT Tel. 0208 327 6957
- Tissues including lymph nodes and biopsies.	Sterile plastic universal	12 weeks	AAFB microscopy will be performed and reported on day of receipt.	Not required	
- Blood and bone marrow	Lithium heparin vacutainer	12 weeks	The Microbiology Department is unable to process blood or bone marrow samples for		Scottish Mycobacterial Reference Laboratory Dept of Laboratory Medicine

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	(sterile) Phone Microbiology to request for container to be sent/collected.		Mycobacterial culture on site. A minimum volume of 2ml is required collected into lithium heparin vacutainer. Samples should be sent to Microbiology at GOSH as soon as possible before being referred to the Mycobacterial Reference Laboratory for culture.		Royal Infirmary of `Edinburgh 51 Little France Crescent Old Dalkeith Road Edinburgh EH16 4SS Tel 0131 242 6016 Email LOTHIAN.SMRL@nhs.net
<u>Screening Cultures:</u>					
- MRSA screening	Charcoal swab	2- 5 days	Please state screening site on swab.	Not required	
-β-haemolytic Streptococcus screening (Nose and throat)	Charcoal swab	2- 5 days	Please state screening site on swab.	Not required	
- Resistant gram-negative screening	Charcoal swab/ Faeces or urine in sterile plastic universal	2- 5 days	Please discuss with Microbiology/Infection Control clinicians before screening. Contact details above.	Not required	
- Vancomycin-Resistant Enterococci screening	Charcoal swab/ Faeces or urine in sterile plastic universal	2- 5 days	Please discuss with Microbiology/Infection Control clinicians before screening. Contact details above.	Not required	
<u>Referred Cultures:</u>					
- Mycoplasma/Ureaplasma (Urine, CSF, sputum)	Sterile plastic universal	7 days		Not required	Bacteriology Reference Department (RVPBRU) 61 Colindale Avenue London NW9 5HT Phone +44 (0)20 8327 7887 Website: www.gov.uk/phe

- <i>Francisella tularaemia</i>	Sterile plastic universal / charcoal swab	7 – 14 days	Please label with hazard stickers. <u>Category 3 organism.</u>	Not required	Rare and Imported Pathogens Laboratory (RIPL) Public Health England Porton Down Salisbury Wiltshire SP4 0JG Telephone: 01980 612348 Email: ripl@phe.gov.uk
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Laboratory Investigations – Bacteriology – Antimicrobial Agent Assays Performed at GOSH

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	Test Schedule
<u>Amikacin:</u>					
- Blood	0.5ml heparinised blood. Orange bottle.	6 hours	On the request form please include: -Date and time antimicrobial last given.	Yes, if urgent only.	Non-urgent levels routinely performed at approximately: 10:30
- CSF	Sterile plastic universal. Minimum 0.3ml.	6 hours	-Date and time sample taken. -Dosage of antimicrobial last given.	Yes, if urgent only.	15:30 20:30 00:00
<u>Gentamicin:</u>					
- Blood	0.5ml heparinised blood. Orange bottle.	6 hours	On the request form please include: -Date and time antimicrobial last given.	Yes, if urgent only.	Non-urgent levels routinely performed at approximately: 10:30
- CSF	Sterile plastic universal. Minimum 0.3ml.	6 hours	-Date and time sample taken. -Dosage of antimicrobial last given.	Yes, if urgent only.	15:30 20:30 00:00

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<u>Tobramycin:</u>					
- Blood	0.5ml heparinised blood. Orange bottle.	6 hours	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Yes, if urgent only.	Non-urgent levels routinely performed at approximately: 10:30 15:30 20:30 00:00
- CSF	Sterile plastic universal. Minimum 0.3ml.	6 hours		Yes, if urgent only.	
<u>Vancomycin:</u>					
- Blood	0.5ml heparinised blood. Orange bottle.	6 hours	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Yes, if urgent only.	Non-urgent levels routinely performed at approximately: 10:30 15:30 20:30 00:00
- CSF	Sterile plastic universal. Minimum 0.3ml.	6 hours		Yes, if urgent only.	

Laboratory Investigations – Bacteriology – Antimicrobial Agent Assays Sent to Reference Laboratories

There may be no weekend or Bank Holiday Reference Laboratory service, levels received after 16:00 Thursday may not be processed until the following Monday or Tuesday.

Please note: This list is not exhaustive. If an antimicrobial agent assay is required for an agent not present on this list, please contact Microbiology on the details provided above.

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	External referrals
Amphotericin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. <u>-Any other antifungals previously administered.</u>	Not required	Mycology Reference Centre Leeds Teaching Hospital NHS Trust The General Infirmary, Leeds, LS1 3EX Telephone: 0113 392 6787 Dr Richard Hobson: 0113 392 2835 Dr Richard Barton: 0113 392 3390
Ceftazidime	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. 24 hour notice must be given before sample sent for testing.	Yes	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Chloramphenicol	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654

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Ciprofloxacin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Colistin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Cycloserine	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Daptomycin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Fluconazole	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. <u>-Any other antifungals previously administered.</u>	Not required	Mycology Reference Centre Leeds Teaching Hospital NHS Trust The General Infirmary, Leeds, LS1 3EX Telephone: 0113 392 6787 Dr Richard Hobson: 0113 392 2835 Dr Richard Barton: 0113 392 3390

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Flucytosine	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. <u>-Any other antifungals previously administered.</u>	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Itraconazole	1ml clotted blood. Brown, serum gel bottle.	72 hours	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. <u>-Any other antifungals previously administered.</u>	Not required	THE DOCTORS LABORATORY (TDL) The Halo Building 1 Mabledon Place London WC1H 9AX Tel: 020 7307 7373
Meropenem	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. 24 hour notice must be given before sample sent for testing.	Yes	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Posaconazole	1ml clotted blood. Brown, serum gel bottle.	72 hours	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	THE DOCTORS LABORATORY (TDL) The Halo Building 1 Mabledon Place London WC1H 9AX Tel: 020 7307 7373
Rifampicin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654

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Streptomycin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Teicoplanin	1ml clotted blood. Brown, serum gel bottle.	7 days	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given.	Not required	Antimicrobial Reference Laboratory Dpt. of Medical Microbiology Lime Walk Building North Bristol NHS Trust Southmead Hospital Bristol, BS10 5NB General enquiries:0117 323 5698/5654
Voriconazole	1ml clotted blood. Brown, serum gel bottle.	72 hours	On the request form please include: -Date and time antimicrobial last given. -Date and time sample taken. -Dosage of antimicrobial last given. <u>-Any other antifungals previously administered.</u>	Not required	THE DOCTORS LABORATORY (TDL) The Halo Building 1 Mabledon Place London WC1H 9AX Tel: 020 7307 7373

Laboratory Investigations – Bacteriology – Serology (Antibody) Processed at GOSH

Serum concentrations of antibody to infective agents.

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	Test Schedule
Anti-Streptolysin O / DNAase B	1ml clotted blood. Brown, serum gel bottle.	7 days		Not required	Samples tested once weekly, usually Thursday PM.
Borrelia burgdorferi (Lyme disease)	1ml clotted blood. Brown, serum gel bottle.	<7 days	This test is now provided by Virology.	Not required.	
Syphilis (ESPLINE)	1ml clotted blood. Brown, serum gel bottle.	1 day	This test is now provided by Virology	Not required	Daily.

Laboratory Investigations – Bacteriology – Serology (Antibody) Sent to Reference Laboratories

Serum concentrations of antibody to infective agents

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	External Reference
Anaplasma	1ml clotted blood. Brown, serum gel bottle.	7 – 14 days		Not required	Rare and Imported Pathogens Laboratory (RIPL) Public Health England Porton Down Salisbury, Wiltshire SP4 0JG Telephone: 01980 612348 Email: ripl@phe.gov.uk
Bartonella (Cat Scratch Fever)			This test is sent to France via TDL which makes the test expensive and has a long turnaround time. Please contact Microbiology consultant before requesting.		Referral bench. THE DOCTORS LABORATORY (TDL) The Halo Building 1 Mabledon Place London WC1H 9AX Tel: 020 7307 7373
Brucella	1ml clotted blood. Brown, serum gel bottle.	7 days		Not required	Brucella Special Diagnostic Unit Liverpool Clinical Laboratories Virology Department 8th floor Duncan Building Royal Liverpool & Broadgreen Hospital Prescott Street, Liverpool L7 8XP Phone: 44 (0)151 529 4900/ 44 (0)151 706 4404/4782
Campylobacter	1ml clotted blood. Brown, serum gel bottle.	Up to 14 days		Not required	Preston Microbiology Services, Royal Preston Hospital, PO Box 202, Sharoe Green Lane Fulwood, Preston, Lancs PR2 9HT Email: LabFwePreston@phe.gov.uk

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					Telephone 01772 523116
<i>E. coli</i> 0157	1ml clotted blood. Brown, serum gel bottle.	7 – 14 days		Not required	Bacteriology Reference Department GBRU 61 Colindale Avenue London NW9 5HT Phone: +44 (0)20 8327 7887
Helicobacter Antibody - serum	1ml clotted blood. Brown, serum gel bottle.	7 -14 days		Not required	Bacteriology Reference Department GBRU 61 Colindale Avenue London NW9 5HT Phone: +44 (0)20 8327 7887
Antigen - Faeces	Faeces in sterile universal/stool pot	2-4 days			Department of Microbiology, St Helier Hospital Epsom & St Helier University Hospitals NHS Trust Wrythe Lane Carshalton, Surrey SM5 1AA
Antigen Biopsy	Sterile universal container	15 days	Avoid sending samples on Friday		Bacteriology Reference Department GBRU 61 Colindale Avenue London NW9 5HT Phone: +44 (0)20 8327 7887
Legionella	See comment	7 -14 days	Serum test no longer available. Please send a urine sample for Legionella antigen.	Not required	Bacteriology Reference Department (RVPBRU) 61 Colindale Avenue London NW9 5HT

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Leptospira	1ml clotted blood. Brown, serum gel bottle.	7 -14 days	It is necessary to examine at least 2 serum specimens taken at least 7 days apart.	Not required	Rare and Imported Pathogens Laboratory (RIPL) Public Health England Porton Down Salisbury Wiltshire SP4 0JG Telephone: 01980 612348 Email: ripl@phe.gov.uk
Neisseria meningitis functional antibody to serogroups ACYW135 or B	1ml clotted blood. Brown, serum gel bottle.	28 days		Not required	PHE Meningococcal Reference Unit Manchester Medical Microbiology Partnership (MMMP) Clinical Sciences Building 2, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL Tel: +44 (0)161 276 8788/6757
Streptococcus Antibody	1ml clotted blood. Brown, serum gel bottle.	7 -14 days		Not required	Bacteriology Reference Department (AMRHAI) 61 Colindale Avenue London NW9 5HT Phone: +44 (0)20 8327 7887
Yersinia enterocolitica and pseudo-tuberculosis			The reference laboratory no longer offers this service. Any specimens sent for this test will be saved for 6 months only. Please contact the Microbiology clinical staff (details above) for further information.		

Laboratory Investigations – Bacteriology – Serology (Antigen Detection) Processed at GOSH

Rapid antigen screens can be performed as urgent investigations and results are available via the Pathology Results Browser as soon as the test is completed. Positive results will be telephoned to the requesting clinician.

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	Test Schedule
<i>E. coli</i> Type K1	1ml clotted blood. Brown, serum gel bottle. 1ml body fluid including CSF.	Same day.		Yes	Urgent request only.
B-haemolytic Streptococci Group B (<i>Streptococcus agalactiae</i>)	1ml clotted blood. Brown, serum gel bottle. 1ml body fluid including CSF.	Same day.		Yes	Urgent request only.
<i>Haemophilus influenzae</i> Type B	1ml clotted blood. Brown, serum gel bottle. 1ml body fluid including CSF.	Same day.		Yes	Urgent request only.
<i>Neisseria meningitidis</i> Groups A,B,C,W ₁₃₅	1ml clotted blood. Brown, serum gel bottle. 1ml body fluid including CSF.	Same day.		Yes	Urgent request only.
<i>Streptococcus pneumoniae</i>	1ml clotted blood. Brown, serum gel bottle. 1ml body fluid including CSF.	Same day.		Yes	Urgent request only.

Laboratory Investigations – Mycology Serology

Please note that the stated times until results are available of antibody tests performed externally to GOSH are a guide only - serology testing is performed on a batch basis and results may be available sooner (or later) than stated.

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	External Reference
Aspergillus Antibody	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe
Aspergillus Antigen (Galactomannan)	Broncheolavage	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe
Aspergillus Antigen (Galactomannan)	2ml clotted blood. Brown, serum gel 2ml clotted blood. bottle. (Minimum 1ml serum required).	72 hours		Not required	THE DOCTORS LABORATORY (TDL) The Halo Building 1 Mabledon Place London WC1H 9AX Tel: 020 7307 7373

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Beta-d Glucan	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	72 hours		Not required	THE DOCTORS LABORATORY (TDL) The Halo Building 1 Mabledon Place London WC1H 9AX Tel: 020 7307 7373
Blastomyces Antibody	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe
Candida Antibody	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe
Candida Antigen (Mannan)	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe
Cryptococcus Antibody	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe

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Cryptococcus Antigen	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe
Histoplasma capsulatum Antibody	2ml clotted blood. Brown, serum gel bottle. (Minimum 1ml serum required).	7 – 14 days		Not required	Mycology Reference Laboratory PHE South West Laboratory Myrtle Road, Kingsdown Bristol BS2 8EL Phone +44 (0)117 342 5028 www.gov.uk/phe

Laboratory Investigations – Parasitology

Please note that the stated times until results are available of antibody tests performed externally to GOSH are a guide only - antibody testing is performed on a batch basis and results may be available sooner (or later) than stated.

Test	Sample requirements	Turnaround time	Additional information	Contact the laboratory to arrange the test in advance	External Reference
<p><u>Ova, cysts and parasites:</u></p> <p>Microscopy for the detection of:</p> <ul style="list-style-type: none"> - Giardia - Entamoeba - Ascaris - Capillaria - Clonorchis - Hookworm - Cryptosporidium 	Unfixed faeces sample.	2 days	<p>Some ova, cysts and parasites cannot be excluded from a single sample and so sequential stool testing may be necessary. Please discuss with the Microbiology Clinicians (see details above) for guidance.</p> <p>Worms and worm segments Adult worms and tapeworm segments should be sent without preservative in a sterile universal container. If there is likely to be a delay of more than 24 hours, then 10% formol water should be added to the specimen.</p>	Not required	
<p><u>Acanthamoeba:</u></p> <p>-Microscopy and culture</p>	<p>contact lens and/or wash fluids</p> <p>corneal scrapes, biopsies, swabs</p>	5-7 days, all positive results telephoned in interim		<p>Yes. Special transport media requirement. Needs discussion with Microbiology medical staff.</p>	<p>Diagnostic Parasitology Laboratory Faculty of Infectious and Tropical Diseases London School of Hygiene & Tropical Medicine Keppel Street London WC1E 7HT</p> <p>Tel: +44 (0)20 7927 2427</p>

- PCR	CSF, biopsy material	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Ameobic Serology / ID	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Angiostrongyloides	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Babesia	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Needs to be discussed HTD only test after discussion	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418

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Cysticercosis	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days	Please note: Intestinal infections with <i>Taenia solium</i> or <i>saginata</i> will usually give negative results by Serology. Please contact the Microbiology clinical staff (details above) for further information.	Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
<u>Cryptosporidium:</u> -Microscopy	Unfixed stool sample	2 days		Not required	Cryptosporidium Reference Unit (CRU) Public Health Wales Microbiology ABM Singleton Hospital Sketty Swansea SA2 8QA
-PCR	Unfixed stool sample	7 – 14 days		Not required	
Fasciola	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Filaria	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Needs to be discussed HTD only test after discussion	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418

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Hytatid	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Leishmania – Serology	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days	Note: negative serology does not exclude the diagnosis of visceral leishmaniasis, particularly in sera from HIV positive patients. Serology is not helpful in the diagnosis of cutaneous infections. In mucocutaneous leishmaniasis serology is usually seropositive except in early cases.	Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Culture/PCR	1ml Bone marrow should be collected into EDTA tube	Up to 20 days			
Schistosoma	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418

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Strongyloides	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days	Note: There is known to be cross reaction between filaria and strongyloides in ELISA tests.	Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Toxocara	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days	A negative serum result does not exclude ocular toxocariasis. Vitreous sampling may be necessary to exclude ocular toxocariasis.	Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Trichinella	1ml clotted blood. Brown, serum gel bottle. (0.5ml serum required)	7 – 14 days		Not required	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418
Trypanosoma	A minimum of 2ml of EDTA anti-coagulated blood(Red EDTA bottle) and a minimum of 0.5ml of serum (Brown serum gel) is required.	7 – 14 days	Trypanosomes disintegrate rapidly on removal from the body, therefore it is vital that EDTA whole blood must be examined within 24 hrs.	Yes, for urgent referral	National parasitology reference laboratory (NPRL) Department of Clinical Parasitology, Hospital for Tropical Diseases 3rd floor Mortimer Market Centre Mortimer Market London WC1E 6JB Telephone: 020 344 75418

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Molecular Microbiology

Broad-range bacterial 16S rDNA PCR

Some bacterial species are difficult to isolate, or grow slowly in the laboratory due to stringent growth requirements, while others may not grow due to prior empirical treatment of patients with anti-microbial agents.

Molecular diagnostic techniques, such as PCR, aid in the diagnosis of bacterial infection by detecting bacterial genetic material.

Broad range assays are based on ribosomal genes (rDNA). Bacterial rDNA consists of highly conserved nucleotide sequences that are shared by all bacterial species, interspersed with variable regions that are genus or species specific.

By using PCR primers that are targeted at conserved regions of rDNA it is possible to design broad-range PCRs capable of detecting DNA from almost any bacterial species. The identity of the bacterium captured is revealed by nucleotide sequencing of the PCR product followed by comparison of this sequence with known sequences located in Genbank or other databases.

Suitable specimens:

Broad range 16S PCR may be performed on specimens from any normally sterile site e.g. empyema, pericardial fluid, joint aspirate, CSF, tissue and pus. Please discuss any requests with a Consultant Microbiologist or Clinical Scientist. Positive results will be telephoned to discuss significance.

Broad-range PCR and sequencing for identification of bacterial and fungal isolates

PCR and sequencing of 16S rDNA (bacteria) and Internal Transcribed Spacer Region – 1 (ITS-1) (fungi) may be used to confirm the identity of isolates which would previously have been referred to a reference laboratory. This provides a more rapid accurate service. Certain strains may be reported as 'identity to follow' pending the 16S rDNA and ITS-1 sequencing results.

Particular strains, for instance *Burkholderia*, are always confirmed by PCR.

***Bordetella pertussis* (Whooping Cough)**

Rapid diagnosis of *B. pertussis* infection is essential for patient management and especially infection control. This bacterium has fastidious growth requirements and laboratory culture is slow (up to 5 days). Detection of *B. pertussis* genomic DNA by PCR is rapid and the preferred method of detection for this organism.

Suitable specimens: Pernal swabs or NPAs. Please discuss this request with a Microbiologist first.

***Tropheryma whippelii* (Whipple's Disease)**

T. whippelii is a recently characterised bacterium that is the aetiological agent of Whipple's disease. First characterised by its 16S rDNA sequence, it has recently been propagated in continuous cell-culture and the entire genome sequenced. Detection of *T. whippelii* by cell-culture is not a practical diagnostic test and routine serological assays are not yet available. Amplification of nucleic acid by PCR remains the preferred detection method for this organism.

Suitable specimens: Preferred specimens are CSF, blood, duodenal biopsy. Please discuss this investigation with a Microbiologist first.

Streptococcus pneumoniae

Our laboratory has shown that diagnosis of *S. pneumoniae* infection can be improved by utilising molecular methods in addition to culture.

A real-time PCR to detect *S. pneumoniae* has been developed in our laboratory which offers greater sensitivity than the broad-range 16S rDNA PCR and can deduce susceptibility to penicillin via sequence polymorphisms in the *S. pneumoniae* penicillin binding protein (PBP)- 2b.

Suitable specimens: Preferred specimens are CSF, blood, pleural fluid, joint fluid and tissue. However, other specimens may also be suitable, please discuss request with a Microbiologist.

Neisseria meningitidis

Neisseria meningitidis is the major cause of bacterial meningitis in the UK, in both adults and children. It is also a cause of septicaemia. Rapid diagnosis is critical for patient management and also for implementation of public health measures. This real-time PCR assay targets the meningococcal *ctrA* gene to detect *Neisseria meningitidis* DNA in clinical material more rapidly than culture-based methods. PCR is also frequently positive in culture-negative samples. This real-time PCR assay can detect *N. meningitidis* is most commonly applied to blood and CSF samples but can be used on any sterile site sample.

***Streptococcus agalactiae* (Group B Streptococcus)**

Streptococcus agalactiae (Group B Strep, GBS) is the leading cause of septicaemia and meningitis in the newborn infant, and can result in serious morbidity and mortality. Empirical antibiotic treatment may result in failure to culture this organism and real-time PCR can then be used to obtain a diagnosis. This real-time PCR assay targets the *sip* gene which codes for a surface antigen protein in *Streptococcus agalactiae*. The assay is most commonly applied to blood and CSF samples but can be used on any sterile site sample.

Kingella kingae

Primary osteoarticular infections (OAI) in children must be diagnosed and treated urgently because of the risk of prolonged morbidity and crippling long-term sequelae. Isolation of the causative organism is the traditional way to confirm diagnosis. *K. kingae*, a member of the HACEK group of organisms, is now considered to be the leading cause of OAI in young children and can also be the cause of other infections, most notable infective endocarditis. However, its prevalence is underestimated as it frequently fails to culture due to its fastidious nature. PCR based methods are essential for the diagnosis of *K. kingae* OAI and other infections. This real-time PCR assay that can detect *K. kingae* from a range of clinical samples, in particular joint fluids and tissue from young children.

Staphylococcus aureus

Staphylococcus aureus is a catalase-positive, gram-positive coccus that may form part of the normal flora of the skin and other sites such as the upper respiratory tract. *S. aureus* causes a wide range of major and minor infections, including wound infections, abscesses, bacteraemia, osteomyelitis, pneumonia and endocarditis. Production of the enzyme *coagulase* is its main distinctive diagnostic feature. Molecular methods of detection are used to diagnose *S. aureus* infection when cultures are negative and a range of different target genes have been utilised. The identification of methicillin-resistant *S. aureus* (MRSA) is based on detection of the *mecA* gene target.

The assay targets the gene coding for the coagulase enzyme (*coa*) and can be applied to any sterile site sample. A second assay can also detect methicillin resistance by simultaneously detecting a *mecA* gene target and another *S. aureus* specific gene target (Sa442). This assay is primarily used on pure cultures of *S. aureus* to confirm methicillin resistance. Simultaneous detection of the *mecA* Sa442 targets at similar CT values directly from clinical samples implies (but does not confirm) infection with a methicillin-resistant *S. aureus* (MRSA).

***Streptococcus pyogenes* (Group A Streptococcus)**

Streptococcus pyogenes (Group A Strep, GAS) can produce a spectrum of clinical syndromes in humans that range from superficial infection of the pharyngeal mucosa to invasive infection of deep tissues or the blood stream. Empirical antibiotic treatment may result in failure to culture this organism and real-time PCR can then be used to obtain a diagnosis.

This real-time PCR assay targets the gene coding for the CsrR protein, which is part of a regulatory system that controls expression of several virulence determinants in *Streptococcus pyogenes*. The assay can be applied to any sterile site sample.

***Mycobacterium tuberculosis* and Atypical Mycobacteria**

Bacterial culture is the gold-standard method for diagnosis of Mycobacterial infection. Molecular methods can be used to detect Mycobacterial DNA directly from clinical specimens. They can also be used to identify cultured organisms, in particular from liquid culture media from positive flagging bottles, providing accurate identification of acid-fast organisms several days before traditional methods. Several different regions in the Mycobacterial genome are targeted in two multiplexed real-time PCR assays that can detect all clinically relevant *Mycobacteria species*, quickly differentiating *M. tuberculosis* complex from NTM and also RGM from the slow-growing NTM. Sequencing of amplicon can further identify organisms to species level. We have implemented this assay to allow us to do the following:

- Rapidly confirm (same day) whether liquid cultures that flag positive contain *M. tuberculosis* complex.
- Differentiate BCG strains from other members of the *M. tb* complex using an *ESAT-6* target (Present in all *M.tb* complex strains but absent in BCG strains).
- Rapidly and accurately identify Mycobacteria to species level when they are isolated by liquid culture or on solid media.
- Primary detection of *Mycobacterium spp.* from a range of clinical samples, in particular CSF, tissue and respiratory samples. (This is always in addition to culture).

Enterobacteriaceae

The Enterobacteriaceae are a large family of gram negative rods that include a number of pathogenic species e.g. *E. coli*, *Shigella sp.*, *Klebsiella sp.*, *Enterobacter sp.* and *Salmonella sp.* Many members of the family are part of the normal human gut flora but can also cause a range of significant infections ranging from sepsis to joint infections. They are of particular interest in our patient population as they are a common cause of neonatal sepsis and meningitis.

Molecular methods can be used to detect Enterobacteriaceae DNA directly from clinical specimens. This assay is primarily for us on culture negative samples for diagnosing infection with bacteria in this family. Additionally the assay can be used to further identify Enterobacteriaceae detected by the broad-range 16S rDNA PCR as sequencing of the *dnaK* target provides better discrimination and can identify many members of this family to genus level.

Tests, sample volumes, containers and turnaround times - Molecular Microbiology

Please note that the stated times until results are available are a guide only.

Investigation	Container	Min. volume	Turnaround Time
Broad-range bacterial 16S rDNA PCR & sequence – sterile site fluids	Any sterile, dry container (no added fluids or transport media).	0.5ml	<7 days
Broad-range bacterial 16S rDNA PCR & sequence - tissue	Any sterile, dry container (no added fluids or transport media)	50 mg	<7 days
<i>Bordetella pertussis</i> (whooping cough) PCR	NPA or pernasal swab		24 hours
Specific bacterial real-time PCR	Any sterile, dry container (no added fluids or transport media) EDTA blood		<7 days

Department of Microbiology location and contact details are [here](#).

The range of specific diagnostic PCRs offered by ourselves and other reference laboratories is increasing, we are happy to discuss the availability of diagnostic molecular tests whenever appropriate.

Please note the 24 hour turnaround at GOS and Reference Laboratories is not available at weekends. Specimens received after 16:00 Thursday may not have results available until the following Monday or Tuesday.

References

Forms and documents are available on the hospital intranet (GOSweb) and Qpulse, the Trust's Quality Management System
Clinical guidelines are also available on the hospital website <http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/>

Forms

Antibiotic Assay Request Form (External users only)	http://www.labs.gosh.nhs.uk/media/760961/RequestFormAntimicrobialAssays.pdf
PCR Request Form	http://www.labs.gosh.nhs.uk/media/760967/RequestFormPCR.pdf
PIMS downtime form	http://goshweb.pangosh.nhs.uk/corporate/ict/Getting_help/PIMS_Information/PIMS_downtime_procedures/Documents/Forms/AllItems.aspx

Documents

Patient Identification Policy	http://goshweb.pangosh.nhs.uk/search/Pages/results.aspx?k=patient%20identification%20policy&s=All%20Sites
Accepting Unlabelled and Mislabelled Samples Policy	http://goshweb.pangosh.nhs.uk/document_library/_layouts/OSSSearchResults.aspx?k=unlabelled&cs=This%20Site&u=http%3A%2F%2Fgoshweb.pangosh.nhs.uk%2Fdocument_library
AQU 013 Laboratory User Satisfaction and Complaints Procedure	Available on Qpulse
Trust Complaints Policy	http://goshweb.pangosh.nhs.uk/document_library/_layouts/OSSSearchResults.aspx?k=complaints%20policy&cs=This%20Site&u=http%3A%2F%2Fgoshweb.pangosh.nhs.uk%2Fdocument_library
Information Governance Policy	http://goshweb.pangosh.nhs.uk/document_library/_layouts/OSSSearchResults.aspx?sq=1&k=information%20governance%20policy&cs=This%20Site&u=http%3A%2F%2Fgoshweb.pangosh.nhs.uk%2Fdocument_library
PHE User Manual	https://www.gov.uk/government/publications/bacteriology-reference-department-brd-user-manual

Clinical Procedure Guidelines	
Blood Tests: requesting, labelling and sampling requirements	http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/blood-tests-requesting-labelling-and-sampling-requirements/
Blood Sampling, Neonatal Capillary	http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/blood-sampling-neonatal-capillary/
Blood sampling from central venous access devices (CVADs)	http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/blood-sampling-from-central-venous-access-devices/