PATHOLOGY

Title	Clinical Microbiology User Handbook		
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Owner	**Dr F Lim**	**Author**	**Daxa Patel**

University Hospitals of Leicester
NHS Trust

Caring at its best

Clinical Microbiology User Handbook

A user guide for UHL Clinical Microbiology Pathology services

1 Contents

2 Purpose of Document

This handbook is intended to serve as a quick user guide to the services available from the Clinical Microbiology Laboratory of the University Hospitals of Leicester NHS (National Health Service) Trust. It is aimed at all staff groups involved with the Microbiological investigations. It is reviewed on an annual basis, or sooner where significant changes are made to the service.

3 Measurement Uncertainty and Risk Management

Within any laboratory process or procedure there will always be a degree of variability. These will vary with specimen type and test. Test-specific information on the processes we use to minimise this variability is available on request.

4 Overview of Services

The Clinical Microbiology Laboratory is part of Pathology service within the University Hospitals of Leicester NHS Trust. The postal address is:

Clinical Microbiology
University Hospitals of Leicester NHS Trust
Level 5 Sandringham Building
Leicester Royal Infirmary
Infirmary Square
Leicester LE1 5WW

DX number: 6770100
Exchange: LEICESTER 90 LE

The Laboratory provides the following services:

- Diagnostic services for hospital clinical staff and general practitioners working in the community.
- Antibiotic stewardship and infection management advice.
- Support to Consultants in Communicable Disease Control and their colleagues UKHSA (UK Health Security Agency) (Formerly Public Health England).
- Local surveillance and special studies in infectious disease.
- Investigation and support in community and national outbreaks of communicable disease.
- Independently funded clinical and other related services.

The patients' well-being, safety and rights are our primary consideration. The laboratory achieves this by treating each specimen with a quality centred approach from specimen receipt to final report.

The Clinical Microbiology Laboratory is situated at the Leicester Royal Infirmary (LRI). Some urgent SARS-CoV-2 testing is also undertaken at Glenfield Hospital (GH). The services provided within the department are Bacteriology, Virology / Molecular, Mycology & Parasitology. Please note that examination of blood samples for malarial parasites is undertaken by Haematology.

5 Quality & Governance

The Microbiology Laboratory participates in a full range of National Quality Assurance Schemes. It is a UKAS accredited medical laboratory No. 8605, currently accredited to ISO 15189:2012. Evidence of accreditation to this standard, along with details of our Schedule of Accreditation outlining the scope of work for which have been assessed is available at:
https://www.ukas.com/download-schedule/8605/Medical

5.1 Complaints Procedure

A complaint may be made via any normal means of communication:

- To the NHS Trust – normally via the Patient Information Liaison Service (PILS), who will direct relevant aspects of any complaint to the laboratory. PILS can be contacted on 0808 178 8337 or pils@uhl-tr.nhs.uk.
- To the laboratory directly via 0116 2586542 – normally to the Head of Service or General Manager.

5.2 Confidentiality

The laboratory is committed to maintaining patient confidentiality and practices Caldicott principles. At times this will mean that electronic communications (phone or email) to and from the laboratory may be constrained by protocols intended to preserve patient confidentiality. These controls will be in accordance with professional and regulatory guidance.

Microbiology sample requests are an agreement between the service and the user for the testing and confirmation of the infection markers indicated.

6 Key Personnel

- Head of Service / Consultant – Dr F. Lim
- General Manager (12-month secondment from September 2024) – Abigail Thornbury
- IT Manager – Karan Gorania
- Scientific Laboratory Manager (Bacteriology), Health & Safety – vacant (Purvi Shah appointed but on Maternity Leave and will officially commence in post on October 21st 2024)
- Scientific Laboratory Manager (Virology) - vacant
- Quality & Service Improvement Manager – Daxa Patel

- Quality Leads – Judi Gardener, Karan Gorania
- Health and Safety Lead – vacant (Arjun Bhatt appointed but will officially commence in post on October 21st 2024)
- Training Lead – Richard Halliwell
- Consultants

Microbiology: Dr S.S. Bukhari, Dr D. Jenkins, Dr S. Koo, Dr F. Lim, Dr R Saunders, Dr J. Veater, Dr D. Modha, Dr G Hill

Virology: Dr J. Tang, Dr O. Toovey

Professor of Clinical Microbiology and Honorary Consultant: Prof. M. Barer

7 Opening Hours and Telephone Numbers

Laboratory Hours – Non-Medical Staff (normal working for 'routine' work)

	Virology	Bacteriology
Monday – Friday	**07:00 – 22:00**	**08:00 - 20:00**
Saturday	**07:00 – 22:00**	**08:00 - 20:00**
Sunday	**07:00 – 22:00**	**08:00 - 20:00**

The general contact number (automated service) which can be used to access all Clinical Microbiology Departments is: **0116 2586542 (ext. 16542 from within UHL)**

Urgent Bacteriology Samples	**16520**
Virology Department	**16522**
Medical Staff (for advice)	**Refer to Section 8**

Monday to Friday, please ensure that Bacteriology specimens arrive in the laboratory before 15:30 (wherever possible) to ensure adequate time for microscopy and culture preparation.

Respiratory samples for Virology are expected to be reported within 24 hours of receipt in the laboratory.

Please telephone the laboratory on 16520 (immediate/urgent bench) to warn of the arrival of any urgent Bacteriology specimen during normal working hours, and 16522 for any urgent Virology specimens during normal hours.

Outside these hours for urgent Bacteriology specimens, please contact the on-call Microbiology technician – Biomedical Scientist (BMS) via the LRI switchboard.

[*see page:* urgent specimens/out of hours specimens*]*

For all results please refer to ICE or Nervecentre – do NOT contact the laboratory (unless exceptional circumstances e.g. urgent result not available on ICE or Nervecentre)

Results enquiries are available until 17:00 on weekdays and on weekend mornings. Please note there is no results enquiries service outside normal laboratory hours.

8 Results Telephoned to Clinicians by Medical Staff

The following results are telephoned to clinicians by the Microbiology Medics (or Biomedical Scientist):

Bacteriology

- **Blood Cultures:**
  - Potentially significant blood culture Gram stain results
  - Significant positive blood culture results if they require a change in patient management or further microbiology input
- **CSF Samples:**
  - Positive CSF microscopy results (Biomedical Scientist)
  - Significant positive CSF culture results
  - Cryptococcal antigen positive results
- **Eye Samples:**
  - Positive vitreous fluid cultures results
  - Acanthamoeba positive results
  - Corneal scrapes with positive fungal results
  - Positive eye sample results for *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*
- **Respiratory samples for inpatients:**
  - Positive *Legionella* culture or urinary antigen results
  - Positive *Bordetella pertussis* culture results
  - Smear-positive or PCR-positive TB sample results
  - *Pneumocystis jirovecii* PCR positive results
- Outside the normal working hours of the Infection Prevention and Control team, any new result that requires a change in source isolation precautions

Virology

- **Positive CSF Viral PCR results**
- **Positive Viral PCR eye swab results** where it is not evident from ICE that the patient is on appropriate treatment
- **Acute Hepatitis A, B and E**

9 Medical Advice

Laboratory Hours – Medical Staff		
	Normal Working Hours	**On-Call Microbiologist**
Monday – Friday	**09:00 – 17:00**	**17:00-09:00**
Saturday – Sunday		**All day**

Bank Holidays		All day

Advice on patient diagnosis, specimens to be taken, the interpretation of results and the use of antibiotics can be obtained from the laboratory during normal working hours (see contact details above). An updated copy of the on-call rota is kept on Medirota and accessed by UHL switchboard.

Please note that many queries regarding empiric antimicrobial prescribing are addressed on UHL Microguide Website or for GPs – Leicester, Leicestershire and Rutland: Antimicrobial Policy and Guidance for Primary Care' produced by Leicester, Leicestershire and Rutland Area Prescribing Committee
- please check the appropriate sites BEFORE contacting Microbiology.

UHL Microguide Website

Leicester, Leicestershire and Rutland Area Prescribing Committee page

9.1 During Normal Working Hours

For clinical microbiology advice on patients who do not have red flag sepsis please send a service request for Microbiology Clinical Advice on ICE between 09:00 and 16:00. Please provide a clear summary of the patient, the issue you wish to be addressed and your direct contact details (mobile phone or bleep number). These requests are triaged and we aim to respond within 4 working hours. Please note that requests must be made prior to 16.00 in order to facilitate a same-day response. If you need same-day Microbiology advice between 16:00 and 17:00, or urgent advice (e.g. red flag sepsis) between 09:00 and 17:00, please ring the Microbiology Doctors on **07811 024829**.

9.2 Out of Hours

Please contact the UHL Switchboard and ask for the “On Call Microbiology Registrar”. This will be either a Registrar or a Consultant. In most cases it would be appropriate for a clinician of similar grade to seek advice. After midnight, calls should only be made by doctors of ST3 grade and above.

Before you phone, please make sure you are aware of the patient's medical history, including details of any antibiotic therapy over the past 2 weeks and the results of any microbiological investigations (please see check list below).

When contacting the on call medical microbiologist, please state the following:

- Your name
- Your grade
- Your bleep or mobile number and where you are calling from
- Please also make sure you have the following information available:
  - The reason for your call
  - The patient identifiers (name, date of birth, hospital number)
  - Any recent procedures (including insertion of vascular catheters).
  - Current antibiotic regimen and previous antibiotic courses during this inpatient stay (if relevant)
  - Relevant microbiology (blood cultures, swab results, MSU results, CSF counts and culture results)
  - Any known allergies
  - Current blood results including renal function

Please be aware that the ‘on-call’ microbiologist does not have access to laboratory results out of hours.

Please note that Cyclosporin, Gentamicin, Tobramycin, Vancomycin, Itraconazole, Posaconazole and Voriconazole assays are undertaken by Blood Sciences department and are reported after the test is complete. Most questions regarding interpretation of results are answered on the UHL Microguide website:

UHL Microguide Website

10 Urgent Specimens & 'Out of Hours' Specimens

- **During working hours** – All non-COVID-19 specimens are processed at the Leicester Royal Infirmary site. Before any urgent specimens are sent, the laboratory must be phoned on extension 16520 for Bacteriology and 16522 for Virology.
- **Urgent COVID-19 only** samples from GGH are tested at GGH - ring 13805 if necessary.
- Please write a bleep or extension number on the form to allow the staff to contact you with the result.
- **Mark or flag the request as 'URGENT' on the request form/electronic request.**
- It is the requestor's responsibility to arrange transport of the specimen/s out of hours for delivery to Clinical Microbiology Level 5 Sandringham Building LRI. Indicate on the specimen bag "For delivery to Microbiology Level 5 Sandringham"

10.1 Bacteriology

Outside normal working hours, at weekends and on Public Holidays the on-call "Microbiology Technician" (Bacteriology Biomedical Scientist (BMS)) **must** be contacted via the switchboard **once the specimen has been obtained**.

Out of hours examinations are offered for the following specimen types: CSFs (Cerebrospinal Fluid), PD fluids, urgent surgical specimens (e.g. aspirates - joint, ascitic and pleural fluids), tissues, broncho-alveolar lavages and corneal scrapes. Please note PD fluids, joint fluids and ascitic fluids are not routinely tested after midnight. Ascitic fluids may be screened for evidence of infection (raised white cell count) using urinary dipsticks. If these are required to be processed after midnight, contact the doctor on call for Microbiology.

In general, urine, sputum, faeces and most swabs do not require out of hours examination. Urine dipsticks that detect bacteria by the nitrite reaction and white cells by leucocyte esterase are available on the wards. Out of hours urine samples should be collected before antibiotic therapy is started, refrigerated and sent to the laboratory the next day. If urine samples are required to be processed before midnight, for children under the age of 3 years contact the on call "Microbiology Technician" (Bacteriology BMS). After midnight and for patients older than 3 years of age, contact the Microbiology registrar on call to confirm that this is appropriate.

SHOULD YOU WISH TO DISCUSS A CLINICAL PROBLEM A MEMBER OF THE MEDICAL STAFF IS ALWAYS ON CALL.

Please be aware that the 'On-call Microbiology Technician' (BMS) does not have access to laboratory results out of hours. All authorised results can be viewed on ICE, Nervecentre or via GP LIMS (Laboratory Information Management System).

10.2 Virology

There is no Virology on-call service at UHL and out-of-hours testing for organ donors is covered by UKHSA Birmingham or UKHSA Cambridge, Urgent testing for renal transplant recipients is offered during normal working hours – please telephone Virology with details and mark the sample as urgent.

Please note that the Bacteriology BMS staff are unable to process Virology specimens.

11 Request Forms and Specimen Collection

Please keep clinical details brief and include correct antibiotic treatment.

For most routine laboratory procedures, consent will be inferred when the patient sample presents in the laboratory with a correctly filled out request form. Patient consent will be assumed when further testing is requested by telephone or a follow up request form. If the sample is from the source of a needlestick injury, consent for HIV testing will be assumed unless the request form explicitly states otherwise.

11.1 Add-on Tests

Further tests can be added verbally to specimens already in the lab, BUT the laboratory MUST be contacted and a written request should be forwarded to the lab as soon as possible stating the required test and recording of the name of the laboratory personnel spoken to. Following telephoned requests to add on tests, the laboratory staff can compile a request form if sufficient details are provided (see details required in section 10.2)

The time limit for requesting additional tests is at the discretion of Medical staff from Clinical Microbiology and depends on the clinical scenario discussed.

The primary microbiology samples are generally retained for 7 days. If a sample is rejected (due to no test requested, no requesting location or insufficient patient identifiers) samples will be retained for 3 days. Requests for additional testing cannot be accommodated after this time.

The time limit for additional testing on archived serum samples is 6 months, but this be extended at the discretion of the Consultant Virologist.

11.2 Completion of Request Forms

Please use electronic requesting where available.

Personnel who have undertaken ICE training should complete the forms. However, if ICE is not available or the test you wish to request is not on the ICE, please use a manual form or phone the laboratory for further information.

In all cases please supply the following information:

- **Patient's Full name (Forename(s) & Surname)**
- **Hospital 'S' number (or NHS number)**
- **Date of Birth**

- **Sender's name (consultant), address and contact number (bleep number or telephone number)**
- **Date and time of collection of specimen**
- **Date of onset of symptoms, vaccination, contact etc**
- **Full clinical and epidemiological details including recent travel and case contact history**
- **Indicate tests that are required**

Please ensure that **ALL SECTIONS** are completed.
Repeatable samples will not be accepted without at least 2 patient identifiers (e.g. full name (not forename initials or nickname (e.g. Jim instead of James), date of birth and NHS or S number) or without a legible requesting location.

11.3 Hazardous Pathogens

Specimens which are known or suspected to contain hazardous pathogens from patients with typhoid fever, tuberculosis, HIV, hepatitis or blood cultures where patients have had foreign travel outside of Northern Europe should be labelled "HIGH RISK" or with "DANGER OF INFECTION" stickers and placed in biohazard bags.

11.4 Bacteriology/Parasitology/Mycology

Electronic request forms (ICE) **MUST** be used where available. If not available then forms for bacteriology are blue printed on white. Requests for non-viral serology (including syphilis) should be sent to Virology.

Please give clinical details and indicate the antibiotics used/anticipated to enable optimum processing in the laboratory.

Inadequate clinical details may result in inappropriate tests being performed or delay in processing.

Failure to clearly label the form and /or specimen with patient identifiers and sender may result in the specimen being discarded.

11.5 Virology/Molecular

Electronic request forms (ICE) **MUST** be used where available. If not available then forms for "Virology" are printed black on white.

Please give clinical details and date of onset to enable optimum processing in the laboratory. Inadequate clinical details may result in delays and inappropriate tests being performed or delay in processing.

Failure to clearly label the form and /or specimen with patient identifiers and sender may result in the specimen being discarded without testing.

Antenatal screening samples for Infectious Diseases in pregnancy (IDPS) must be sent using the specific Antenatal request form and in addition to the details required as stated above, must also include the name of the person taking the specimen.

12 Specimen Collection - General

Specimen container **MUST** be clearly labelled with:

- **Patient's Full name (Forename (s) & Surname)**
- **Hospital 'S' number (or NHS number)**
- **Date of Birth**
- **Date and time of collection**

These details must match those on the accompanying form, or testing will not be performed.

To enhance survival of microorganisms during transport to the laboratory, specimens should be collected in the appropriate container (see below) and should reach the laboratory as quickly as possible - overnight storage of specimens may lead to loss of some organisms and overgrowth of others.

If a delay of more than a few hours is expected, microbiology specimens should be refrigerated (except blood cultures or Quantiferon tubes - these MUST NOT be refrigerated) and sent to the laboratory as soon as possible.

It is important that all specimens are clearly labelled as most unlabelled/unidentifiable specimens are discarded.

Please ensure that lids on containers are tightened and that specimens are packaged adequately to prevent breakage during transport. Leaking specimens may be discarded. Samples should be placed in a sealable bag and accompanied with the request form.

For the safety of laboratory staff it is essential that specimens which are known or suspected to contain hazardous pathogens (e.g. patients with typhoid fever, tuberculosis, hepatitis or blood cultures where patients have had foreign travel outside of Northern Europe) should be labelled as "**HIGH RISK" or with "DANGER OF INFECTION"** stickers and placed in biohazard bags and that the "High Risk" flag is indicated on the request form. A list of hazardous pathogens and clinical conditions can be located on the 'Hazardous Pathogens and their Associated Conditions' section. Completed forms MUST NOT be placed within the same bag/pouch as the sample apart from Blood Cultures sent in a Blue bag.

12.1 High Risk Specimens

A High Risk specimen is any specimen whether suspected or known to contain hazardous pathogens. Examples of hazardous pathogens include hepatitis B and C, HIV, HTLV, CJD and the causative agents of tuberculosis and typhoid fever. For a complete list refer to the Leicestershire Control of Infection Guidance.

All blood cultures from patients with foreign travel outside Northern Europe in the preceding 3 months are considered high risk specimens.

Cases of suspected Viral Haemorrhagic Fever and other Hazard Group 4 infections MUST be discussed with a consultant in Infectious Diseases before collection of specimens.

All High Risk specimens **MUST** be transported in the sealed section of a **biohazard bag**.

The request form **MUST** be completed with full clinical information (details of foreign travel is essential), the date of onset, also indication of a high risk flag and placed in the outer (open) pocket of the biohazard bag.

Both the sample and request form should be labelled "High risk or with Danger of Infection" sticker.

12.2 SARS-CoV-2 PCR samples

Swabs in Virus Transport medium and other samples for SARS-CoV-2 PCR testing should be transported in the sealed section of a specimen bag. The request form must NOT be placed in the sample section of the bag and should be folded to allow the test required to be read prior to opening the sample bag. This ensures the safety protocols in place for opening the samples can be followed for the protection of the laboratory staff. Request forms with Rapid stickers for agreed rapid pathways will be tested urgently.

13 Specimen Transportation to Microbiology

All specimens should be correctly labelled and packaged and accompanied by a fully completed request form.

Any incidents during the transport of the specimen to the laboratory that might affect the quality of the specimen or the safety of the personnel must be reported.

For arrangements for sending urgent specimens see 'Urgent Specimens & 'Out of Hours' Specimens' section.

13.1 Delivery of samples

13.1.1 Delivery of diagnostic specimens from internal Hospital areas

It is recognised that hospital staff transporting specimens to the Pathology laboratories are usually not managed by Pathology UHL. The following systems are to be adopted by Hospital Staff; serious breaches to be reported to relevant Managers and Datix reported.

Process to follow:

- All specimens to be carried upright in trays and a secondary bag or in individual sealed leak proof bags. The specimens are to be in a separate pocket to request form to avoid accidental contamination of form. Known or query high risk; hazard group 3 or derogated group 3 as Advisory Committee Dangerous Pathogens (ACDP) classification should be delivered in a clearly labelled leak proof biohazard bag with request form labelled appropriately with relevant information for 'Danger of infection' biological risk. This may also include relevant clinical details e.g. travel abroad, febrile etc. All specimens should be transported on/in an appropriate trolley and tray or receptacle that would contain leaks and spills. It is recommended that all trolleys used for conveyance of specimens have available spill kits, including an approved disinfectant and absorbent mopping up material.
- Leaking specimens should not leave the treatment area and should be immediately retaken.
- Specimens should be transported in such a way e.g. in a container or box, to maintain patient confidentiality.
- All specimens to be taken directly from source (or distribution route) to laboratory or laboratory specimen reception area so to be delivered in a timely manner.
- If a specimen leaks into the tray or box, report to the nearest Pathology laboratory reception for assistance if required.
- If a specimen is dropped and spilt, and if a spill kit is not readily available; it must not be touched or left unattended. Send a messenger to the nearest Pathology laboratory reception for assistance.
- Human tissue specimen containers may contain varying quantities of 4 to 10% Formalin. If spilt and not contained, contaminated areas should be cleared and if safe to do so mopped with absorbent material and then mopped up with copious water. For major spills (> 250 mls) after the area has been

cleared the local Histopathology Laboratory should be called for advice and assistance as Personal Protective Equipment and Formalin spill kits may be required for clean-up.

13.1.2 Transportation and delivery of diagnostic specimens by road

All Diagnostic/Biological specimens transported to and from the Pathology Laboratories by road are considered to be Biological substances **Category B** status or below, this is Hazard group 3 pathogens or lower when referencing to ACDP categorisation of Pathogens.

Category A (Hazard Group 4) specimens are not knowingly transported and request for transportation of these would be refused and reported to Pathology Stores/ Logistics line manager or Operational /Deputy Service Manager ASAP. If driver becomes aware that they are carrying such specimens during transit then they are to report this to the Manager as stated. The specimens will be delivered to the Pathology Specimen reception, Level 2 Sandringham Building at LRI, where Notification of Viral Haemorrhagic fever or other Hazard Group 4 procedure , see section 4, will then be invoked.
See: Risk Assessment IN 1478 Pathology Specimens Transport Service
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- All diagnostic specimens must be enclosed in the appropriately labelled bags and transport boxes - these must not be opened unnecessarily by the driver. The laboratory follows UN 3373 (P650 packaging instruction) as European Agreement Carriage of Dangerous Goods by Road (ADR) as regulated by Carriage of Dangerous Goods Regulations as amended.
  Essentially the packaging shall be of a good quality, strong enough to withstand the shocks and loadings normally encountered during transport and have correct UN3373 label.
  The packaging shall consist of 3 main components:
  - •A primary receptacle.
  - •A secondary packaging (sealed bag with absorbent material).
  - •A rigid secure outer package.

- Specimens to be taken <u>directly</u> from route completion, to the appropriate laboratory reception area.

- In the event of a vehicle breakdown or accident, or incident with the conveyance; the specimens must not be touched by unauthorised personnel. Procedures for leakage and spillage are in place with spill kits available. The Pathology Stores /Transport Managers will be informed immediately.
  See *Risk Assessment IN1478 Pathology Specimens Transport Service*

13.1.3 Sending Samples within the LRI Site

Please use the air-tube system if you have a port in your department (see below). The air-tube port in Microbiology is number 5. Other specimens will be collected by the clinical distributor service.

<u>When using the Air tube system</u>:

- No glass containers
- No samples on ice
- No High Risk samples
- No suspected SARS-CoV-2 respiratory samples

NOTE: Blood cultures <u>can</u> be sent via the air tube

13.1.4 Sending Samples from Leicester General or Glenfield Hospital Sites

During “normal working hours” Clinical Distributor staff will deliver your specimens to pathology reception. From there, hourly van services operate to the Royal Infirmary. Outside normal working hours, urgent transport is arranged by the requesting clinician.

During busy periods, including the COVID pandemic, volunteer services such as Blood Bikes, may be utilised to assist in delivery of samples to LRI, but this will be organised in conjunction with Microbiology.

13.1.5 Sending Samples from Non-UHL Sites

From Monday to Friday a daily van collection service operates between GP surgeries and Health Centres to the Royal Infirmary. Other sites will organise their own transportation arrangements.

Samples for Microbiology should be sent in the blue plastic bags provided, and purple or blue and white striped bags for Antenatal.

13.2 Guidance on Transportation of Samples to Microbiology

13.2.1 Samples for Bacteriological Investigations

Sample type	Transport requirements	Exceptions or comments
Blood Cultures	Don’t fridge. Send urgently. Ideally processed within 4 hours of collection	
CSF	Send urgently, ideally to allow microscopy within 2 hours of collection. Don’t fridge prior to microscopy	
Faeces for Culture or PCR	If processing is delayed, store refrigerated, rather than at room temperature (RT)	pH changes during storage (even in fridge) - may affect *Shigella* culture
Faeces for CDT	If processing is delayed, store refrigerated, rather than at room temperature (RT)	
Sellotape Slides	May be stored at RT or refrigerated for up to 48 hours before testing	
Eye Samples Genital Swabs MRSA Screens Cannulae/Tips Ear Swabs Mouth Swabs Throat Swabs LRT Samples - TB CRO screen (rectal swabs)	Test as soon as possible after collection. If processing is delayed, store refrigerated, rather than at (RT)	For *Neisseria gonorrhoeae* culture, ideally inoculate plates at collection and incubate as soon as possible. Eye samples for amoebae should be processed within 8 hours and stored at RT if delayed.

Sample type	Transport requirements	Exceptions or comments
Pus/Wound Swabs Tissue Biopsies Bile Nose Swabs	Test as soon as possible after collection. If processing is delayed, store refrigerated, rather than at (RT)	Longer transport time affects viability of anaerobic bacteria. Test bile within 3 hours of collection ideally. Larger sample size helps preserve anaerobes
Fluids from Normally Sterile Sites	Test as soon as possible after collection, within 4 hours if acute infection is suspected. If processing is delayed, store refrigerated, rather than at (RT)	
Urine Samples	Samples without Boric Acid should be processed within 4 hours or refrigerated for up to 48 hours. In Boric Acid, urine may be processed up to 96 hours from collection if refrigerated.	Urine for *Schistosoma* should be tested as soon as possible after collection. If processing is delayed, store refrigerated, rather than at (RT)
Skin/Hair/Nails for superficial mycoses	Keep sample dry and at RT	
Blood Samples for Quantiferon testing	Samples should be kept between 22 ± 5◦C and arrive a maximum 16hrs post venepuncture.	Do not refrigerate
Blood samples for antibiotic and antifungal levels	Samples may be sent by post. Transport time must be <7 days. Samples should be kept at 4°c if there is a delay in sending	Sample type for Isoniazid testing is whole sodium oxalate blood, is sent by post and must arrive at testing lab within 5 days of collection
Samples for Galactomannan Ag	Serum samples: Unopened samples can be stored at 2-8°C for up to 5 days prior to testing. Bronchial alveolar lavages (BALs): BAL fluid samples must be uncontaminated with fungal spores and/or bacteria. Transport and store samples in sealed tubes, unexposed to air.	

13.2.2 Samples for Virological investigations

Sample type	Transport requirements	Exceptions or comments
Clotted Blood for Serology Testing	The centrifugation step may occur up to 24 hours post draw. Test specimens as soon as possible after collecting. Store processed specimens at 2–8°C if not tested within 24 hours of collection. Samples maintained at room temperature for up to 2 days or refrigerated up to 7 days demonstrated no qualitative differences.	From ADVIA Centaur kit inserts From Diasorin Liaison XL kit inserts

	Blood should be collected aseptically by venepuncture, allowed to clot, and the serum separated from the clot as soon as possible. If the assay is performed within seven days of sample collection, the samples may be kept at 2-8°C.	
Whole blood (EDTA) for BBV viral load testing	Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of collection.	
Swabs in Virus Transport medium for respiratory PCR, including SARS-CoV-2 PCR	Process as soon as possible after collection, storage at 5-25ºC is acceptable.	
Urine for Legionella/ Pneumococcal Ag	Storage at RT for up to 24 hours, or refrigerated up to 1 week prior to testing	
Genital swabs for STD NAAT testing	Transport and store the specimen in the Aptima swab specimen transport tube at 2°C to 30°C for up to 60 days after collection, or in VTM at 2-8ºC for up to 3 days before testing.	
Faeces for viral antigen testing (norovirus, rotavirus or adenovirus)	Process as soon as possible after collection; store refrigerated if not being tested within 1-2 days.	
BAL, ET aspirates	Test as soon as possible after collection. If processing is delayed, store refrigerated, rather than at (RT)	
Tissue biopsies	Test as soon as possible after collection. If processing is delayed, store refrigerated, rather than at (RT)	

A more comprehensive list of Virology samples available in section 19 'VIROLOGY/MOLECULAR TESTS AVAILABLE'.

13.3 Guidance on the Taking of Samples

Procedures associated with taking samples are based on guidelines produced by Public Health England (UKHSA) Standards for microbiology investigations (SMI). These guidelines are the minimum criteria for the taking of samples.

https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

See test/sample specific pages for further details.

13.4 Disposal of Specimen Collection Equipment

Following collection of patient samples of all types, equipment used must be disposed of according to UHL Waste Disposal policy.

http://insite.xuhl-tr.nhs.uk/homepage/management/corporate-directorates/facilities/waster-and-recycling/waste-types

14 Turnaround Times (TAT)

The turnaround times quoted for the most commonly requested tests are from receipt of specimen in the laboratory to the production of the final report. These vary greatly, depending on the tests requested and whether confirmatory tests are required. Positive culture results may take longer due to antimicrobial susceptibility testing required.

The times quoted are those that are normally expected for the majority **(95%)** of our specimen workload processed during normal working days. The TAT quoted is expressed in normal working days. Tests sent to reference laboratories are marked with an asterisk (*). The turnaround times for reference laboratory tests may be longer than stated due to delays encountered at the reference laboratory or by the specimen transport system, especially over weekends or bank holiday periods. The TAT provided by the referral laboratories (from receipt to reporting) is monitored by the Microbiology Quality Team.

If you have any issues with these expected Turnaround Times, please feel free to contact the Microbiology General Manager to discuss them further.

In the following table, tests/samples in **BOLD** font are those for which normal working days are 7 days a week. For all other tests, normal working days are only Monday to Friday. Please take this into account when waiting for results. (# for Respiratory Virus screens, 7-day testing is performed during the winter season, with 6-day (Monday to Saturday) testing during summer)

NOTE: Testing of post-mortem tissues for Virology has an overall TAT of 8-10 working days due to the range of PCR tests required.

Test	Turnaround Time (Normal Working Days)
Aspergillus Galactomannan Antigen	5 days
Aspergillus PCR	6 days
Antibiotic levels: Amikacin levels Teicoplanin levels Colistin, Cycloserine, Daptomycin, Ethambutol, Flucytosine, Rifampicin, Isoniazid, Levofloxacin, Linezolid, Moxifloxacin and Streptomycin levels Pyrazinamide levels	 * 1 day * 2 days *3 days *7 days
Bacterial 16S DNA by PCR	5-8 days
B. cepacia culture	6 days
Beta D Glucan (BDG)	5 days
Blood Cultures	**5 days**
Blood cultures (Paediatric)	**5 days**
Bordetella pertussis Antibody Screen	*14 days
Borrelia burgdorferi IgG Screen (see also Lyme Disease)	5 days
Brucella serology	* 9 days
Chlamydia trachomatis and *Neisseria gonorrhoeae* (GC) DNA by NAAT LGV DNA by PCR	5 days *8 days
***Clostridioides difficile* toxin**	**Next day**
Cryptococcal Antigen	5 days
Cryptosporidia and Giardia antigen	??
CSF Microscopy	**1 day**

Test	Turnaround Time (Normal Working Days)
Cytomegalovirus (CMV): IgM Antibody IgG Antibody DNA by PCR CMV IgG Avidity - only tested after discussion with the Duty Virologist	 5 days 5 days 6 days 3 days
Discharge (HVS)	5 days
Enterovirus: RNA by PCR	 3 days
Epstein-Barr Virus (EBV): EBNA IgG Antibody VCA IgM Antibody VCA IgG Antibody DNA by PCR	 5 days 5 days 5 days 5 days
Faeces Culture	4 days
Fluids and Tissues (Special Cultures)	**6 days**
Fungal Culture if Microscopy Positive (GP Specimens)	14-21 days
Fungal Culture (Dermatology Specimens)	14-21 days
Fungal culture (non-skin/nails)	10 days
Fungal Microscopy	7 days
Atypical Pneumonia Serology	Not currently available
Haemorrhagic Fever (*Discuss with Medical Virologist)*	*16 days
Hepatitis A Virus (HAV): **IgG Antibody** **IgM Antibody**	 **2 days** **2 days**
Hepatitis B virus (HBV): **Hepatitis B Surface Antigen** **Hepatitis B Surface Antibody** **Hepatitis B e Ag/Ab** **Hepatitis B Core Antibody** **Hepatitis B Core IgM** HBV DNA by PCR Antenatal HBsAg Testing and Confirmation/Marker Testing Dried Blood Spot (DBS) Testing for HBsAg	 **3 days** **3 days** **3 days** **3 days** **3 days** 15 days 5 days 5 days
Hepatitis C virus (HCV): **Antibody Screen** Confirmation of Reactive Samples HCV RNA Quantitation (including Vertical Transmission) HCV Genotype Dried Blood Spot (DBS) Testing for HCV Ab Dried Blood Spot (DBS) Confirmation of HCV Ab Reactive Samples	 **2 days** 7 days 8 days *12 days 14 14
Hepatitis D (delta) Virus (HDV): IgM Antibody/Total Antibody	 *28 days
Hepatitis E virus (HEV): IgG Antibody IgM Antibody	 5 days 5 days
Herpes Simplex Type 1 & 2: HSV Serology DNA by NAAT (genital swabs only) DNA by PCR (non-genital samples) – see Non-respiratory virus panel	 5 days 5 days

Test	Turnaround Time (Normal Working Days)
HTLV-1 & 2: Antibody Screen Confirmation of Reactive samples	 5 days *10 days
Human Herpes Virus (HHV) 6,7 and 8 DNA by PCR	 *3 days
Human immunodeficiency virus (HIV-1 & 2) **Antibody Screen:** Further Confirmation and Typing Antenatal Screening and Confirmation Quantitative RNA (Viral Load) Proviral DNA (Vertical Transmission) Resistance Profile/Genotype Dried Blood Spot (DBS) Testing for HIV Ab	 **2 days** 7 days 5 days 7 days *23 days *28 days 5 days
IGRA Testing (TB – QuantiFERON)	7 days
Legionella: **Antigen Detection (Urine)** Antibody Screen for Outbreaks ONLY Confirmation of Urinary Antigen Reactive samples *Legionella* Culture	 **1 day** *12 days *14 days 11 days
Leptospira IgM Antibody	*6 days
Lyme Disease (*Borrelia*) IgG & IgM Antibody (confirmation)	*9 days
Measles Virus: IgG IgM antibody	 5 days *6 days
Meningococcal PCR	*4 days
MRSA screen	**2 days**
Mumps Virus: IgG antibody IgM antibody	 5 days 5 days
Mycoplasma genitalium DNA by PCR	*4 days
Non-Respiratory virus panel by PCR (includes HSV type 1, HSV type 2, VZV, CMV, EBV, enterovirus, parechovirus, adenovirus, Human herpesvirus type 6 (HHV6) and human herpesvirus type 7 (HHV7)	3 days
Parasitic Serology: Amoebic Serology (Schistosomal, Filarial, Strongyloides etc.)	 *5 days *10 days
Parasitology (Microscopy)	2-3 days
Parvovirus B19: IgG antibody IgM antibody DNA by PCR	 5 days 5 days *3 days
Pneumococcus: **Urinary Antigen Detection**	 **1 day**
Pneumocystis jiroveci DNA by PCR	*3 days
Pneumonia panel screen by PCR (includes viral and bacterial targets)	5 days
Polyoma (JC or BK) Virus DNA by PCR	*3 days

Test	Turnaround Time (Normal Working Days)
Respiratory Virus Screen: (Influenza A & B, Parainfluenza types 1-4), RSV, Enterovirus, Rhinovirus, Parechovirus, Metapneumovirus, Non-MERS Coronavirus and Bocavirus RNA, Adenovirus DNA by PCR) MERS Coronavirus SARS-CoV-2 RNA by PCR (including as part of RESP-4 panel) SARS-CoV-2 RNA by PCR (Rapid Screen)	**2 days (#)** *1-2 days 1 days 4 hours
Rubella Virus: IgG antibody IgM antibody	 2 days 2 days
Sputum	4 days
Streptococcal Serology (Anti Streptolysin O Titre)	8 days
Swabs for Routine Bacterial Culture	4 days
TB Culture	7-10 weeks
TB PCR (Reference Laboratory)	*10 days
CTB PCR (GeneXpert)	**Next day**
TB Microscopy	**Next day**
Therapeutic Drug Monitoring	*28 days
Toxoplasma: Antibody Screen Confirmation by Dye Test DNA by PCR	 5 days *9 days *3 days
Treponemal (Syphilis) Serology: **IgG Antibody** Confirmation by TPHA (Treponemal Pallidum Haem Agglutination), RPR (Rapid Plasma Reagin) Antenatal Screening and Confirmation IgM	 **2 days** 9 days 5 days *10 days
Trichomonas Culture	2-4 days
Urine Culture	4 days
Urine Microscopy	1 day
Varicella Zoster Virus: IgG antibody DNA by PCR – see Non-respiratory virus panel	 4 days
Viral Gastroenteritis Screen: Rotavirus and Adenovirus Antigen Detection Norovirus Antigen Detection	 1 days 1 days
Viral Haemorrhagic Fevers (e.g. Ebola, Marburg, Lassa, Crimean-Congo Haemorrhagic Fever, Dengue, Yellow Fever, Junin, Machupo, Guanarito, Sabia, etc.): Serology PCR	*2-5 days *Within 1-2 days - after consultation with the Duty Virologist/ Microbiologist and in accordance with current UKHSA Guidance.
VRE/CRO Screen Culture	**2-3 days**
Direct CRO PCR screen	**1 day**

Test	Turnaround Time (Normal Working Days)
Key to abbreviations in table above	
PCR	Polymerase Chain Reaction
NAAT	Nucleic Acid Amplification Test
TPHA	*Treponemal pallidum* Haemagglutination
RPR	Rapid Plasma Reagin
IGRA	Interferon gamma Related Assay (Quantiferon)
CRO	Carbapenem Resistant Organisms
VRE	Vancomycin Resistant Enterococci

15 Reference Laboratory – Referral List

This table covers most of the 'common tests' – information on other testing which can be sourced, but is not provided on-site, may be available on discussion with medical staff.

All referral laboratories used are listed below, along with their UKAS accreditation status, which is checked annually.

Reference Lab	Test	UKAS Accreditation Number
Anaerobe Reference Unit, University of Wales, Cardiff	Anaerobic Identification	9510
Cardiff Toxicology Laboratory, Penarth	Pyrazinamide Levels	8989
CJD Unit, Western General Hospital, Edinburgh	vCJD Testing	
Clinical Microbiology, Queen's Medical Centre, Nottingham.	*Staphylococcal aureus* PVL *Helicobacter pylori* IgG Mycoplasma IgM	8755
Cryptosporidium Reference Laboratory, Swansea	*Cryptosporidia* Genotyping	9510
HSL (Analytics), LLP	HIV-2 PCR	8059
Lab21, Cambridge	Therapeutic Drug Monitoring (HAART)	9325
Leeds General Infirmary, Leeds	Whipples PCR	8105
Leeds Teaching Hospital	TB PCR, Atypical (Primary Samples)	8157

Reference Lab	Test	UKAS Accreditation Number
Leeds UKHSA Regional Laboratory	*Clostridioides difficile* Ribotyping	10883
Micropathology – Coventry	Adenovirus BK virus HHV6 HHV7 HHV8 Mumps PCR HSV (EDTA only) JC virus *Listeria monocytogenes* Parvovirus B19 *Toxoplasma gondii* VZ (Blood only) DNA by PCR, including quantitation if appropriate. HCV genotyping *Acanthamoeba* PCR *Ureaplasma* PCR Mycoplasma genitalium PCR 16S PCR Coxiella burnetii DNA PCR (confirmation)	9622
Mycology Reference Unit – Bristol	Mycology Identification & Cultures Histoplasma and Coccidioides Serology Yeast ID and Sensitivities 18s PCR	8043
NMRS - Birmingham	TB/Mycobacteria ID and Sensitivity	8213
Source Bioscience	16S sequencing	9571
UKHSA Birmingham, Heartlands Hospital	*E. coli* O157 (Cultures only) Measles PCR B. pertussis PCR	8213
UKHSA Bristol	Syphilis IgG confirmation and IgM LGV PCR and serology	8043
UKHSA Colindale	Enteric Bacterial Pathogens (other than *E.coli* O157)	8197
UKHSA Colindale, Food Safety Microbiology Laboratory	*Listeria* Identification	8197
UKHSA Colindale, Lab of HealthCare Associated Infection	*Burkholderia* Identification, Characterisation and Resistance Staphylococcal Identification Difficult and Fastidious Organism ID	8197
UKHSA Colindale, Laboratory of Gastrointestinal Pathogens	*Helicobacter* culture	8197

Reference Lab	Test	UKAS Accreditation Number
UKHSA Colindale, Respiratory and Systemic Infection Lab	*Bordetella pertussis* Culture and Serology Diphtheria Culture and Serology *Haemophilus* Typing Influenza A Typing *Legionella pneumophila* Serology and Confirmation Streptococcal Cultures	8197
UKHSA Colindale, Sexually Transmitted Bacteria Reference Lab	*Neisseria gonorrhoeae* Isolate Confirmation	8197
UKHSA Colindale, Virus Reference Department	Anti-HSV 1 and 2 IgM/IgG HIV IgG Avidity HIV Proviral DNA HBV Genotyping and Resistance Testing HDV Serology and PCR HTLV 1 & 2 Serology Confirmation Poliovirus Serology Measles IgM	8825
Manchester Medical Microbiology Partnership (incorporating UKHSA Meningococcal Reference Unit), Manchester	Meningococcal PCR and Identification of Isolates Pneumococcal PCR HSV type specific IgG CMV Ganciclovir Resistance	8393
UKHSA North Bristol, Southmead Hospital	Amikacin, Colistin, Cycloserine, Daptomycin, Ethambutol, Flucytosine, Isoniazid, Levofloxacin, Moxifloxacin, Rifampicin, Streptomycin, Ethambutol and Teicoplanin Levels	8099
UKHSA West Midlands, Birmingham, Antiviral Resistance Testing Service	HIV RT, Protease and Integrase Resistance Testing	8213
Rare and Imported Pathogens Laboratory (RIPL), Porton Down	Dengue Serology Leptospiral Serology Borrelia serology (confirmation) Rickettsial Serology All Rare/Imported Virus Serology (incl VHF)	9304
Toxoplasma Reference Unit, Swansea	Toxoplasma Dye Test and IgM	9510
University College Hospital (UKHSA National Parasitology Reference Lab), Hospital for Tropical Diseases, London	Amoebic IFAT and CAP Hydatid, *Toxocara*, *Trichinella*, *Trypanosoma*, *Fasciola*, *Filaria*, *Leishmania*, *Schistosoma*, *Strongyloides*, *Taenia* and Malaria Serology Parasite Identification *Entamoeba histolytica* PCR (confirmation)	9702
Brucella reference Lab: Animal, plant and Health agency (formerly AHVLA)	Brucella Isolate speciation	1769

Reference Lab	Test	UKAS Accreditation Number
University Hospital Aintree, Liverpool	*Brucella* Serology	9755

16 Quality Procedures in Place within Microbiology

Several quality procedures are in place within Microbiology which enable us to have confidence in the results which we produce and the assistance they can give in patient management.

These include:

- **Maintenance of UKAS Accreditation**
- **External Quality Assurance (EQA) schemes** - we participate in EQA from UK NEQAS, QCMD (Quality Control for Molecular Diagnostics). BMSmicro and LabQuality (among others) to provide EQA material for all tests where suitable schemes are available. This enables us to compare the results we get for samples sent to us, with other laboratories using the same, or different platforms and methods. We also participate in sample exchange schemes with other UKAS accredited laboratories for tests where no official scheme exists.
- **Internal Quality Controls (IQC)** - these are independently sourced control material which is run with samples on various tests and is monitored to ensure it gives a result in the expected range. Patient results are not released if the IQC results are not acceptable.
- **Internal Quality Assurance (IQA)** - this is a programme of duplicate testing, whereby we re-test from 0.5-1.0% of all our samples to ensure the repeatability of the results. Any significant deviation is investigated.
- **Acceptance Testing** – this is a process for checking that new lots of reagents and kits are performing as expected. We also perform suitability checks on all suppliers (including the referral laboratories), to ensure they offer a high quality, certified or accredited service.
- **Training and Competence Monitoring** – we have an ongoing programme of competence checks for all staff and all tasks performed within the laboratory. All staff are encouraged to participate in CPD and professional grade staff maintain portfolios.
- **Audits** – all aspects of the laboratory work are audited annually and any non-conformances are investigated and suitable corrective or preventative actions are put in place.
- **Turnaround Times** - are monitored regularly to ensure reasons for any breaches are identified.
- **Incidents are recorded using Datix and are investigated thoroughly** - All non-conformances are investigated and we aim to put appropriate actions in place in a timely manner.
- **User Feedback** - is sought annually and reviewed to enable us to address any issues raised. All complaints and concerns are investigated and responded to within timescales found in Trust guidelines.
- **Staff Suggestions** - on service or quality improvements are welcomed and all possible ways to improve the ways we work are reviewed by teams made up of a range of staff grades and implemented where they give added value.

17 Guidance of Sample Collection and Testing Protocols for Bacterial Investigations

17.1 Blood Cultures

See details in UKHSA SMI B37 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Type	Method for Taking Sample

Blood Cultures	Refer to blue bag for instructions.
	
	1. Equipment preparation
	
	• Clean hands
	• Gather all equipment
	  ○ Blue blood culture collection pack
	  ○ Non-sterile gloves
	  ○ Sharp's bin
	  ○ ANTT tray
	• Clean the ANTT tray with an approved disinfectant e.g. Chlor-clean or Trigene and allow to dry
	• DO NOT REMOVE OR COVER THE BAR CODE LABELS on the blood culture bottles - this is for laboratory use, not for patient records.
	• Check the expiry date and that the bottom and sides of the blood culture bottles are intact, do not use if the coloured spot on the bottom of the bottle is yellow as this indicates the bottle is contaminated
	• Assemble the equipment and place in the ANTT tray, ensuring all key parts are protected
	
	2. Patient preparation
	
	• Clean hands before patient contact
	• Positively identify the patient and obtain consent
	• Identify puncture site. If the intended site is visibly soiled, clean with soap and water and then dry
	• Apply disposable tourniquet and palpate to identify vein
	• Clean hands

	• Remove plastic caps from the blood culture bottles. Clean the newly exposed bottle tops with 2% chlorhexidine/70% alcohol wipe for a minimum of 15 seconds and allow to dry. • Put on non-sterile gloves • Clean patient skin with SEPP (Chloraprep) for approximately 30 seconds and allow to dry (about 30 seconds) **Remember, if it's not dry it's not aseptic** If a culture is being collected from a CVC, clean the access port with a new 2% chlorhexidine/70% alcohol wipe for 30 seconds and allow to dry (about 30 seconds) before blood collection • **Do not palpate the vein again after cleaning the patient's skin** **3. Sample collection** • Insert the winged needle into the prepared site • Place adaptor cap over the blood culture bottle and pierce septum. Fill the aerobic bottle first (to prevent oxygen being added to the anaerobic bottle) • Hold the bottle upright and **use the bottle graduation lines to accurately gauge the sample volume** and collect ideally 10 ml (5-12ml is the acceptable range) into each blood culture bottle • Release the tourniquet • Remove the needle from the vein using the in-vein activator on the collection set • Cover the puncture site with a sterile gauze • Dispose of waste appropriately • Remove gloves, then clean hands

Blood culture to detect bacteraemia is an important investigation with major implications for the diagnosis of patients with infection and the selection of appropriate treatment.

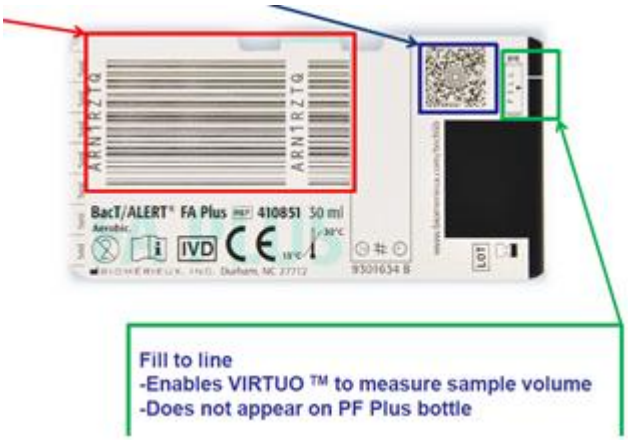
These recommendations aim to ensure that blood cultures are taken:

- For the correct indication
- At the correct time
- Using correct technique to prevent contamination of the sample and minimise risk to patients and staff

A false positive blood culture is defined as growth of bacteria in the blood culture bottle that were not present in the patient's bloodstream and were introduced during sample collection.

Contamination can come from several sources: the patient's skin, the equipment used to take the sample and transfer it to the culture bottle, the hands of the person taking the blood sample, or the general environment.

The Bact/Alert Virtuo blood culture system is currently in use in Leicester. This system uses either aerobic/anaerobic sets for adults or paediatric PF Plus bottles. Ideally, 10ml should be added to each bottle. However, the acceptable range is between 5-12ml. To aid clinicians, fill lines are included on aerobic/anaerobic bottles to prevent underfilling/overfilling of bottles.



Only take blood for culture when there is a clinical need to do so and not as routine

Blood cultures are taken to identify patients with bacteraemia. There are many signs and symptoms in a patient which may suggest bacteraemia and clinical judgement is required, but the following indicators should be considered when assessing a patient for signs of bacteraemia or sepsis:

- Core temperature out of normal range
- Focal signs of infection
- Abnormal heart rate (raised), blood pressure (low or raised) or respiratory rate (raised)
- Chills or rigors
- Raised or very low white blood cell count
- New or worsening confusion

NOTE: Signs of sepsis may be minimal or absent in the very young and the elderly. Blood cultures should be taken after identification of possible bacteraemia or sepsis and **before the administration of antibiotics**.

The manipulation of unidentified organisms on the open bench can lead to laboratory staff being exposed to Category 3 pathogens. Laboratory staff rely on the clinical information when selecting the most appropriate route for the processing of each individual sample. Due to the safety critical processes within the laboratory all requesting clinicians must provide relevant information with each request so that laboratory staff are not exposed to dangerous pathogens.

17.1.1 Blood Cultures from High Risk Patients

- **Any cultures from patients with foreign travel outside of Northern Europe in last three months**
- **Samples suspected or known to contain hazardous pathogens such as Typhoid, Brucellosis, Mycobacteria, Hepatitis B and C, HIV, HTLV or CJD**

Bottles must be transported in a sealed section of a biohazard bag. The form must be completed with full clinical information including use of high risk flag and placed in the outer pocket of the bag. Both the sample and form must be labelled "High Risk or with Danger of Infection sticker".

17.1.2 Competence in Blood Culture Collection

Blood cultures should only be collected by members of staff (medical, nursing, healthcare assistant, phlebotomist or technician) who have been trained in the collection procedure and whose competence in blood culture collection has been assessed. Detailed instructions for blood culture collection are available on the UHL e-learning site. All staff who collect blood cultures must view the video and answer the accompanying MCQs.

Where possible use the blue Blood Culture Collection Pack. This contains all the equipment required to collect blood cultures including a safety blood culture device for inoculating the culture bottles from a peripheral vein. **Make sure the 'blue' information sticker is completed and placed in the patient's records.**

If the blue bag blood culture collection pack is not used (e.g. in paediatrics or specimens collected from lines):

- Ensure the collection site and blood culture bottle tops are prepared appropriately (see below).
- Following inoculation, place the blood culture bottles in to a clear specimen sample bag, attaching the completed request form.
- Document in patient's notes the name of the person taking the blood culture and the date and time.

Samples from High Risk patients (see above) MUST be placed into a separate biohazard bag. Ensure the form and the specimen are clearly marked 'High Risk' or 'Danger of Infection'.

In patients with suspected bacteraemia, it is generally recommended that two sets of cultures be taken at separate times from separate sites.

IMPORTANT: Always make a fresh stab

Do not use existing peripheral lines/cannulae or sites immediately above peripheral lines. If a central line is present, blood may be taken from this and from a separate peripheral site when investigating potential infection related to the central line. The peripheral vein sample should be collected first.

Identify a suitable venepuncture site before disinfecting the skin.

Avoid femoral vein punctures because of the difficulty in adequate skin cleansing and disinfection.

- All bottles **MUST** be clearly labelled with the **Patient's Name, Hospital 'S' number - preferred (or NHS number) and Date of Birth,** along with date and time of collection

- Place the blood culture bottles in to specimen sample bag, attach completed request form then place back into the blue blood culture collection bag. Seal and send to **Clinical Microbiology Laboratory**

Document in patient's notes the name of the person taking the blood culture and the date and time – **DO NOT** remove the **Bar codes** from the bottles, these are for laboratory use only.

Samples from High Risk patients (see above) MUST be placed into a separate biohazard bag. Ensure the form and the specimen are clearly marked 'High Risk' or 'Danger of Infection' before placing back into the blue blood culture collection bag.

If culture for **atypical mycobacteria/MAI** is required, please contact the laboratory (**a different bottle is required** for this investigation). Investigation for atypical mycobacteria/MAI must be indicated on the request form.

Out of hours cultures should be sent as soon as possible to the Clinical Microbiology laboratory for incubation (there is no need to telephone the on-call technician when sending blood cultures).

When a culture appears positive, a Gram stain is made and a medical microbiologist will telephone the patient's doctor to discuss management. Full culture and antibiotic sensitivity is available later and the doctor will again be contacted.

17.2 CSF – Samples for Microbiological Investigation of Meningitis

See details in UKHSA SMI B27 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Type	Method for Taking Sample
Cerebrospinal Fluid (CSF)	• CSF is normally collected sequentially into three or more separate containers which should be numbered consecutively. Collect specimens in appropriate CE marked leak proof containers and transport specimens in sealed plastic bags. • Collection of an additional sample in a container with fluoride for glucose estimation is also recommended, although such tubes should be filled last because they may contain environmental bacteria which might otherwise contaminate samples for culture. • Common practice is to send the first and last specimens taken for microbiological examination and the second specimen for protein. The fluoride or protein sample should not be sent to Microbiology. Ideally testing should be carried out on the last sample with the first one reserved as a backup. • Ideally a minimum volume of 1mL for each tube 1 and 3 taken for microscopy (in adults). When sample volume is below this it is possible to pool samples. • For *Mycobacterium* species, at least 10mL where possible. **NOTE:** The larger the volume, the greater the cultural yield - particularly in relation to *M. tuberculosis* investigations.

All CSF specimens are considered urgent. Always telephone the laboratory before sending the specimen. If out of hours, contact the On-call Bacteriology Biomedical Scientist ("micro tech"; BMS) once the specimen has been taken. It is the requestor's responsibility to ensure arrangements are made for the specimen/s to be delivered to Clinical Microbiology Level 5 Sandringham Building.

- Use plain sterile universal containers. Use three universals.
- If Sub-arachnoid haemorrhage suspected - counts are done on Number 1 and Number 3.
- A separate specimen should go to Chemical Pathology for glucose and protein and for xanthochromia if required. Ensure serum glucose is requested to compare with the CSF glucose.
- The CSF will be cultured and a Gram stain performed.

- If the white cell count is raised a differential count will be performed and a medical microbiologist informed.
- An EDTA blood should be sent in all cases of suspected meningitis.

In suspected **meningococcal disease**, a throat swab should be sent (labelled "? Meningitis"). In cases with a rash, meningococci may be seen and even grown from skin scrapings (please contact the laboratory). It is helpful if these cases are first discussed with a medical microbiologist.

If **viral meningitis** is suspected send a CSF sample to virology. In instances when a CSF cannot be obtained another specimen such as a blood sample (PCR), throat swab or faeces should be sent to Virology.

In cases of possible **TB meningitis**, organisms are rarely seen. Please discuss with the medical microbiologist as methods for rapid detection may be available.

Bacteria may not grow from the CSF if antibiotics had been given prior to lumbar puncture.

If a lumbar puncture is contraindicated, the organism may still be recovered from **blood culture which should be taken in all cases of meningitis.**

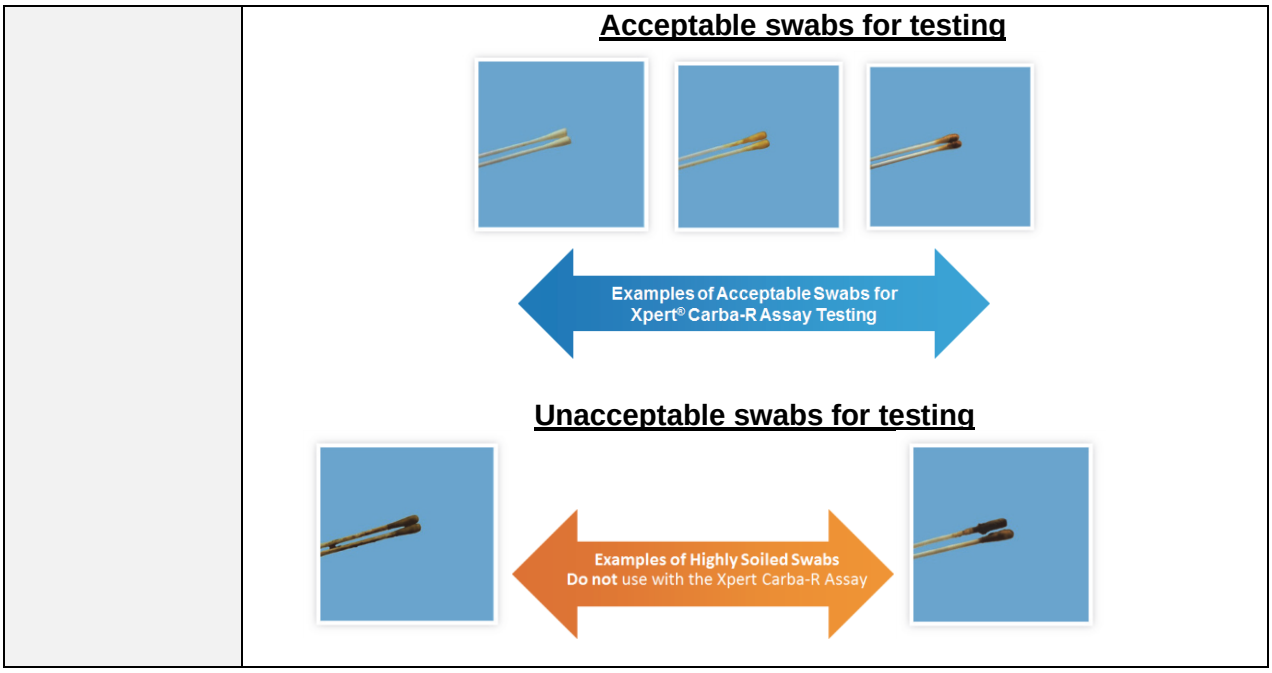
NOTE: **All cases of bacterial meningitis and meningococcal septicaemia must be notified to the CCDC (Tel: 0844 2254524 or OUT OF HOURS via 0115 9675099 and ask for UKHSA On Call).**

17.3 Faeces/Rectal Swabs and Samples for Parasitology

See details in UKHSA SMI S7 (routine), B10 (CDT) and B31 (parasites) found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
Faeces - Routine	• Collect specimens soon as possible after onset of symptoms. • Collect specimens before antimicrobial therapy where possible. • Specimen may be passed into a clean, dry, disposable bedpan or similar container and transferred into an appropriate CE marked leak proof containers and place in sealed plastic bags. The specimen is unsatisfactory if any residual soap, detergent or disinfectant remains in the pan. • Faecal samples should be liquid or semi formed (i.e. take the shape of the container).
Faeces - *Clostridioides difficile* toxin (CDT)	• Collect specimens before antimicrobial therapy where possible. • Specimen may be passed into a clean, dry, disposable bedpan or similar container, and transferred into a CE marked leak proof container. The specimen is unsatisfactory if any residual soap, detergent or disinfectant remains in the pan.

	• Formed stools are unsuitable for investigation for *C. difficile*. These should be rejected with the appropriate comment appended to the report. • A liquid specimen of 1-2ml is sufficient for culture and toxin detection. Repeat testing of samples if there is no indication within a 28 day period. This applies to repeat testing of positive results. On the contrary, a negative test, if symptoms persist, should be re-tested as it is known that a one-off negative can occur, after 3 days of initial test.
Faeces (Parasites)	• Faeces should be collected before antimicrobial or anti-diarrhoeal therapy where possible and between 10pm and midnight, or early in the morning, before defecation or bathing. • Fresh faeces specimens are essential for the examination of trophozoites. • Faeces may be passed directly to a sterile wide-mouthed CE marked leak proof container or may be passed to a clean, dry bedpan or similar container and transferred to a CE marked leak proof container. • Fresh, unpreserved specimens should be transported immediately. Cysts will not form once the specimen has been passed. • Protozoan trophozoites will not survive if the specimen dries out. Liquid stool should therefore be examined ideally within 30 minutes from the time of collection Soft stools (which may contain both trophozoites and cysts) should preferably be examined within 1hr of passage. • Formed specimens (less likely to contain trophozoites) can be kept for up to one day, with overnight refrigeration if needed, prior to examination. **Microscopy for *E. vermicularis* ova - Sellotape slide** • Apply clear Sellotape to the perianal region, pressing the adhesive side of the tape firmly against the left and right perianal folds several times; the tape can be wrapped around a tongue depressor to aid specimen collection. Smooth the tape back on the slide, adhesive side down. **Perianal swab** • Perianal specimens are best obtained in the morning before bathing or defecation. Three specimens should be taken on consecutive days before pinworm infection is ruled out. • Cotton-wool swab in a dry container should be used for collection. • Spread buttocks apart, and rub the moistened cotton wool swab over the area around the anus, but do not insert into the anus. Place cotton wool swab back in its container (no transport medium required). Occasionally, an adult worm may be collected from a patient and sent in saline or water for identification.
Rectal Swab - Direct CRO PCR	• Collect a sample using the paired (red topped) swab by carefully inserting both swab tips approximately 1cm beyond the anal sphincter and rotate gently. Ensure the swab does not have too much faecal matter on it see below.



See page: ***'Investigation of Sporadic Diarrhoea in Patients Greater than Two Years Old' – below***

- CDT testing on patients under the age of 2 will **NOT** be performed. These samples will have faecal culture. Should the investigation of an organism other than those routinely screened for be required, please contact the Microbiology Department.
- (2ml) is the acceptable minimum volume for examination. A larger volume should be submitted if possible in a dedicated faeces pot, but do not overfill.
- **Formed stools are unlikely to contain bacterial pathogens and are not processed, so should not be sent.**
- Vomit is not useful and will not be processed. In an outbreak of winter vomiting, a faeces sample should be sent to the Virology department.
- If viral source is suspected a **separate sample and form** should always be sent for virological investigations to the Virology department.
- Rectal swabs are not a good substitute for faeces for routine examination and will not be processed.
- Rectal swabs can be used for screening of resistant organisms such as Carbapenem Resistant Organisms (CRO) and Vancomycin Resistant Enterococci (VRE), see above for sample collection.
- The clinical information on the form is crucial in directing the examination (i.e. patient age and symptoms, history of travel, duration of illness or antibiotic usage).
- Ova, cysts and parasites may be sought in the faeces specimens, but this request must be accompanied with relevant details such as travel history.
- If threadworms are suspected (perianal itching), sellotape should be applied to the perianal skin and sent stuck flat onto a microscope slide in a box. Alternatively, a sterile swab of the sample, in a dry container (swab with no transport media), should be sent for examination.

17.3.1 Urines - For the detection of Schistosoma haematobium

Results may take 2-3 days

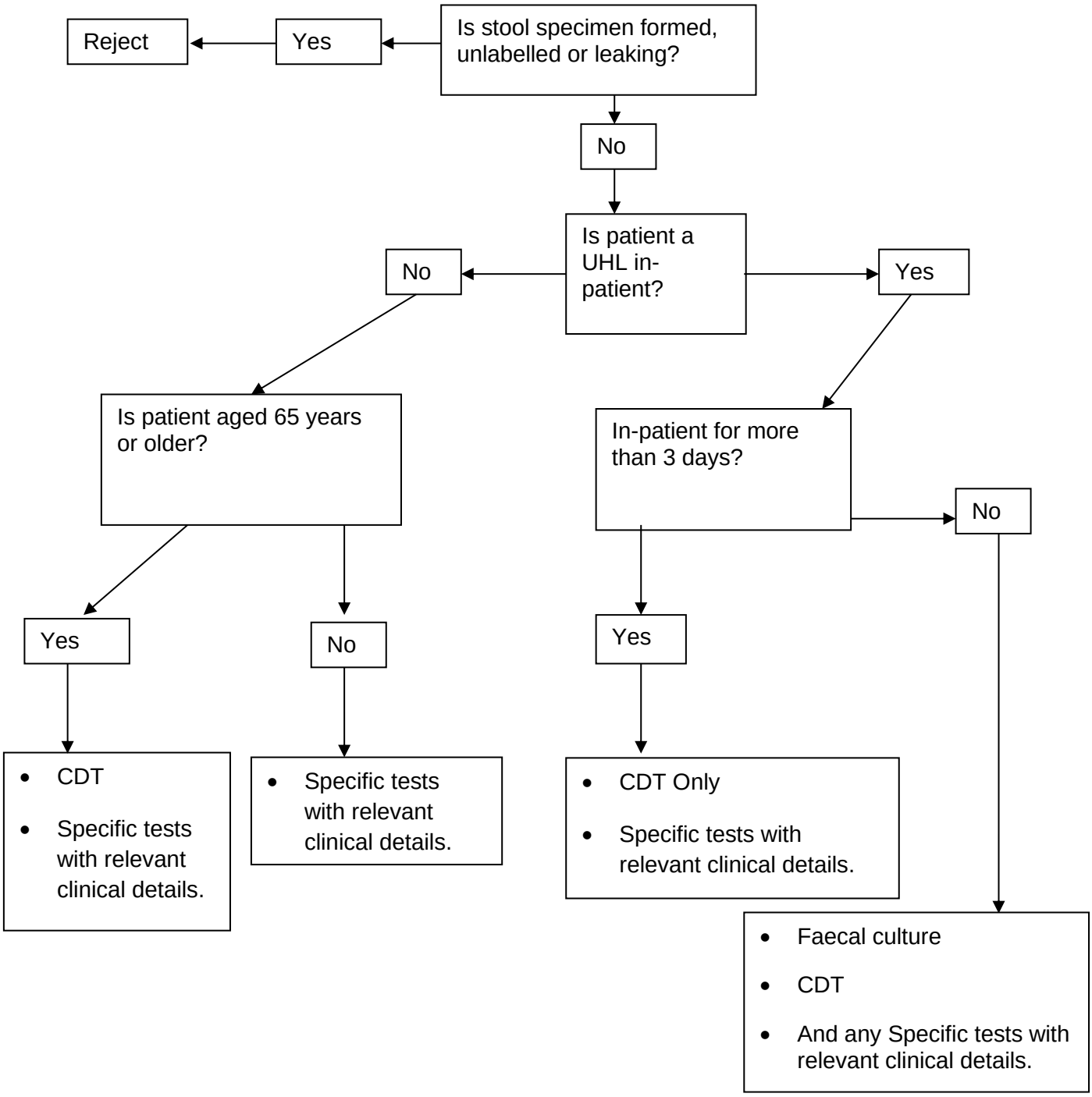
See details in UKHSA SMI B31 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
Urine (for *S.haematobium*)	• In urinary schistosomiasis, very few ova are present in the urine. The number of ova in the urine varies throughout the day, being highest in urine obtained between 10am and 2pm. In patients with haematuria, eggs may be found trapped in the blood and mucus in the terminal portion of the urine specimen. It is therefore preferable to obtain total urine collected over the time period between 10am and 2pm. Alternatively, a 24hr collection of terminal samples of urine may be helpful. • Sterile containers without boric acid must be used. Specimens should be collected aseptically and placed in a CE marked leak proof container without preservatives in a sealed plastic bag

NOTE: Cases of food poisoning and dysentery should be notified by the clinician to the 'proper officer' at their local health protection team (HPT) in East Midlands. If there is a hospital outbreak, the Control of Infection Officer or Control of Infection Nurse and the laboratory should be contacted as soon as possible (see separate Control of Infection Guide).

East Midlands HPT Centre Telephone: 0344 225 4524
Institute of Population Health
Nottingham City Hospital
Hucknall Road, Nottingham NG5 1QP

17.3.2 Bacteriological Investigation of Sporadic Diarrhoea in Patients >2 Years of Age



If case is part of a cluster or outbreak contact Infection Control if the patient is an inpatient or Health Protection Team/UKHSA if patient is in the community

Faecal Culture: Culture for *Salmonella*, *Shigella*, *Campylobacter*, *E coli* O157 and detection of *Cryptosporidium*, *Giardia and Entamoeba histolytica*
CDT: *Clostridioides difficile* toxin

17.4 Lower Respiratory Tract Bacterial Infections

- **Sputum** – please ensure that freshly expectorated sputum with minimal salivary contamination is sent. Salivary specimens will be rejected. Purulence is assessed by visual appearance. The specimens should be sent to the laboratory **without delay.**
- **Broncho-alveolar lavage or brushings** – The specimens should be sent to the laboratory **without delay.**
- **Endotracheal aspirates -** The specimens should be sent to the laboratory **without delay.**
- **Cough Swabs –** These should only be sent where sputum cannot be obtained and generally applies to CF and PCD patients.
- **Culture for *Burkholderia species*, *Legionella* and fungi are available**
- **Atypical pneumonia** – serological tests available - See 'Virology tests available'
- ***Pneumocystis jiroveci*** is detected by PCR - See 'Virology tests available'
- **Pulmonary tuberculosis** – See section 'Investigation for *Mycobacterium*'
- **Nasopharyngeal aspirates (NPA's)** - NPA's should be submitted to Virology only for the detection of respiratory viruses. These are unsuitable specimens for bacterial investigations other than for *Bordetella species*.

17.4.1 Sample collection

See details in UKHSA SMI B57 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
Expectorated Sputum Samples	• Sputum samples are known to have issues with contamination. Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. • The sputum is collected directly in a CE marked leak proof container.
Broncho-Alveolar Lavage (BAL)	• A segment of lung is 'washed' with sterile saline after insertion of a flexible bronchoscope, thereby allowing recovery of both cellular and non-cellular components of the epithelial surface of the lower respiratory tract. It is a reliable method for making a definitive aetiological diagnosis of pneumonia and other pulmonary infections. • The BAL is collected in a CE marked leak proof container.
Bronchial Aspirate	• Bronchial aspirates are collected by direct aspiration of material from the large airways of the respiratory tract by means of a flexible bronchoscope. • The bronchial aspirate is collected in a CE marked leak proof container.
Bronchial Washings	• Bronchial washings are collected in a similar fashion to bronchial aspirates but the procedure involves the aspiration of small amounts of instilled saline from the large airways of the respiratory tract. • The bronchial washing is collected into a CE marked leak proof container.
Tracheal/Endo-tracheal aspirate	• Tracheal aspirates are collected via the endotracheal tube. They are subject to the same limitations as sputum specimens. The sample is collected in a CE marked leak proof container.

Naso-pharyngeal Aspirates (NPA)	• Not recommended for culture of bacteria • For Virology, collect the NPA in a CE marked leak-proof container

17.5 Tuberculosis & Atypical Mycobacteria

See details in UKHSA SMI B40 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
All Specimens	• Collect specimens before antimicrobial therapy where possible. • For the initial diagnosis of mycobacterial infection all specimens should be fresh and taken, whenever possible, before anti-tubercular treatment is started. 'Other' antimicrobials may also have significant anti-mycobacterial activity, notably the fluoroquinolones such as ciprofloxacin, levofloxacin or moxifloxacin and the macrolides such as clarithromycin or azithromycin. • All samples must be labelled as high risk.
Sputum	• Sputum specimens should be fresh (less than 1 day old) to minimise contamination. Purulent specimens are best. Two to three samples of ≥5mL should be collected approximately 8-24 hours apart with at least one from early morning. • Samples taken early morning (that is, shortly after patient waking) have the greatest yield. When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline ('sputum induction') before expectoration may be helpful.
Broncho - alveolar Lavage/ Bronchial Washings	• These may be sent if spontaneous or induced sputum is unavailable or if such specimens are AFB smear negative. **NOTE:** Contamination of the bronchoscope with tap water, which may contain environmental *Mycobacterium species*, should be avoided. Minimum sample size is preferably 5mL.
Gastric Washings	• Gastric washings are usually used for children where there are problems obtaining sputum. Young children will often swallow their respiratory secretions rather than cough them up. Induced sputum is considered preferable to gastric washings, if possible. • Collect samples early in the morning (before breakfast) on 3 consecutive days. • Preferably, a minimum volume of 5mL should be collected. • Aspirates should be promptly delivered and processed to avoid acidic deterioration of organisms.

	• Results of direct microscopy on gastric washings can be misleading because other acid-fast bacilli are normally present in the stomach.
Sterile Site Body Fluids	• Collect aseptically as much (for example >6mL in adults) CSF sample as possible into a CE marked leak proof container in a sealed plastic bag. If only a small volume is available after initial lumbar puncture, and the findings of cell counts and protein suggest TB meningitis, a second procedure should be considered to obtain a larger volume to improve chances of achieving positive cultures. • It should be noted that pleural or pericardial fluids are not very sensitive samples for the detection of *M. tuberculosis,* and that a concurrent pleural or pericardial biopsy taken with the fluid is more useful. A negative result on these fluids does not rule out the diagnosis.
Urine Specimens	• Urine specimens should be collected in the early morning on three consecutive days in a CE marked leak proof container (that does not contain boric acid), and placed in a sealed plastic bag. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.
Skin, Bone, and Tissue including Post Mortem Specimens	• Specimens of such type should be homogenised, apart from bone. It may be necessary to select and cut out a suitable piece of tissue if a large piece is received. Similarly, some pieces of tissue may need to be 'minced' using sterile scissors and forceps before they can be successfully homogenised. • Specimens should be collected aseptically and placed in a CE marked leak proof container without preservatives in a sealed plastic bag and sterile distilled water added to prevent desiccation. • A caseous portion should be selected if possible. The majority of organisms will be found in the periphery of a caseous lesion. **NOTE: Tissue biopsy specimens received in formalin are unacceptable and are not be processed.**
Pus or Pus Swabs	• Pus, or pus swabs should be collected aseptically and the largest practical sample submitted in CE marked leak-proof container in a sealed plastic bag. Pus is the sample type of choice. Swabs are less preferable as mycobacteria, if present, may adhere to the swab rather than be transferred successfully to the culture media.
Bone Marrow and Blood	• As large a sample of bone marrow as possible should be aspirated and added directly to the culture medium in accordance with the manufacturer's instructions. • 1- 5ml blood inoculated into MycoF/lytic blood culture bottle, should be transported and loaded into the automated culture system as soon as possible – follow method of taking sample highlighted in blood culture section.
Faeces	• Not recommended – contact laboratory for advice.

17.5.1 Pulmonary and Extra pulmonary Tuberculosis

Sputum specimens should be fresh (less than 1 day old) to minimise contamination. Purulent specimens are best. Two to three samples of ≥5mL should be collected approximately 8-24 hours apart with at least one from early morning.

Samples taken early morning (i.e. shortly after patient waking) have the greatest yield. When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline ('sputum induction') before expectoration may be helpful.

If a patient cannot expectorate then a gastric aspirate should be sent in a plain universal container. Minimum sample size volume is preferably 5mL.

Renal tuberculosis - As the organisms are excreted intermittently, three consecutive early morning urines are required in sterile universal containers **without boric acid.**

TB meningitis - Cerebrospinal fluid (CSF) collected aseptically should be submitted to the laboratory. If rapid testing is required please discuss with a Medical Microbiologist.

Tissue and aspirates - send in sterile universal containers. It should be noted that mycobacteria are often not recovered from pleural or pericardial fluid. A concurrent pleural or pericardial biopsy taken with the fluid is more useful. A negative result on these fluids may not rule out the diagnosis.

Blood cultures - may be helpful when looking for atypical mycobacteria - please discuss with the laboratory. Special blood culture bottle are available.

Faecal specimens - The isolation procedure is unreliable and has a low success rate due to the heavy contamination with other bacteria; hence culturing faecal samples for mycobacteria is not recommended.

Direct microscopy – in most cases acid-fast bacilli will be looked for by direct microscopy and a preliminary report issued. Microscopy of urine and faeces however is usually not helpful as non-pathogenic mycobacteria may be present. Microscopy of swabs and gastric aspirates is also not helpful due to the small number of mycobacteria which may be present.

TB PCR – The GeneXpert MTB RIF Ultra is used to test all primary samples that are new AAFB smear positive and on clinical request. Requests for this test should be made by ICE referral to the microbiology medical team.

Positive cultures for Mycobacteria may take several weeks to grow. When cultures are positive, they are initially reported as '*Mycobacterium species* isolated'. All isolates are referred to the National Mycobacteria Reference Laboratory for identification and susceptibility testing. Pigmented strains are not tubercle bacilli but may cause disease.

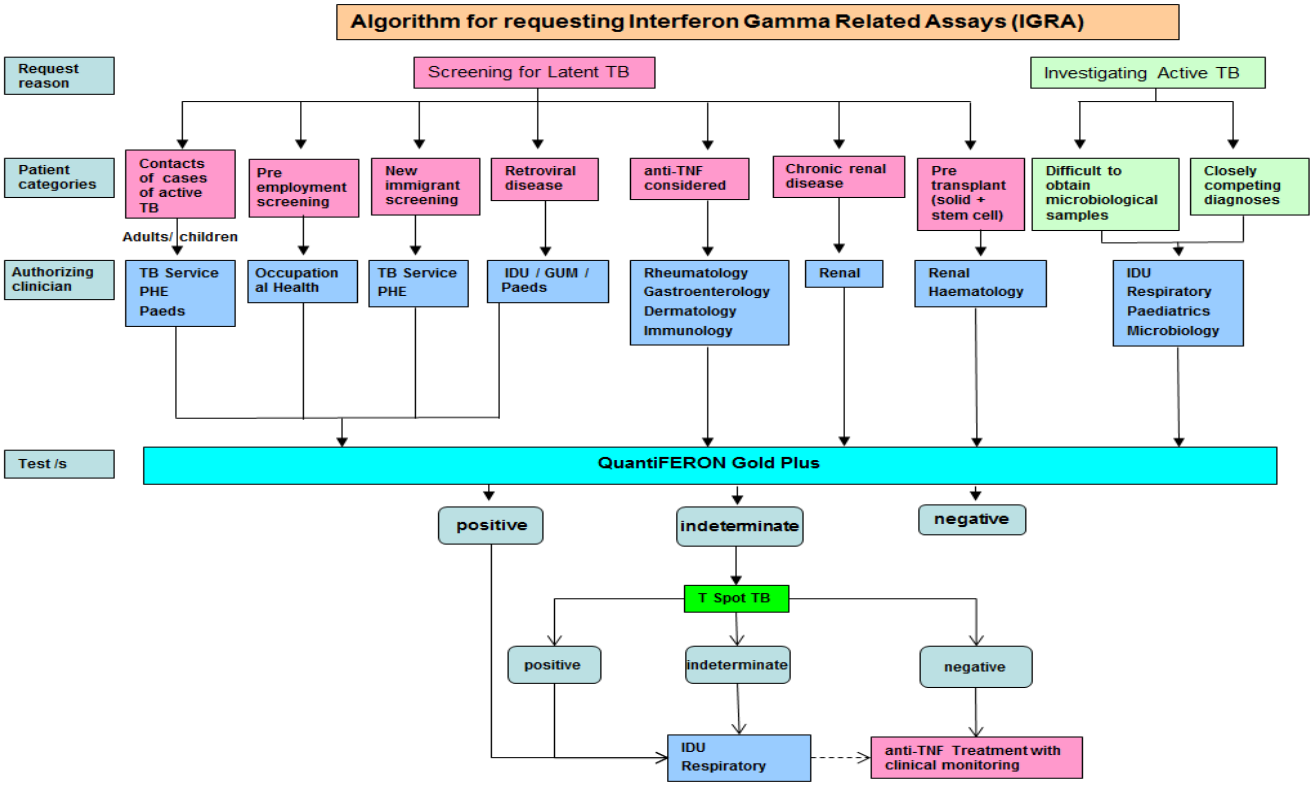
New developments in culture, identification and sensitivity testing mean that the laboratory handling of these specimens is improving all the time. Please discuss any difficult or urgent cases with a microbiologist.

17.6 QuantiFERON TB Gold & T Spot TB – Interferon Gamma Testing

17.6.1 Algorithm for IGRA testing

UHL offers QuantiFERON TB Gold and T Spot TB testing for diagnosis of latent infection due to *M. tuberculosis*. These tests detect if a person has been infected with *M. tuberculosis*, but does NOT distinguish between active and latent tuberculosis. The patient categories that will benefit from these tests are outlined in the algorithm below. Please refer to this when requesting the test/s:

Algorithm for requesting Interferon Gamma Related Assays (IGRA)



17.6.2 QuantiFERON TB Gold Plus test

This test is available from the Department of Microbiology. Request can be made on ICE or completion of the supplied request form. QuantiFERON blood collection tubes can be obtained from the Microbiology department.

Blood Sample Collection is into QFT tubes (4 tubes - green, yellow, purple and grey top) OR **1 Lithium Heparin LiHe) tube** – THE LiHe TUBE IS FOR AUTHORISED USERS ONLY (authorisation to be obtained from the Microbiology Department).

NOTE: The QFT test is unique in its methodology. Please ensure this method is strictly adhered to, to avoid re-bleeding of the patient.

Samples may be rejected for testing if:

- Any of the blood collection tubes are not received by the laboratory within 16 hours of venepuncture.
- If QFT blood collection tubes are overfilled OR under-filled.
- If there is <4.5ml blood filled in the LiHe tube, when used as an alternative to the 4 tubes.
- If there are insufficient patient identifiers or requesting sender's details, as required in general for performance of all laboratory tests

17.6.2.1 Instructions for use

Label the blood collection tubes completely. Include full name, patient number, date of birth and very importantly – the date and time of blood collection. Complete all relevant sections of the form. Maintain tubes at 22°C ± 5°C. Do not fridge or freeze tubes once full. Filled tubes need to be transported to the laboratory rapidly as they need to be incubated at 37ºC within 16 hours of collection.

QuantiFERON TB Gold Plus test (4 tube test)

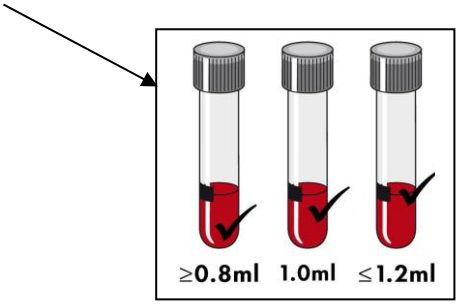
Tubes should be at 17-25°C at the time of blood filling.

1) Collect 1ml of blood by venepuncture into each of the QFT tubes. DO NOT TRANSFER BLOOD FROM ONE TUBE TO ANOTHER: Nil tube - grey cap, TB1 tube - green cap, TB2 tube – yellow cap and Mitogen tube – purple cap.

2) Tubes fill slowly, hold tube on needle for 2-3 seconds after flow ceases. As a guide to the fill volume, each tube has a bold black line approximately 25mm from the base of the tube – this is the desired fill level. If blood level is not close to the black mark, obtain another sample. (See diagram below).

NOTE: If using a "Butterfly needle" – prime tubing with a 'purge' tube (not supplied) before filling QFT tubes.

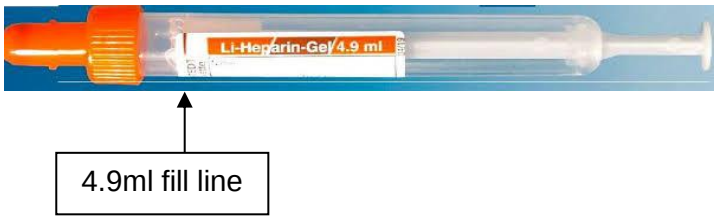
Acceptable fill levels for all QFT blood collection tubes



3) Once filled, invert the tubes 10 times, just firmly enough to ensure the inner surface of the tube is coated in blood. NOTE – Over energetic shaking may cause gel disruption and could lead to aberrant results.

Lithium Heparin (LiHe), Blood Collection (General Practitioners and Authorised Users Only)

If blood is collected in a single generic blood collection tube that contains lithium heparin as the anticoagulant, the minimum volume required is 4.5 ml. To ensure this, aim to fill to the 4.9ml line. Gently mix by inverting the tube several times to dissolve the heparin. Please note, use only lithium heparin as a blood anticoagulant, as other anticoagulants interfere with the QFT Plus assay.



QFT tests must be received within the lab between 09:00 to 17:00 Mon-Fri. Deviation from these times can only be made after discussion with lab staff. The lab does not take responsibility for samples received outside of these hours. Samples should not be sent on Saturday or Sunday.

17.7 T Spot TB Test

This test is available from the Department of Immunology. Please contact Immunology department on extension 16713 or refer to Immunology User's Handbook.

17.8 Ophthalmology Investigations & Specimens

17.8.1 General Information

1) Media sourced from Microbiology is stored in eye casualty (OPD) sufficient for 2 weeks supply. However, during normal working hours the laboratory can be contacted on ext. 6533 if there is insufficient media. Viral transport medium can be obtained from the Microbiology stores department (ext. 6538). Out of hours the on call Microbiology Technician (MMS) can be contacted via the LRI switchboard.

2) Aqueous/vitreous fluid and corneal scrape specimens are processed as "Urgent" by Bacteriology (see Out-of-hours guidelines). It is important to notify the laboratory once the culture media/slides have been inoculated.

See details in UKHSA SMI B2 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
Eye swabs, Canalicular Pus, Aqueous/Vitreous Humour, Corneal Scrapes, Contact Lens Cases, Cleaning Fluid	• Contact lens cases and cleaning fluid are only investigated for *Acanthamoeba*. • Use aseptic technique.

	• Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags. • Collect swabs into black top charcoal swabs in sealed plastic bags. • Corneal scrapings and intraocular fluids will be collected by an ophthalmic surgeon. Because of the small amounts of material involved, inoculation of plates and preparation of slides may need to be done at the patients' side. • Any available pus should be sampled as well as the lesion of interest. • If processing is delayed, refrigeration is preferable to storage at ambient temperature. • If specimens for investigation for amoebae cannot be processed within 8hr, it is preferable to store them at ambient temperature.

17.8.2 Inoculation of Media/Slides

1) It is **imperative** that aseptic techniques are used and **sterile gloves worn** to prevent contamination of the culture media.
2) The following numbers of blades/needles are required depending on what tests are required:
    a. Bacteriology (including Gram film) - 3
    b. Virology - 1
    c. Fungi - 1

    NOTE: If all investigations are required a total of 5 blades/ needles will be required.

3) The order of inoculation is important to achieve the best use of material available. Please inoculate a small area of the agar plates gently (the laboratory will spread the inoculum on arrival) – do not break surface if possible.

Do not use plates that already have any growth present. Plates should be smooth and the surface unblemished.

Do not use a blade/needle again if it has already been used in the eye previously (follow instructions below).

The recommended order of inoculation is as follows:

Order	Media
1	Chocolate Agar – Once used place needle / blade in Robertson's cooked meat (RCM) broth.
2	Smear for Gram stain on glass slide (discard needle in sharps bin after placing smear on slide). Mark with pencil the side of slide inoculated
3	Robertson's Cooked Meat Broth *
4	Blood Agar - Once used place needle / blade in nutrient broth (NB).
5	Sabouraud's Agar - Once used place needle / blade in Robertson's Cooked Meat Broth
6	Nutrient Broth *

7	Viral Transport broth
8	Send dry swab for *Acanthamoeba* testing by PCR

* To avoid excessive scrapes being taken, the broths are inoculated following inoculation of 1&2 and 4&5.

4) Clearly label **ALL** plates and broths with the patient's information. For bacteriology/fungi complete appropriate request on ICE and include all relevant details. Use a pencil to write patients details on the slide. Ensure that labels are not placed across the centre of the agar plate.
5) Ensure the culture media / slide are delivered urgently to the Clinical Microbiology Department (Level 5 Sandringham Building) via porters.
6) The Bacteriology department will telephone a Gram film result as soon as possible. All Bacteriology culture media will be incubated. Virology samples will be stored and dealt with during normal working hours only.
7) Request *Acanthamoeba* PCR on ICE and include relevant clinical details and risk factors.

17.9 Urine Sampling & Advice

See details in UKHSA SMI B41 and B31 (schistosomiasis) found at:
https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Type	Sample Taken	Method for Taking Sample
Urine – Routine Culture	**Mid-Stream Urine (MSU)**	• Peri-urethral cleaning is recommended (water is considered sufficient). • The first part of voided urine is discarded and, without interrupting the flow, approximately 10mL is collected into a CE marked leak proof container. The remaining urine is discarded. **If boric acid preservative is used, the container is filled up to the mark in a similar manner and the contents mixed well.**
	Clean-Catch Urine	• A reasonable alternative to MSU. • Peri-urethral cleaning is recommended. The whole specimen is collected and then an aliquot sent for examination in a CE marked leak proof container.
	Supra-Pubic Aspirate (SPA)	• Urine is obtained aseptically, directly from the bladder by aspiration with a needle and syringe. The use of this invasive procedure is usually reserved for clarification of equivocal results from voided urine (e.g. in infants and small children). • Ultrasound guidance should be used to show presence of urine in the bladder before carrying out SPA.
	Catheter Urine (CSU)	• The sample may be obtained either from a transient ('in and out') catheterisation or from an indwelling catheter. In the latter case, the specimen is obtained aseptically from a sample port in the catheter tubing or by aseptic aspiration of the tubing.

		• The specimen should not be obtained from the collection bag. **If boric acid preservative is used, the container is filled up to the mark in a similar manner and the contents mixed well.** • Specimens should be collected aseptically and placed in a CE Marked leak proof container in a sealed plastic bag.
	Bag Urine	• Used commonly for infants and young children. The sterile bags are taped over the freshly cleaned and dried genitalia, and the collected urine is transferred to a CE marked leak proof container. • There are frequent problems of contamination with this method of collection.
	Pad Urine	• An alternative to collecting bag urine from infants and young children. After washing the nappy area thoroughly, a pad is placed inside the nappy. As soon as the pad is wet with urine (but no faecal soiling), push the tip of a syringe into the pad and draw urine into the syringe. • Transfer specimen to a CE marked leak proof container. If difficulty is experienced in withdrawing urine, the wet fibres may be inserted into the syringe barrel and the urine squeezed directly into the container with the syringe plunger.
Urine for *S. haematobium*	**Urine**	• In urinary schistosomiasis, very few ova are present in the urine. The number of ova in the urine varies throughout the day, being highest in urine obtained between 10am and 2pm. In patients with haematuria, eggs may be found trapped in the blood and mucus in the terminal portion of the urine specimen. It is therefore preferable to obtain total urine collected over the time period between 10am and 2pm. Alternatively, a 24hr collection of terminal samples of urine may be helpful. • Sterile containers without boric acid must be used. Specimens should be collected aseptically and placed in a CE Marked leak proof container without preservatives in a sealed plastic bag.

17.9.1 Urine Testing Protocols

The starting point for submitting urine samples to the laboratory is always the clinical presentation. There is generally no rationale in sending specimens from asymptomatic patients. The only exceptions to this would be pregnancy (screening for asymptomatic bacteriuria) or patients about to undergo surgical procedures on the urinary tract. It therefore follows that 'screening' samples are rarely justified and might risk unnecessary treatment of patients with asymptomatic bacteriuria.

The same principles apply to the use of urinary dipsticks for leucocyte esterase (LE) and nitrites. Specifically, they should NOT be used in:

1. **Older patients** - without specific urinary symptoms or generalised features of infection. Many older patients have asymptomatic bacteriuria which does not require treatment.

2. **Catheterised patients** - Most catheterised patients will have positive dipstick results and many will have positive cultures but treatment (and hence investigation) is only indicated if the patients is systemically unwell.

3. **Pregnancy** - to screen for bacteriuria. Urine culture is required.

Dipsticks and urine cultures are not recommended in women of child-bearing age who present with the typical features of acute uncomplicated cystitis. Such patients should be treated empirically with a 3-day course of antibiotics unless they are pregnant.

In **elderly/institutionalised women** dipsticks may have some value in patients with a new onset of fever or UTI (urinary tract infections) symptoms where negative LE +/- nitrites will indicate that a UTI is unlikely. In such patients, positive dipstick results may justify empiric treatment after sending an MSU for culture.

In **men** with suspected UTI, urine cultures should always be sent. In patients with typical/severe symptoms dipstick testing is unhelpful as such patients require empiric treatment whatever the dipstick shows. In men with mild/non-specific symptoms a negative LE and nitrite dipstick can usually exclude a UTI.

In **children** urine cultures are generally required to exclude UTI although negative dipstick LE/nitrite results in patients over 3 years of age would exclude a UTI.

- Urine containers containing boric acid are ideally used.
- If filled to the indicated volume, boric acid will prevent the overgrowth of contaminating bacteria during transit to the laboratory.
- Low volume of urine (<5ml) should be sent in a plain sterile container as high concentrations of Boric acid may prove toxic to some organisms – for this reason low volume urines (<1ml) may NOT be tested, including paediatric urines received in boric acid.
- Contamination of urine with flora from the perineum can give misleading results.
- A well taken mid-stream urine or SPA should be sent.
- A bag urine may be satisfactory if removed from the skin as soon as the child has voided.
- Clinical and patient information is important as ordinarily, urines are screened for evidence of infection by an automated urine analyser and are only cultured when analysis of both white cells and bacteria indicate evidence of urinary tract infection.
- At present Haematology, Renal, Pregnant and Children under the age of 3 years are cultured regardless of the screening result.
- Manual microscopy for the presence of casts is only carried out on specific request.
- Traditionally a pure culture of $>10^5$ cfu/ml of bacteria in the absence of contamination is indicative of infection, however, lower numbers are seen in some infections. Appropriate antibiotics are reported. Please indicate on the form which antibiotic the patient is starting.

17.9.2 Non-Visible Haematuria

Dipstick testing should be used to investigate haematuria and urine microscopy should not be used to confirm dipstick results (NICE guidance). Automated red cell counts do not correlate with dipstick testing.

17.9.3 Catheter Urines

This should be taken by needle aspiration from the tubing, not from the bag where contaminants can multiply. Bacteria may be present in a CSU with no ill effect. The decision as to treat is a clinical one. Using antibiotics indiscriminately leads to resistance or fungal superinfection. A CSU sent routinely at catheter removal is not helpful, nor are specimens sent because the urine is cloudy or strong-smelling**. The actual urinary catheter is an inappropriate specimen and must not be sent to the laboratory.**

17.9.4 Prostatic Massage (VB1,2,3, EPS)

Each specimen has microscopy, a quantitative culture and antibiotic sensitivities if appropriate. Please ensure Prostatic Massage is clearly written on the request form.

17.10 Gynaecological/Genital Infections

See details in UKHSA SMI B28 found at https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
High Vaginal (HVS) Swab, **Vaginal Discharge,** **Vulval Swab,** **Labial Swab,** **Cervical Swab,** **Endocervical Swab,** **Penile Swab,** **Urethral Swab** **Genital Ulcer Swab,** **Semen,** **Screening swabs for *N. gonorrhoeae,*** **Aspirates from Bartholin's gland/Fallopian tube,** **Tubo-ovarian abscess**	• Collect specimens before antimicrobial therapy where possible. • Ideally transport time for specimens for *N. gonorrhoeae* should be as short as possible. • Collect swabs into black top charcoal swabs in sealed plastic bags. • Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags. • Separate samples should be collected into appropriate transport media for detection of viruses or *C. trachomatis.* • **Genital tract swabs** (e.g. cervical and high vaginal swabs) should be taken with the aid of a speculum. It is important to avoid vulval contamination of the swab. For *Trichomonas*, the posterior fornix, including any obvious candidal plaques should be swabbed. If pelvic infection, including gonorrhoea, is suspected, the cervix should be swabbed. • **High vaginal swabs -** After the introduction of the speculum, the swab should be rolled firmly over the surface of the vaginal vault. • **Cervical swabs -** After introduction of the speculum to the vagina, the swab should be rotated inside the endocervix. • **Urethral swabs -** Contamination with micro-organisms from the vulva or the foreskin should be avoided. Thin swabs are available for collection of specimens. The patient should not have passed urine for at least one hour. For males, if a discharge is not apparent, attempts

	should be made to "milk" exudate from the penis. The swab is gently passed through the urethral meatus and rotated. • **Intrauterine contraceptive devices (IUCDs) –** Currently not examined • **Rectal swabs -** Rectal swabs are taken via a proctoscope. • **Fluids and pus** - These are taken from the fallopian tubes, tubo-ovarian and Bartholin's abscesses during surgery. Preferably a minimum volume of 1mL.

- In cases of vaginal discharge an HVS is adequate unless gonorrhoea is suspected when an endocervical swab is needed (please request GC culture).
- If full culture is required (e.g. post-operative, post-natal) then a cervical swab is required.
- In cases of suspected pelvic inflammatory disease an endocervical swab for both gonococci and *Chlamydia* molecular testing is required and must be sent to Virology.
- Do not send IUCD (Intrauterine contraceptive devices) as these are unsuitable for culture

A useful link for guidance on Management & Laboratory diagnosis of abnormal vaginal discharge:
SMI B 28: Investigation of genital tract and associated specimens - Publications - GOV.UK

17.10.1 Vaginal Discharge – Guidance for GP's

Abnormal vaginal discharge is a common complaint in primary care. These notes are intended to help with diagnosing the common causes of this problem.

The normal physiological discharge is usually non-irritant, clear and variable in amount through the menstrual cycle. Symptoms of abnormal discharge may include colour change, irritation/soreness, increase in volume, odour and/or pain on penetration.

The two commonest causes of vaginal discharge are Bacterial Vaginosis and Candidiasis. In both conditions the normal vaginal microbial flora is replaced by overgrowth of organisms that are carried without symptoms by many women. It is important to identify the clinical syndromes produced by that overgrowth as simple cultures merely indicate the presence or absence of an organism - not if it is present behaving as a commensal or a pathogen. This limits the value of the high vaginal swab in diagnosis.

17.10.2 Bacterial Vaginosis (BV)

Bacterial Vaginosis is a clinical syndrome caused by depletion of the normal vaginal *Lactobacillus* population accompanied by overgrowth of anaerobes and *Gardnerella vaginalis*. Studies of unselected populations show prevalence rates of 10 to 20%. It is not a sexually transmitted infection. It may spontaneously arise after menstruation and resolve itself by mid-cycle.

Symptoms/Signs

- Increased volume of discharge, an offensive fishy odour that is worse after sex, minimal soreness, itching or irritation

- Women who are familiar with *Candida* infection will report that "it is not like thrush".
- No vulvitis, vaginitis or cervicitis, a thin grey/white frothy discharge This absence of inflammatory changes is important to note in women complaining of discharge.

Diagnosis

Up to 50% of normal women may carry *Gardnerella vaginalis* so culturing this organism from a high vaginal swab means nothing. The "Amsel" criteria are used for diagnosis and 3 of the following must be present:

- A thin white-grey homogeneous discharge
- pH of vaginal fluid >4.5
- Release of a fishy odour on adding alkali (10% KOH)
- "Clue cells" on microscopy

In primary care only the first two criteria may be possible to comment on. Studies have shown that clinicians can recognise the discharge accurately with a false positive rate of 3% and sensitivity of 69%. Vaginal pH is highly sensitive at 97% but with a false positive rate of 47%. None of the criteria achieve 95% sensitivity and specificity so there is no "gold standard" available for diagnosis. **Most cases of Bacterial Vaginosis can be diagnosed on clinical criteria alone. There is no case for routinely sending specimens to the laboratory to establish this diagnosis.**

Treatment

Treatment is indicated for:

- Symptoms, women undergoing surgery involving the vagina, asymptomatic pregnant women with histories of second trimester loss or pre-term delivery with no known cause.

  Regimes include:
  - Metronidazole 400mg bd for 5 days
  - Intra-vaginal metronidazole gel (0.75%) once daily for 5 days
  - Intra-vaginal clindamycin cream (2%) once daily for 5 days

  Partners do not need treatment.

17.10.3 Candidiasis

Vulvo-vaginal candidiasis is caused by overgrowth of *Candida species* - mainly *C. albicans*. 10-20% of asymptomatic women carry *Candida* and the following may predispose to overgrowth:

- Antibiotics
- Hormonal change e.g. pregnancy
- Diabetes

It is not sexually transmitted.

Symptoms/Signs

Symptoms may be worse just before a period and resolve after the period.

- Vulval itching/soreness
- Vaginal discharge - may be curdy (non-offensive)
- Superficial dyspareunia

- External dysuria
- Vulvitis - sometimes with linear fissuring
- Satellite skin lesions
- Vulval oedema

None of these symptoms or signs is specific for the diagnosis of candidiasis. Other conditions such as allergic reactions, eczema or psoriasis can cause similar vulvitis although usually not with a discharge.

Diagnosis

Diagnosis is usually clinical based on signs and symptoms. *Candida* cultured from the vagina means nothing in the absence of signs and symptoms.

Treatment

There are many creams and pessaries available for topical treatment. Oral treatments with Itraconazole and Fluconazole are not safe in pregnancy or breastfeeding. Asymptomatic male partners do not need treatment.

17.10.4 Trichomonas infection

Trichomonas vaginalis is a flagellated protozoan that is almost exclusively sexually transmitted. Although it is possibly the commonest STD in the world it is not very common in Leicestershire.

Symptoms/Signs

10 - 50% of cases are asymptomatic when diagnosed. 5-15% of women will have no abnormalities on examination.

- Vaginal discharge (may vary from thin and scanty to profuse and thick; the classical discharge of frothy yellow occurs in 10-30% of women)
- Vulval itching
- Dysuria
- Offensive odour
- Vaginal discharge
- Vulvitis
- Vaginitis
- Cervicitis

Diagnosis

- Direct observation by a wet smear will diagnose 40 - 80% cases.
- Culture media are available and up to 95% of cases can be diagnosed by culture.

Trichomonads are sometimes reported on cervical cytology, where the sensitivity is approx. 60 - 80%. But, there is a false positive rate of about 30%. A diagnosis must be confirmed by another method before telling a woman that she has a sexually transmitted infection via a cervical cytology result.

Every woman with *Trichomonas* infection must be screened for other STD's - especially gonorrhoea and chlamydia.

Treatment

Metronidazole 400mg bd for 5 days. In early pregnancy metronidazole may be too emetic as well as there being caution in treating women in the first trimester. Betadine vaginal cream may be used to control symptoms until metronidazole can be safely used. Sexual partners must be seen and treated.

17.10.5 Other Problems Presenting as Discharge

Retained Tampons

Women with retained tampons may be unaware that the tampon is there. They usually have a profuse vaginal discharge with an offensive odour. The diagnosis will be made on examination when removal of the tampon will produce a cure.

Cervicitis

Gonorrhoea and *Chlamydia* produce a cervicitis and may present as an increased volume of non-irritant discoloured mucous discharge. Examination will show a muco-purulent cervical discharge with contact bleeding. Appropriate endocervical tests should be taken for gonorrhoea and chlamydia.

National Guidelines

National Guidelines for the management of all Genital infections can be found at:
http://www.bashh.org/guidelines

17.11 Wound Infections

Aspirated pus is always more useful than a superficial swab that may be contaminated by surface organisms. Please send pus in a dry sterile universal container.

See details in UKHSA SMI B14 (pus) and B11 (skin swabs) found at:
https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Type	Sample Taken	Method for Taking Sample
Pus and Exudates	**Pus, Abscess Swab, Pus swab**	• Collect specimens before antimicrobial therapy where possible. • **Samples of pus are preferred to swabs**. However, pus swabs are often received. When using a swab disinfect the superficial areas first. The deepest part of the wound should be sampled, avoiding the superficial microflora. • Ideally, a minimum volume of 1mL of pus should be submitted. • Swabs should be well soaked in pus. • Cleaning the site with sterile saline or 70% alcohol is recommended by some sources. • Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags. • Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in black top charcoal swabs.

Swabs from leg ulcers and pressure areas should only be taken if there are signs of infection (cellulitis etc). They often yield heavy mixed bacterial flora which may mask the infecting organism. Careful cleaning of the skin and then swabbing or, ideally, aspirating from the edge of the ulcer may be helpful.

Sample Type	Sample Taken	Method for Taking Sample
Investigation of Swabs from Skin and Superficial Soft Tissue Infections	**Skin swab, Swab from Superficial, Non-surgical and Surgical Wounds, Swabs of Pus**	• Collect specimens before starting antimicrobial therapy where possible. • Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in black top charcoal swabs. • If only a minute amount of pus or exudate is available it is preferable to send a pus/exudate swab in transport medium to minimise the risk of desiccation during transport. • Sample a representative part of the lesion. Swabbing dry crusted areas is unlikely to yield the causative pathogen. • If specimens are taken from ulcers, the debris on the ulcer should be removed and the ulcer should be cleaned with saline. **NOTE:** Samples of pus/exudate, if present, are preferred to swabs

Please note that swabs/pus from sinus tracts may also provide misleading culture results. Operative specimens (tissue/bone) are generally required to identify the pathogens causing deep-seated sepsis.

17.12 Superficial Mycoses

See details in UKHSA SMI B39 found at: https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample Taken	Method for Taking Sample
Skin, Nail, Hair	• Care should be taken if using a sharp scalpel blade or scissors to collect samples. • Specimens should be collected into folded paper squares secured and placed in a plastic bag or in commercially available packets (e.g. Dermapak) designed specifically for the collection and transport of skin, nail and hair samples. • Transport specimens in CE marked container in sealed plastic bags. • Specimens adherent to Sellotape should not be sent. • **Skin -** Patients' skin and nails can be swabbed with 70% alcohol prior to collection of the specimen, this is especially important if creams, lotions or powders have been applied. The edges of skin lesions yield the greatest

quantities of viable fungus. Lesions should be scraped with a blunt scalpel blade. Samples in containers achieve the optimum results.

- **Nail -** Good nail samples are difficult to obtain. It should be specified whether the sample is from the fingernails or toenails. Material should be taken from any discoloured, dystrophic, or brittle parts of the nail. The affected nail should be cut as far back as possible through the entire thickness and should include any crumbly material. Nail drills, scalpels and nail elevators may be helpful but must be sterilized between patients. When there is superficial involvement (as in white superficial onychomycosis) nail scrapings may be taken with a curette. If associated skin lesions are present samples from these are likely to be infected with the same organism and are more likely to give a positive culture. Samples from associated sites should be sent in separate packets.

- **Hair -** Samples from the scalp should include skin scales and hair stumps. Cut hairs are not suitable for direct examination as the infected area is usually close to the scalp surface. Scraping for direct examination is the preferable sample collection method, however plastic hairbrushes, scalp massage pads, swabs or plastic toothbrushes may be used to sample scalps for culture where there is little obvious scaling. If sufficiently long, hairs should be plucked with forceps and wrapped in black paper or commercial transport packs together with flakes of skin. Collect specimens other than swabs into appropriate CE marked leak-proof containers and place in sealed plastic bags.

If fungal infection of any site is suspected, please request 'fungal examination'. Please include as much clinical details as possible as this helps the laboratory in selecting the best way of processing the sample. Information such as foreign travel (stating country visited), immune status, repeat sample, diabetes and area of body specimen is taken are especially important. Cleared preparations are examined for hyphae and a report issued.

17.13 Specimens from General Practice

If the microscopy is negative for fungal hyphae, culture will not be attempted. If the microscopy is positive, culture will be performed for the presence or absence of *Candida species* only. If foreign travel is relevant, culture may be carried out if an unusual pathogen is suspected.

17.13.1 Specimens from Dermatology Clinics

These specimens will be cultured and identification performed if possible.

17.13.2 Miscellaneous Sample Collection for Bacterial Culture/Testing

See details in UKHSA SMI B29 (MRSA Screening), B17 (Tissues), B26 (Fluids from Normally Sterile Stes), B20 (Tips and Cannulae), B15 (Bile), B6 (Pertussis), B1 (Ear), B9 (Throat), B4 (Mouth) and B5 (Nose) found at: https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

Sample /Test type	Sample Taken	Method for Taking Sample

Antibiotic assays	**Blood**	• The **Blood Science Laboratory**, Level 4 Sandringham Building, LRI routinely assays **Gentamicin, Vancomycin and Tobramycin, Itraconazole, Posaconazole and Voriconazole.** • Please use combined chemistry/haematology request forms for these requests. State the current dose regimen along with the time of the dose and the time the sample was taken. • If the 7mg/kg regimen is used for Gentamicin a single timed sample taken between 6 and 12 hours after the beginning of the infusion should be sent. For other Gentamicin regimens (e.g. in neonates or the treatment of endocarditis) please send samples before (pre or trough level) and 1 hour after the dose (post or peak levels). **Please ensure that pre- and post-dose Gentamicin samples are clearly labelled as such on both the specimen tubes and the request form.** • For Vancomycin assays only pre-dose levels are monitored. • For Tobramycin assays in patients receiving 10 mg/kg once daily please send a pre-dose level • Blood samples must be collected in brown top serum gel tubes (code Z 4.9ml for adults, 2.7ml for paediatrics). • **Other antimicrobials** (e.g. Cycloserine, Netilmicin, Streptomycin, and Teicoplanin) may also be measured **by arrangement with the microbiology laboratory**, Level 5 Sandringham Building, LRI. These requests are sent to a reference laboratory and there is a 1- or 2-day delay hence please avoid taking these on Fridays or Saturdays (or just prior to Bank Holidays). Please use Clinical Microbiology Request form • Isoniazid levels must be sent using sodium oxalate bottles (grey tops) available on request from Microbiology stores **Samples for antibiotic assays must *not* be taken from a line through which the drug is infused**.
MRSA samples	**Perineum Swab, Nose Swab, Urine, Sputum, Open Wounds**	• Collect specimens before antimicrobial therapy where possible. • Specimens should be transported and processed as soon as possible. • If processing is delayed, refrigeration is preferable to storage at ambient temperature. • Swabs should be placed in appropriate transport medium. Sputum and urines should be collected in the same way as routine samples.
Aspergillus DNA by PCR	**BAL**	• Part of pneumonia PCR panel; all positives confirmed by further in-house PCR assay

Aspergillus Galactomannan Antigen and Beta D Glucan	**Serum.** **Bronchial Alveolar Lavage (BAL) for Galactomannan requests only**	• For investigation and monitoring of invasive aspergillosis • Collect specimens as outlined elsewhere, send plain clotted blood sample (use serum Z/ 9ml Sarstedt collection tube).
Fluids from Normally Sterile Sites	**Amniotic Fluid, Pericardial Fluid, Peritoneal Fluid (Ascites), Pleural Fluid, Synovial (Joint) Fluid, Bursa Fluid**	**Note -** Blood, cerebrospinal fluid, continuous ambulatory peritoneal dialysis (CAPD) fluid, Pouch of Douglas fluid, bile and urine are dealt with elsewhere. • Collect specimens before antimicrobial therapy where possible. • Samples of fluid rather than swabs of the fluids are the preferred specimen type to facilitate comprehensive investigation. • Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags. • Ideally, a minimum volume of 1mL. • Large volume specimens such as peritoneal fluid and ascitic fluid may contain very low numbers of organisms which require concentration in order to increase the likelihood of successful culture. • Small volume fluids such as synovial fluids may be received in insufficient volumes. This may impede the recovery of organisms. • The number and frequency of specimens collected depends on the clinical condition of the patient. • Specimens should be transported and processed as soon as possible • If processing is delayed, refrigeration is preferable to storage at ambient temperature.
Intra-Vascular Cannulae and Assoc'd samples	**Line tips e.g. CVP or Hickman Lines, Swabs of Cannula Sites**	**Note:** Peripheral lines are not suitable specimens for cultures and should not normally be sent to the laboratory for testing. • **Cannulae -** Disinfect the skin around the cannula entry site, remove cannula using aseptic technique, and ideally cut off 4cm of the tip into an appropriate CE marked leak proof container using sterile scissors. • Place in sealed plastic bags for transport. **Note:** Cannulae should only be sent if there is evidence of infection. • **Swabs -** Sample the inflamed area / exudate around the catheter insertion site using an appropriate swab**.**

Bile	**Bile**	• Collect specimens before antimicrobial therapy where possible. • Bile may be collected in theatre or from a closed drainage system by aspiration with a needle and syringe. Ideally, a minimum volume of 1mL. • Collect specimens into appropriate CE marked leak proof containers and place in sealed plastic bags.
***Bordetella pertussis* and *Bordetella para-pertussis* Whooping cough**	**Pernasal swab**	• Collect specimens before antimicrobial therapy where possible • A pernasal swab (Dacron or rayon with flexible ultrafine wire shaft) is inserted through a nostril and advanced along the floor of the nose until it reaches the nasopharynx. It has been suggested that the swab is held against the posterior nasopharynx for up to 30s or until the patient coughs. In practice, it is more likely that a patient will only be able to tolerate this for a few seconds.
Ear Infections and Associated Specimens	**Ear Swab, Middle Ear Effusion**	• Use aseptic technique. • Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags. • Collect swabs into black top charcoal swabs in sealed plastic bags.
Throat Related Sample	**Throat Swab, Pharyngeal/ Naso-pharyngeal Swab, Pharyngeal washings, Pus, Aspirate, Oro-pharyngeal Swab**	• Collect specimens before antimicrobial therapy where possible. • Collect swabs into black top charcoal swabs in sealed plastic bags • Throat swabs should be taken from the tonsillar area and/or posterior pharynx, avoiding the tongue and uvula. • Throat culture should not be taken if the epiglottis is inflamed as sampling may cause serious respiratory obstruction. • Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.
Superficial Mouth Samples	**Mouth Swab**	• Collect specimens before starting antimicrobial therapy where possible. • Collect swabs into black top charcoal swabs in sealed plastic bags

		• To assure that the preconditions of the sampling for oral infections are comparable it is advised that patients should not: 1. Eat or drink within 2 hours 2. Brush their teeth within 2 hours 3. Use any mouth rinse of disinfectant within 2 hours prior to sampling • If possible samples should be taken in the morning under fasting conditions. • Sample any lesions or inflamed areas using cotton tipped swabs. Samples of denture fitting surfaces should also be swabbed as these are more sensitive sites than the palatal mucosa to recover *Candida* species. • The use of a tongue depressor or spatula may be helpful.
Nasal samples	**Nose swab, Antral Washout, Sinus Aspirate, Sinus Washout**	• Collect specimens before antimicrobial therapy where possible. • Collect swabs into black top charcoal swabs in sealed plastic bags • Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

18 Antimicrobial Testing Advice

The laboratory follows the latest European Committee in Antimicrobial Susceptibility Testing (EUCAST) guidelines for both the reporting and interpretation of bacterial/fungal susceptibilities.

18.1 Minimum Inhibitory Concentration (MIC)

Antimicrobial susceptibility results are based on the minimum inhibitory concentration (MIC) – the lowest concentration of antimicrobial which prevents bacterial growth. The MIC of clinical isolates is often inferred from the size of the zone of inhibition on an agar plate - the area around an antimicrobial disc where the bacteria cannot grow.

The exact MIC/zone size at which a bacterial isolate is considered susceptible or resistant is known as the ‘breakpoint’. The breakpoint varies considerably for each agent and organism. Breakpoints are decided by EUCAST and are based on the available pharmacokinetics and clinical outcomes data.

For many organisms the ‘susceptible’ breakpoint does not match the ‘resistant’ breakpoint. For example the Meropenem MIC susceptible breakpoint for *Acinetobacter species* is ≤2mg/L while the resistant breakpoint is >8mg/L. Organisms with a MIC which falls between the breakpoints were previously reported as ‘intermediate’.

18.2 Interpretation of Antimicrobial Results

EUCAST guidelines on the interpretation of antimicrobial results have recently changed. In particular, this has impacted on the interpretation of 'intermediate' susceptibility.

In previous versions of the EUCAST guidelines, 'intermediate' susceptibility was defined as 'a level of antimicrobial agent activity with uncertain therapeutic effect'. However, this did not distinguish between an antimicrobial agent which would still be effective at a higher concentration, versus an agent where there is genuine uncertainty on clinical outcome. In practical terms this meant when an agent was reported as 'intermediate', it was generally avoided as a treatment option.

In the latest guidelines, EUCAST have attempted to address this issue around 'intermediate' susceptibility. The following interpretation criteria are now used by the laboratory:

Interpretation of Antimicrobial Results (from February 2021)		
Category		**Definition**
S	Susceptible, standard dosing regimen	High likelihood of therapeutic success with the agent
I	Susceptible, increased exposure	Increased exposure is required for therapeutic success. 'Increased exposure' usually means an increased dose, though it can also mean increased frequency (i.e. from 3x daily to 4x daily) or altering mode of administration (i.e. prolonged or continuous infusion instead of bolus).
R	Resistant	High likelihood of therapeutic failure

18.3 Reporting of Antimicrobial Results

Unfortunately, the in-use laboratory IT system can only currently report isolates as being 'Susceptible', 'Intermediate' or 'Resistant' - instead of 'Susceptible'(S), 'Susceptible Increased Exposure' (I)and 'Resistant'(R). Where an organism is reported as 'intermediate' to an agent, then the 'high dose' regime should be used – whilst also adjusting for renal function etc.

To aid interpretation of results, from February 2021, the department utilises the following standardised report comment:

> *The criteria for reporting antimicrobial susceptibilities has recently changed.*
>
> *From the end of January 2021 agents reported as 'intermediate' can still be considered effective, provided that the organism has increased exposure to the agent. Increased exposure may be achieved by either increasing the dose, frequency, or mode of administration. A further explanation can be found in the Microbiology handbook. Clinical antimicrobial advice pertaining to this should be sought from the Microbiology team as per usual instruction.*

18.4 Procedure to Use When Considering Using a 'Susceptible Increased Exposure' Regime

When a higher dose regimen is needed for treatment, clinical advice from the Microbiology team should continue to be sought via the electronic referral system (or where urgent by phone). The

Microbiologist will issue a code that indicates a discussion has taken place and Pharmacy will then dispense the antimicrobial agent according to that code.

19 Virology/Molecular Tests Available

The following is a list of the tests available from the department. The tests marked with an asterisk (*) require a ***freshly taken sample*** to reach the laboratory within 4 hours. Tests marked with a hash (#) require prior arrangement with the laboratory. All significant positive results will be notified by telephone.

The following link provides advice and instructions on collection of blood samples using the Sarstedt monovette system in use at UHL:

Sarstedt monovette

Test	Samples Required	Notes
Adenovirus Faecal Antigen	Faeces	For diagnosis of viral gastroenteritis only
Adenovirus DNA by PCR	Respiratory Swab/Secretions, BAL, Endotracheal Secretions, Nasopharyngeal Aspirate (NPA), Throat Swab or Combined Nose and Throat Swabs, Eye Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples
Antibiotic Levels	Serum	Reference laboratory tests NB Isoniazid levels must be sent using sodium oxalate bottles (grey tops) available on request from Microbiology stores
Bocavirus DNA by PCR	Respiratory Swab/Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Eye Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples
Bordetella pertussis DNA by PCR	BAL	Part of pneumonia PCR panel but all positives confirmed by Reference Laboratory test
Bordetella pertussis Serology	Serum	Reference Laboratory test

Test	Samples Required	Notes
Borrelia burgdorferi **Serology**	Serum	For investigation of Lyme disease. Reference Laboratory confirmation of positives.
Brucella Serology	Serum	Reference Laboratory test
Chlamydia psittaci and pneumoniae DNA by PCR	BAL	Part of pneumonia PCR panel
Chlamydia trachomatis **DNA Detection by NAAT**	Urine, Throat Swab, Vulvo-Vaginal Swab, Rectal Swab, Eye Swab	Testing is performed for both Chlamydia trachomatis and Neisseria gonorrhoea on all submitted samples. *See *Sexually transmitted infection sample collection for details of swab/urine collection.*
CMV IgG	Serum	Screening test for exposure to the virus. Testing in parallel to investigate possible seroconversion during pregnancy.
CMV IgG Avidity Test	Serum	Mainly for pregnant women, to exclude or confirm recent primary infection
CMV IgM	Serum	Suggestive of recent infection, or reactivation, if positive.
CMV DNA by Quantitative PCR	EDTA, Urine, Throat Swab, Amniotic Fluid	Test for active infection, especially in immuno-compromised patients, monitoring levels
CMV DNA by PCR	BAL, Biopsy Tissue (e.g. Placental), CSF	Test for active infection, especially in immuno-compromised patients
Coronavirus (Non-MERS/SARS) RNA (Types NL63, OC43, HKU1, 229) by PCR **SARS-Cov-2 RNA by PCR**	Respiratory Swab/Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Eye Swabs, Tissue	In-house respiratory panel. Variety of in-house PCR and molecular assays available
Coxiella burnetii **DNA by PCR**		Part of pneumonia PCR panel but all positives confirmed by Reference Laboratory test
Coxiella burnetii **(Q Fever) Serology**	Paired Serum	Currently unavailable
Coxsackie Virus		*See* ***Enterovirus***
Cryptococcus antigen	Serum, CSF	Investigation of possible cryptococcal meningitis in immunocompromised patients
Cryptococcus neoformans DNA by PCR	BAL	Part of pneumonia PCR panel but all positives confirmed by Reference Laboratory test
Dengue Virus Serology **Dengue RNA by PCR**	Serum EDTA	Reference Laboratory Test.
Diphtheria Serology	Serum	Reference Laboratory test
Echo Virus (including Parechovirus)		*See* ***Enterovirus***

Test	Samples Required	Notes
Enterovirus RNA by PCR (including Poliovirus*, Coxsackie, Echo and Parechovirus)	CSF Respiratory Swab/Secretions, BAL, Endotracheal Secretions, NPA, Throat and/or Nose Swabs, Tissue Faeces	*To exclude wild poliovirus, all Enterovirus positives from neurological patients are sent to reference laboratory. It is advised to send Faeces and EDTA blood samples on receipt of a positive result by PCR if clinical suspicion of Enterovirus infection exists (e.g. Sepsis, increased irritability, or poor feeding in infants)
Epstein Barr Virus (EBV) anti-Viral Capsid Antigen (VCA) IgM	Serum	Suggestive of recent infection or reactivation, if positive
Epstein Barr Virus (EBV) anti-Viral Capsid Antigen (VCA) IgG	Serum	Screening for exposure to the virus
Epstein Barr Virus (EBV) anti-Nuclear Antigen (EBNA) IgG	Serum	Tests for convalescence post EBV infection
Epstein Barr virus (EBV) DNA gby PCR	EDTA, Urine, Throat Swab	Test for active infection, especially in immuno-compromised patients, monitoring levels
Gonococcal (*Neisseria gonorrhoeae*) DNA Detection by NAAT	Urine, Throat Swab, Vulvo-Vaginal Swab, Rectal Swab, Eye Swab	Testing is performed for both Chlamydia trachomatis and Neisseria gonorrhoea on all submitted samples. *See *Sexually transmitted infection sample collection for details of swab/urine collection*
Hepatitis A IgM	Serum	Indicative of acute infection if positive.
Hepatitis A Total Ab	Serum	Immunity screen; past infection /immunisation
Hepatitis B (HBV) Serology Screening With HBsAg Total AntiHBc, HBc IgM, HBeAg And AntiHBe Markers	Serum, Including Antenatal Serum, Dried Blood Spots (DBS)	Screening for infection, including Infectious Diseases in pregnancy screening (IDPS), monitoring disease progression, determination of acute/recent infection, previous exposure/infection and levels of infectivity. All reactive samples undergo in-house confirmatory testing. A second blood sample is required to confirm identity of patient following positive result.
Hepatitis B Surface Antibody (HBsAb, Anti-HBs)	Serum	Indicates immunity post vaccination or, if HBcAb positive in the absence of surface antigen, resolved infection.
HBV DNA by NAAT	Serum/Plasma	Monitoring infectivity in response to treatment, measure of infectivity and

Test	Samples Required	Notes
		decision-making in relation to prevention of vertical transmission.
HBV Resistance Profile and Genotype	Serum/Plasma	Reference Laboratory Test for monitoring resistance
Hepatitis C (HCV) Serology	Serum, DBS	Screening for infection/exposure. All reactive samples undergo in-house confirmatory testing. A second blood sample is required to confirm identity of patient following positive result.
HCV RNA by NAAT	Serum, DBS	Acute or chronic infection, monitoring treatment effectiveness
HCV Genotype	Serum	Reference laboratory
Hepatitis D (Delta) Serology, HDV Antigen/ RNA By PCR	Serum	Available after consultation with the laboratory, and only appropriate for HBV positive patients
Hepatitis E (HEV) Serology IgG/IgM	Serum	HEV IgG and/or IgM testing available
HEV RNA by PCR and Genotyping	Serum	Reference lab test for acute infection of transplant patient monitoring and epidemiology
HIV 1 & 2 Serology (HIV Ag-Ab and Typing)	Serum, Including Antenatal Serum, DBS	Screening for infection, including Infectious Diseases in pregnancy screening (IDPS). All reactive samples undergo in-house confirmatory testing. A second blood sample is required to confirm identity of patient following positive result.
HIV RNA by NAAT	EDTA	Viral load for monitoring response to treatment in known positive patients or for early-stage screening in high-risk exposure incidents
HIV Proviral DNA	Whole EDTA	Must be received by 4pm on day of collection. For evaluation of vertical transmission. Reference laboratory
HIV Resistance Profile (Reverse Transcriptase, Protease and Integrase) HIV Tropism	EDTA	Reference laboratory test. Only available if viral load >200 copies/ml
HIV Therapeutic Drug Monitoring (HAART)	EDTA	Include specific form and drug/dose details
HTLV I/II Serology	Serum	Reactive specimens require confirmation at reference lab.
Herpes Simplex Virus (HSV) IgG	Serum	Evidence of past exposure
HSV DNA by PCR	CSF, Eye Swabs, Tissue EDTA	Diagnosis of herpes infection, including meningitis Monitoring levels

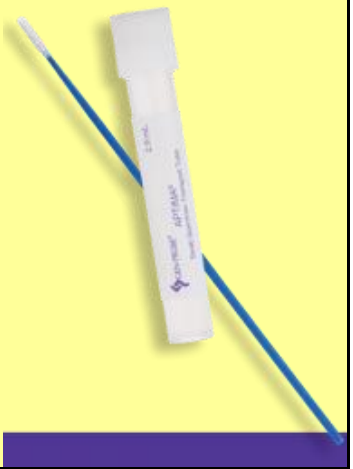
Test	Samples Required	Notes
HSV DNA by NAAT	Genital, Skin,	Diagnosis of herpes infection, primarily genital infections. *See *Sexually transmitted infection sample collection for details of swab/urine collection*
Influenza Types A and B Virus (including Typing for H1N1 Pdm09 And H3) **Influenza A further Typing for H5, H7, H9 other Avian Influenza Viruses** **Influenza A Sequencing and Oseltamivir Resistance Testing**	Respiratory Swab/ Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples Positive Influenza samples are referred for epidemiological sequence analysis during the Winter season. Reference laboratory testing following positive Influenza A result and discussion of risk with the Clinical Virologist
***Legionella pneumophila* and *L. longbeachae* DNA by PCR**	BAL	Part of pneumonia PCR panel
Leptospira IgM/IgG	Serum	Reference Laboratory test. For investigation of Weil's disease. Seroconversion may take up to 6 weeks.
Measles IgG	Serum	Immunity screen
Measles IgM	Serum	Diagnosis of acute/recent infection. Reference Laboratory test
Measles RNA by PCR	Urine, EDTA, CSF	Diagnosis of acute/recent infection in immunocompromised patients
Meningococcal Antigen/ PCR	EDTA Blood	Reference laboratory test
MERS Coronavirus	Sputum, Acute Serum and Duplicate Nose and Throat Swabs	Reference laboratory: Contact the Clinical Virologist to discuss potential cases prior to taking specimens
Metapneumovirus RNA Types A and B by PCR	Respiratory Swab/ Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples
Mumps IgM	Serum	Acute infection
Mumps IgG	Serum	Past infection or response to immunisation
Mumps RNA by PCR	Urine, EDTA	Acute infection

Test	Samples Required	Notes
Mycoplasma Genitalium DNA by PCR	Genital Swabs, Urine	Reference laboratory test
Mycoplasma pneumoniae DNA by PCR	BAL	Part of pneumonia PCR panel
Mycoplasma Pneumonia Serology	Serum	Currently unavailable
***Neisseria gonorrhoeae* (GC) DNA Testing by NAAT**	Urine, Throat Swab, Vulvo-Vaginal Swab, Rectal Swab, Eye Swab	Testing is performed for both Chlamydia trachomatis and Neisseria gonorrhoea on all submitted samples. *See *Sexually transmitted infection sample collection for details of swab/urine collection*
Norovirus Antigen	Faeces	Please send loose, **UNFORMED** stools only, taken **WITHIN THREE DAYS of ONSET** of symptoms. For outbreaks of viral gastroenteritis contact lab. Always send separate samples for Virology and bacteriology if possible.
Parainfluenza (PIV) Types 1 To 4 RNA by PCR	Respiratory Swab/ Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples
Parasitic Serology including Amoebic (Entamoeba histolytica), Fasciola, Filaria, Hydatid, Leishmania, Malaria, Schistosoma, Strongyloides, Toxocara, Trichinosis and Trypanosoma	Serum	Reference Laboratory Tests.
Parvovirus B19 IgG and IgM	Serum	Past or recent infection. Testing in parallel to investigate possible seroconversion during pregnancy.
Parvovirus B19 DNA by PCR	Serum/EDTA	Reference laboratory test
***Pneumocystis jiroveci* DNA by PCR**	BAL or Induced Sputum only, Do NOT Send EDTA Blood	Part of pneumonia PCR panel but all positives confirmed by Reference Laboratory test
Poliovirus		*See **Enterovirus***
Polyomavirus **BK DNA by PCR** **JC DNA by PCR**	 Urine, EDTA CSF, EDTA	 Reference laboratory test Reference laboratory test

Test	Samples Required	Notes
Respiratory Syncytial Virus (RSV) Types A and B RNA By PCR	Respiratory Swab/ Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples
Rhinovirus (Hrv) RNA by PCR	Respiratory Swab/Secretions, BAL, Endotracheal Secretions, NPA, Throat Swab or Combined Nose and Throat Swabs, Eye Swabs, Tissue	Part of respiratory virus PCR panel; please do not send sputum samples
Rickettsial Serology	Serum	Reference laboratory test
Rotavirus	Faeces	Please send loose, UNFORMED stools only, taken WITHIN THREE DAYS of ONSET of symptoms. For outbreaks of viral gastroenteritis contact lab. Always send separate samples for Virology and bacteriology if possible.
Rubella IgG	Serum	Past infection or immunisation
Rubella IgM	Serum	Indicative of acute infection/re-infection.
Streptococcal Serology	Serum	ASOT Test
***Toxoplasma gondii* IgG**	Serum	Past or recent infection screen
***Toxoplasma gondii* IgM, IgA and Dye Test**	Serum	Reference laboratory test
Toxoplasma gondii DNA by PCR	CSF, EDTA	Reference laboratory test
***Treponema pallidum* (Syphilis) IgG Screen**	Serum, Including Antenatal Serum	Screening for infection, including Infectious Diseases in pregnancy screening (IDPS).
***Treponema pallidum* confirmation by TPHA and RPR**	Serum, Including Antenatal Serum, CSF	Current/active infection or re-infection. RPR may be used to investigate possible vertical transmission. CSF may be tested to investigate possible neurosyphilis if serum is positive.
Treponema Pallidum IgM and Status Confirmation	Serum, Including Antenatal Serum	Reference laboratory test

Test	Samples Required	Notes
Ureaplasma urealyticum by PCR	Genital Swabs, Urine	Reference laboratory test
Varicella Zoster (VZ) IgG	Serum	Past infection/immunisation. Contact Virology to arrange booking blood testing following exposure during pregnancy.
Varicella Zoster DNA by PCR	Lesion/Skin Swab, CSF, Eye Swab, Tissue	Acute chickenpox infection/ confirmation of shingles
Varicella Zoster DNA by PCR	EDTA/BAL	Reference Laboratory test
Viral Haemorrhagic Fever Virus Testing, Including Ebola Virus PCR/Serology	Various	Reference laboratory: Contact the Clinical Virologist to discuss potential cases prior to taking specimens
Zikavirus IgM And IgG Serology (Convalescent Phase / Exposure Assessment) **RNA by PCR (Acute Phase)**	Serum Urine, Semen	Reference laboratory test following discussion with the Consultant Clinical Virologist Requesting is based on travel to endemic areas and is focussed on infection of pregnant patients.

20 Sexually Transmitted Infection Sample Collection

DESCRIPTION OF PRODUCT	APTIMA URINE COLLECTION DEVICE	APTIMA MULTITEST SWAB COLLECTION DEVICE	APTIMA UNISEX (CX / URE) SWAB COLLECTION DEVICE
TUBE TYPE			
SUPPLIER PRODUCT CODE	301040	PRD-03546	301041
PURPOSE OF COLLECTION KIT	**For Urines only**	**For Vaginal, Throat, Rectal, Penile Meatal, Eye swabs, HSV swabs**	**For Endocervical and urethral swabs**

21 Virology Clinical Algorithms

21.1 Post-mortem Samples

***Please note that as tissue samples are predominantly post-mortem, the turnaround times for results are expected to be 8-10 days, regardless of the combination of tests requested.**

Post-mortem tissue samples are processed for a panel of Virology tests as advised by the Consultant Clinical Virologist according to the algorithm below:

Sample Type	Test Code	Additional Considerations	Testing Laboratory
Heart	HSV type 1&2, VZV, enterovirus, adenovirus and CMV PCR	If Myocarditis, request Parvovirus B19 PCR for heart tissue	Parvovirus request to be sent to Colindale if necessary
Spleen/Liver Tissue	HSV type 1&2, VZV and CMV PCR	N/A	All in-house
Lung/Tracheal Tissue	Respiratory PCR panel	Add HSV 1&2, VZV & CMV PCR for immunocompromised patients	All in-house
Post-Mortem Respiratory samples (including Nasal/Throat/Oro-pharyngeal swabs, ET aspirates, BALs)	Respiratory PCR panel	Add HSV1C, HSV2C, VZVC & CMVDNA for immunocompromised patients	All in-house
Brain Tissue	CSF	Add CMV, EBV adenovirus, JC & HHV-6 (Human herpesvirus type 6) DNA for immunocompromised patients	If JC & HHV6 DNA required, this is to be sent to Micropathology
Post-mortem CSF / Meningeal swab	CSF	Add CMV, EBV adenovirus, JC & HHV 6 DNA for immunocompromised patients	If JC & HHV6 DNA required, this is to be sent to Micropathology
Post-Mortem Bowel Biopsy / Tissue	Enterovirus RNA, adenovirus and CMV DNA	For **non-PM bowel** Biopsy/Tissue CMV PCR **ONLY** (if requested) otherwise refer to Medics	Send Bowel biopsies to Micropathology
Other	N/A	Refer to Medics for Guidance	Dependant on sample type

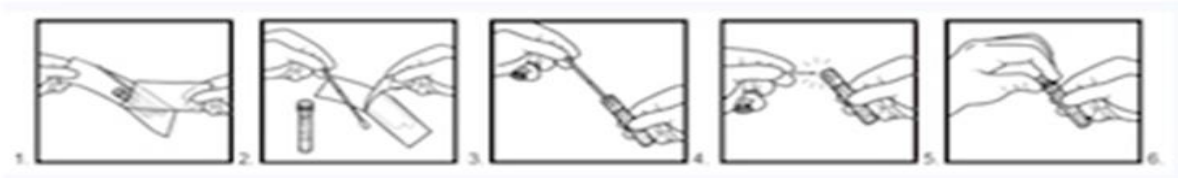
21.2 Eye Swabs/Samples

Eye swab samples are processed in Virology dependant on the clinical presentation as follows:

Sample Type Required	Initial Investigation	Additional Clinical Details
Routine Eye or Conjunctival swabs in VTM	**Adenovirus PCR** and **HSV PCR**	• Add **VZV PCR** if clinical details include chicken pox or shingles. • Add **Enterovirus PCR** if clinical details state haemorrhagic.
Conjunctivitis in neonates ≤ 1 month: as above **plus** additional Eye or Conjunctiva swab in **Aptima Collection Tube**	**Chlamydia and *N. gonorrhoeae* NAAT and confirmation of GC positives as necessary**	• Laboratory requests an Eye swab in Aptima collection tube for Chlamydia if only VTM sample tube received.
Vitreous Fluid or Tissue from the Eye (e.g. Corneal Biopsy)	Send to Reference laboratory for **Adenovirus PCR** and **HSV PCR**	• Add **VZV PCR** if clinical details include chicken pox or shingles. • Add **Enterovirus PCR** if clinical details state haemorrhagic conjunctivitis.
Suspected Trachoma: Eye or Conjunctiva swab in **Aptima Collection Tube**	**Chlamydia NAAT**	• Laboratory requests an Eye swab in Aptima collection tube for Chlamydia if only VTM sample tube received.

22 Specimen Collection for Specific Virology Tests Requested

Specimen	Container	Notes
CSF	Plain sterile universal	**See CSF samples page for details**
Faeces for Norovirus, Rotavirus, Adenovirus or Enterovirus PCR	Dedicated sterile faeces pot 	Please send loose, **UNFORMED** stools only, taken **WITHIN THREE DAYS of ONSET** of symptoms For outbreaks of viral gastroenteritis contact lab.
Chlamydia DNA and GC	Urine in plain sterile universal or transferred into Aptima collection tubes Swabs (Genital, Throat, Rectal and Eye) in special Chlamydia collection kits	Available from laboratory: Aptima collection tubes MUST be filled to between the lines as indicated on the tube
HIV RNA and Resistance Profile, HBV DNA and Genotype	2 x 4.9 ml EDTA blood **Samples must be received in the lab within 6 hours of collection**	Use 1.2ml monovette for paediatric EDTA samples.

Specimen	Container	Notes
HIV proviral DNA	4.9 mL EDTA blood **Must arrive by 3pm on day of collection for forwarding to reference laboratory**	Use 1.2ml monovette for paediatric EDTA samples
Broncho-Alveolar Lavage, Endotracheal Secretions, Induced Sputum	Plain 60ml container or sterile universal (to lab within 2 hours)	Induced sputum is only suitable for Pneumocystis PCR
Sterile Samples for Bacterial 16S DNA Detection and Sequencing	As appropriate for sample type (see above)	Only available after discussion with a Consultant Microbiologist
Tissue	Plain sterile universal, with or without saline	Do not send tissue in formalin as this is unsuitable for Virology testing
Urine for Pneumococcal and Legionella Urinary Antigen Testing	Either plain sterile container or universal, or boric acid container is suitable.	Fill to indicated line if using boric acid container.
Viral and Bacterial Serology	Plain clotted blood sample (serum Z/7.5mL); serum gel tubes are accepted	Use 1.2ml monovette for paediatric samples
Infectious Diseases in Pregnancy (IDPS) Antenatal Screening	Plain clotted blood sample (serum Z/7.5mL); 4.9mL serum gel tubes are accepted	A minimum of 5mL of clotted blood is required for testing of all markers.
HCV RNA/Genotype	Plain clotted blood sample (serum Z/7.5mL) **Samples must be received in the lab within 6 hours of collection**	Use 1.2ml monovette for paediatric samples
Viral Nucleic Acid Detection (Throat, Eye, Skin Swabs, Vesicular Aspirates etc)	Virus Transport Medium (VTM) 	Available from laboratory NB From May 2020, considering the SARS-CoV-2 pandemic, other types of Virus Transport medium have been validated for use in Virology testing. Please contact the laboratory with any queries.
		
Viral Nucleic Acid detection (Urine, NPA, BAL, fluid)	Plain sterile container or universal	Urine for virology must NOT be sent in boric acid container.
Viral Nucleic Acid Detection in blood	4.9 or 7.5 ml EDTA blood	Use 1.2ml monovette for paediatric EDTA samples

23 Hazardous Pathogens & their Associated Clinical Conditions

Most infections which constitute a high risk are acquired outside the UK. Diseases marked with an asterisk (*) may rarely be acquired in this country. Cases of suspected **Viral Haemorrhagic Fever** or other hazard group 4 viruses must be discussed with a Consultant in Infectious Diseases and/or the Consultant Medical Virologist before admission and collection of specimens.

Clinical Condition	Hazardous Pathogen
	Bacteria
*Anthrax	Bacillus anthracis
Brucellosis	*Brucella spp.*
Tularemia	*Francisella tularensis*
*Tuberculosis/atypical mycobacterial disease	*Mycobacterium spp.*
Glanders	*Burkholderia mallei*
Melioidosis	*Burkholderia pseudomallei*
Typhus	*Rickettsia spp.*
	Erlichia sennetsu (*Rickettsia sennetsu*)
*Typhoid fever	*Salmonella Typhi*
*Paratyphoid fever	*Salmonella Paratyphi* A/B/C
Plague	*Yersinia pestis*
Severe bacillary dysentery	*Shigella dysenteriae*
*Bloody diarrhoea/HUS	*E. coli* O157
Psittacosis	*Chlamydophila psittaci (avian strains)*
Q Fever	*Coxiella burnetii*
	Fungi
Blastomycosis	*Blastomyces dermatitidis*
Histoplasmosis	*Histoplasma spp.*
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*
Coccidiosis	*Coccidioides immitis*
	Cladophialophora bantiana
	Talaromyces marneffei (Penicillium marneffei)
	Parasites
*Hydatid disease	*Echinococcus spp.*
Leishmaniasis	*Leishmania spp.*
*Amoebic meningoencephalitis	*Naegleria spp.*
South American trypanosomiasis	*Trypanosoma cruzi*
	Naeglaria fowleri
	Plasmodium falciparum
Cysticercosis	*Taenia solium*
	Viruses
	*Human Immunodeficiency Virus (HIV)
	*Human T-cell Leukaemia Virus-1 & 2 (HTLV)
	*Hepatitis B
	*Hepatitis C
	*Hepatitis D/E
	Herpes Virus B
	Rabies
	Arthropod borne Encephalitis

	Dengue Yellow Fever West Nile Fever SARS SARS-CoV-2 (??) *CJD/variant CJD and other TSEs Alphaviruses Arenaviruses Bornaviridae Bunyaviridae Flaviviruses Hantaviruses, Rhabdoviridae, Togaviridae

24 Notifiable Diseases

The doctor who makes a clinical diagnosis of a notifiable infectious disease is responsible for the notification as a statutory duty and should contact the UKHSA's Health Protection Team (HPT) in the East Midlands. Those conditions highlighted in Section 23.1 with an *asterisk are considered **urgent** and should be telephoned to the HPT. For non-urgent cases, the notification form can be downloaded from https://www.gov.uk/guidance/contacts-phe-health-protection-teams#east-midlands-hpt

East Midlands UKHSA Health Protection Team

UK Health Security Agency
Seaton House
City Link
Nottingham
NG2 4LA
Telephone: 0344 225 4524 (option 1)

If the laboratory identifies the causative organism this is reported directly to the UKHSA.

24.1 Diseases Notifiable by Law under Health Protection (Notification) Regulations 2010

Acute Encephalitis	Food Poisoning (*if part of cluster or outbreak)	Plague*
Acute Meningitis (*if suspected bacterial)	Haemolytic Uraemic Syndrome (HUS)*	Rabies*
Acute Poliomyelitis*	Infectious Bloody Diarrhoea*	Rubella
Acute Infectious Hepatitis*	Invasive Group A Streptococcal Disease* and Scarlet Fever	Severe Acute Respiratory Syndrome (SARS)*
Anthrax*	Legionnaires' Disease*	Smallpox*
Botulism*	Leprosy	Tetanus (*if associated with injecting drug use)
Brucellosis (*if UK-acquired)	Malaria (*if UK-acquired)	Tuberculosis
Cholera*	Measles*	Typhus

Diphtheria*	Meningococcal Septicaemia*	Viral Haemorrhagic Fever (VHF)*
Enteric Fever* (Typhoid or Paratyphoid Fever)	Mpox (Monkeypox)*	Whooping Cough (*if diagnosed in acute phase)
	Mumps	Yellow Fever (*if UK-acquired)