Alder Hey Children's NHS
NHS Foundation Trust

A User's guide to The Medical Microbiology Department

An accredited laboratory, under the United Kingdom Accreditation Service

Laboratory Number: 9091

1. Contents

1. Introduction

This handbook is intended to give an overview of the microbiology service at Alder Hey, and to provide outline guidance for the collection of samples for the diagnosis of infection. It is not intended to be a complete guide to all situations and advice can always be sought from the microbiology consultants or scientists.

A proportion of our testing is sent to external laboratories- Liverpool Clinical Laboratories provide the majority of our virology services. Where samples are processed by external providers, the details are recorded on the Alder Hey report. If additional tests are required on samples processed elsewhere please approach the Alder Hey laboratory rather than going directly to the external laboratory. The abbreviations used for the external laboratories can be found at the end under “List of Referral Laboratories”.

We welcome suggestions on how this handbook can be improved; please contact the Lead Biomedical Scientist Mrs Janine Jackman (see the section “Contact Details” below).

COVID-19

For information on the transport and collection of specimens for COVID-19 (SARS Cov-2) please refer to the COVID-19 hub on the Trust intranet

1.1. Useful websites

- Alder Hey Laboratory Medicine (Intranet): http://intranet/ClinicalSupport/SitePages/Pathology.aspx
- Alder Hey Antimicrobial and Infection Guidance (Intranet): http://intranet/DocumentsPolicies/SitePages/Antimicrobials.aspx
- Public Health England (External): PHE provides information to both the public and healthcare professionals in respect to infectious diseases. https://www.gov.uk/government/organisations/public-health-england
- The European Committee on Antimicrobial Susceptibility Testing (External): EUCAST deals with antimicrobial breakpoints and technical aspects of susceptibility testing. www.eucast.org
- COVID -19 HUB on intranet https://alderheynhsuk.sharepoint.com/sites/COVID19/SitePages/COVID-19-INFORMATION-HUB.aspx

  UKAS: ISO 15189 Scope of accreditation

- https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/9091-Medical-Single-1.pdf

2. Notifiable Infections

The following diseases are notifiable under the Health Protection (Notification) Regulations 2010:

Acute encephalitis	Measles
Acute infectious hepatitis	Meningococcal septicaemia
Acute meningitis	Mumps
Acute poliomyelitis	Plague
Anthrax	Rabies
Botulism	Rubella
Brucellosis	Severe Acute Respiratory Syndrome (SARS)
Cholera	Scarlet fever
Diphtheria	COVID-19 (SARS CoV-2)
Enteric fever (typhoid or paratyphoid fever)	Smallpox
Food poisoning	Tetanus
Haemolytic uraemic syndrome (HUS)	Tuberculosis
Infectious bloody diarrhoea	Typhus
Invasive group A streptococcal disease	Viral haemorrhagic fever (VHF)
Legionnaires' disease	Whooping cough
Leprosy	Yellow fever

Malaria

Report other diseases that may present significant risk to human health under the category 'other significant disease'.

A positive culture / PCR / serology result is not required for notification; notification should be performed as soon as possible if any of the above are clinically suspected. The Cheshire and Merseyside Health Protection Team can be contacted on 0344 225 0562.

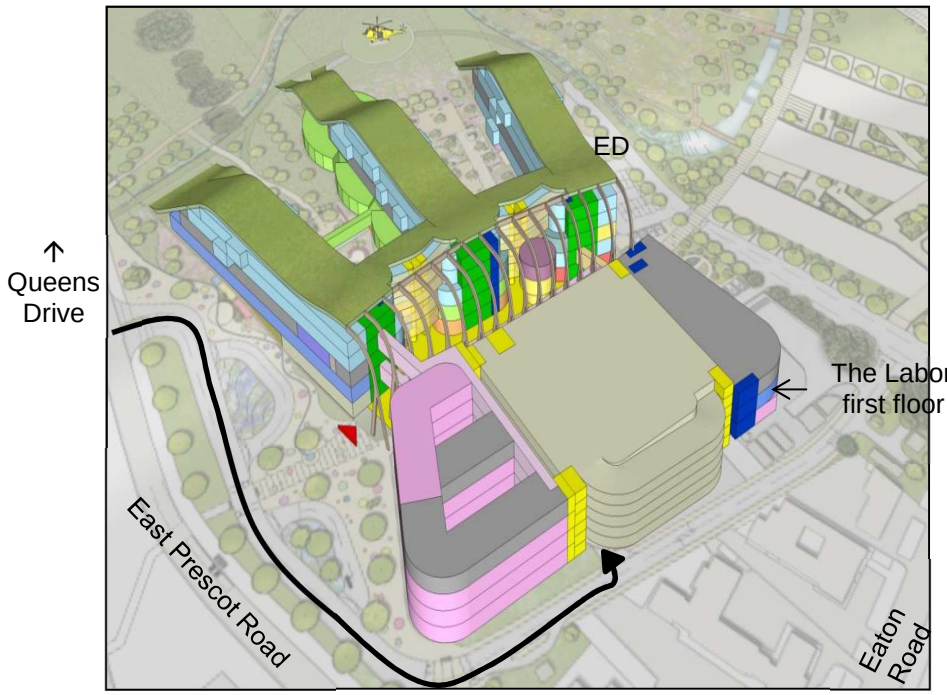
3. General Information

The Alder Hey Children's Medical Microbiology Department is a specialist paediatric Microbiology Laboratory which serves both the clinicians and patients of the hospital as well General Practitioners from Liverpool and the surrounding areas.

The aim of the laboratory is to provide an efficient patient-centred microbiology service which improves the investigation and management of infectious diseases in children.

Any samples that cannot be processed on-site are referred to UKAS approved laboratories, with the exception of Bartonella serology which is sent to a referral laboratory in Marseilles, France.

3.1. **Where to find us:**



The entrance to the multi-story visitor car park is located off East Prescot Road. There is a drop off point for A&E patients at the Eaton Road entrance.

External visitors should exit the atrium via the rotating doors next to WHSmith; there is an intercom button that connects to specimen reception next to the double doors to the right.
Staff can access specimen reception on the first floor next to the large lecture theatre.

3.2. Contact Details

Postal Address	Microbiology Department Alder Hey Children's NHS Foundation Trust Eaton Road West Derby Liverpool L12 2AP
Sat-Nav Postcode	L14 5AB
Telephone	Main switchboard - 0151 228 4811 then request extension as indicated below
Hays DX Address	DX6961702 Old Swan 90L
E-mail	microbiology@alderhey.nhs.uk Please note this is a generic e-mail address which may be seen by any of the microbiology medical or senior scientific staff. Please do not use for urgent clinical queries.

3.3. Results / Enquiries

- **Monday – Friday: 8.30am – 5pm**
  Contact the pathology reception on ext.3591 - direct line – 0151 293 3591
  Any other time contact the laboratory on ext. 2268

Please note: Results cannot be given to or discussed with family members.

4. Principal Services

Access to consultative and principal diagnostic services are available on a 24 hour basis.

4.1. Clinical Service

4.1.1. Clinical Microbiology Consultants

5. Staff member	Title	Ext.	Direct (0151)	Email
Dr Mukul Acharya	Locum Consultant Medical Microbiologist			Mukul.Acharya@alderhey.nhs.uk
Prof. Nigel Cunliffe	Consultant Medical Microbiologist (Part-time)			NigelC@liverpool.ac.uk
Dr Anna Smielewska	Consultant Virologist & Strategic Lead for Virology	4082	Tuesdays	Anna.Smielewska@liverpoolft.nhs.uk
Dr Beatriz Larru	Consultant Paediatric Infectious Diseases. Trust Infection Control Doctor & DIPC			Beatriz.Larru@alderhey.nhs.uk

5.1.1. Availability of Medical Consultant Services

Advice is available from either a Consultant Medical Microbiologist or a Consultant in Infectious Diseases and Immunology seven days a week. The Consultant Medical Microbiologists can be contacted using the telephone numbers above or via the hospital switchboard. The Paediatric Infectious Diseases and Immunology team can be contacted via switchboard or on bleep 244.

The out–of–hours clinical service is provided jointly by the Consultant Medical Microbiologists and the Paediatric Infectious Diseases Consultants.

- **A Consultant is available at all times for urgent clinical advice – contact is via switchboard.**

5.2. Diagnostic Service

The department provides a comprehensive microbiological service in medical bacteriology, mycology, virology, parasitology and serological investigations. Advice on the selection of appropriate diagnostic specimens, their collection and transport is available.

5.2.1. Senior Laboratory Scientists

Staff member	Title	Ext.	Direct (0151)
Mrs Janine Jackman	Lead Biomedical Scientist	2267	252 5267
Mrs Fiona Shaw	Senior Biomedical Scientist	2268	252 5268
Mrs Kathryn Ball	Senior Biomedical Scientist	2268	252 5268

5.2.2. Laboratory Hours

Routine laboratory hours are Monday to Friday, 9 am to 5.30 pm, with a reduced service at weekends. An on-site service is available between 5.30 pm and 11 pm during the week and between 9 am and 11 pm at weekends and bank holidays to process any samples that are considered urgent. The BMS must be contacted in the laboratory on extension 2268 (or via mobile number through switchboard after 5.30pm), with the details of the request and how the specimen is being transported to the laboratory.

5.2.3. Urgent samples

Requests for urgent specimens to be processed after 11pm should be directed to the on-call Biomedical Scientist through switchboard. The following requests will be processed out-of-hours:

- Urine samples on children less than 6 months of age for microscopy, culture ± direct sensitivity (if positive for leucocyte esterase or nitrites on dipstick).
    - NB. Urines should be screened by dipstick by the requestor.
    - BMS staff are only expected to process dipstick positive samples out of hours.
- Urine samples from patients with known renal problems if the dipstick is positive – no age limits apply.
- CSF microscopy and culture
    - Please ensure the sample is ready to send to the laboratory before you contact the BMS or call them in from home.
- Material from sterile sites, e.g. synovial fluid, peritoneal fluid
- Pus from deep seated abscesses

Other requests can be discussed on a case-by-case basis with the Consultant on duty.

5.2.4. Results of Particular Clinical Significance

Significant results are phoned through to the ward or relevant medical staff, irrespective of whether the original request is marked as urgent or routine. Significant results are also passed to the Paediatric Infectious Diseases team.

5.2.5. Delays in the Examination Process

In the event of a significant delay in the examination of any sample, a comment will be added to the sample on Meditech and the Lead Biomedical Scientist or Consultant Microbiologist will inform the requesting doctor by email. Delays may be due to technical failure, failure of equipment or failure to supply by the manufacturer.

In the event of an extended delay samples will be sent to an accredited external laboratory for processing.

5.2.6. Teaching and Training

The Department of Microbiology supports scientific and professional training for its staff, as well as the teaching of science students attending local universities and colleges.

The department welcomes enquiries from staff members who require a basic insight into microbiology services. Please contact the Lead Biomedical Scientist, Janine Jackman, with any enquiries.

5.3. **Environmental Microbiology**

The Microbiology department undertakes a number of screening programmes throughout the Trust. This includes aerobiology of high risk areas. Please note these procedures are not covered by United Kingdom Accreditation Service (UKAS)

5.4. **Epidemiology**

The laboratory contributes to national epidemiological surveillance via Public Health England. As standard, invasive strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Pseudomonas aeruginosa* are referred for strain typing.

5.5. **Principal working relationships**

5.5.1. Infection Prevention and Control

The Director of Infection Prevention and Control (DIPC) is the Medical Director, Dr Beatriz Larru. For day-to-day infection control matters, the Infection Prevention and Control Team (IPCT) should be contacted.

The Infection Prevention and Control Team:

Role	Name		Contact
DIPC/Infection Control Doctor Alder Hey	Dr Beatriz Larru		Beatriz.Larru@alderhey.nhs.uk
IPC line manager / Deputy Director of Allied Health Professionals	Veronica Greenwood		veronica.greenwood@alderhey.nhs.uk
IPC Lead Nurse	Jo McLean	2338	josephine.mclean@alderhey.nhs.uk
IPC Nurse Specialist	Tracey Styles		Tracy.Styles@alderhey.nhs.uk
IPC Nurse Specialist	Uzoamaka 'Anita' Ukah		uzoamaka.ukah@alderhey.nhs.uk
IPC Nurse Specialist	Lindsay Case		lindsay.case@alderhey.nhs.uk
IPC Assistant Practitioner	Vickie Lam		Vickie.lam@alderhey.nhs.uk
IPC Programme Coordinator	Natalie Murphy	2485	Natalie.murphy1@alderyhey.nhs.uk

- The IPCT can be contacted by:
  - Email; infection.control@alderhey.nhs.uk,
  - Telephone; 4175 / 2485 / 2338 (Direct dial 0151 252 5485)
  - Bleep; 138
- Working hours 8am-5pm Monday-Friday
- The IPCT office is located in room 01.02.0003 (zone 1, level 2, close to the 'treehouse' in the former patient flow offices)

Where appropriate (e.g. respiratory viruses, multi-resistant Gram-negative isolates etc.) the microbiology report contains brief details about the appropriate infection control precautions to be taken. These comments were developed in consultation with the IPCT and approved by the previous DIPC. Further details regarding the isolation procedures can be found on the Trust intranet.

Electronic notification is in place to alert the IPCT organisms that need IPCT intervention, such

as patients identified as carriers of MRSA. Electronic alerting is also in place for samples sent for the diagnosis of Measles, Mumps, Rubella, or Chickenpox/Varicella infections, where contact tracing may be indicated. The IPCT is also informed of the positive blood cultures which fall under Public Health England's monitoring schemes, and work in collaboration when supporting the ward areas.

The IPCT liaises directly with the Microbiology Department in outbreak situations so the laboratory is aware of the volume of tests to expect. The department supports the IPC when the team needs to perform environmental testing for investigation into cases of organism transmission from patient to patient.

For patients who have been admitted for more than 3 days, stool samples must be discussed with the IPCT before they are sent to the laboratory for testing.

5.5.2. Paediatric Infectious Diseases and Immunology Team

The microbiology consultants work in cooperation with the Infectious Diseases clinicians, for example at multi-disciplinary meetings. It is important to note however that the two specialities have separate training requirements and that the Consultant Medical Microbiologists have not specifically trained in paediatrics.

The Infectious Diseases consultants have access to the complete microbiology results (including suppressed antibiotic tests) in order to provide clinical advice, however they are not able to amend or re-issue microbiology reports. The Consultant Medical Microbiologists may not be aware of discussions between clinical teams and the Infectious Diseases team; please contact the Consultant Microbiologists if such discussions require the issuing of an amended report (e.g. if an antibiotic has been recommended but has not been reported).

The Infectious Diseases team can usually be contacted on bleep 244.

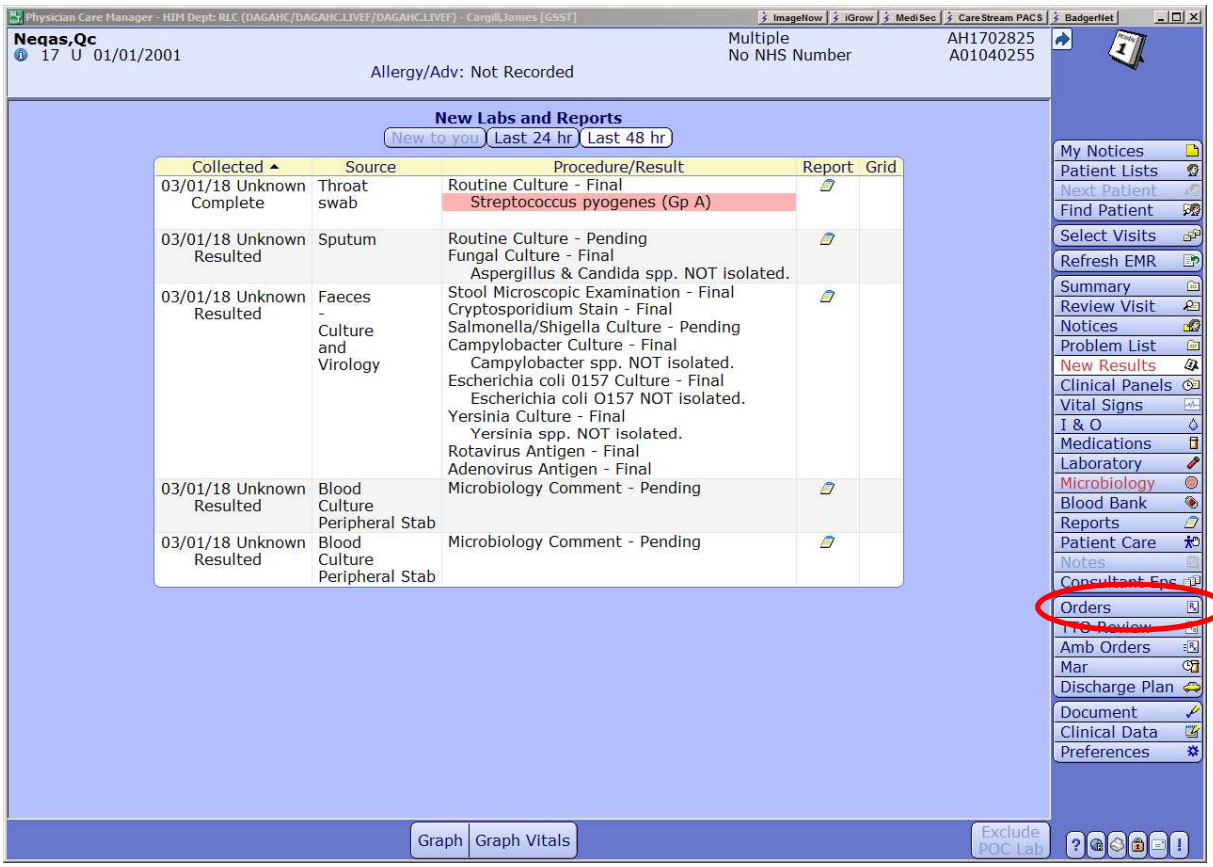
6. Microbiology Requests

6.1. Meditech Codes and Test Information

On Meditech 6 most tests can be identified on the “New Orders” screen by typing in the first few letters of the test name (see the tables under the individual specimen types). Additional tests may be available for specific infections; if the clinical condition under investigation is not listed in the tables then additional testing can be discussed with the microbiologists.

6.1.1. Ordering microbiology tests

1. From the Electronic Medical Record (EMR) select the orders tab from the right-hand options list:

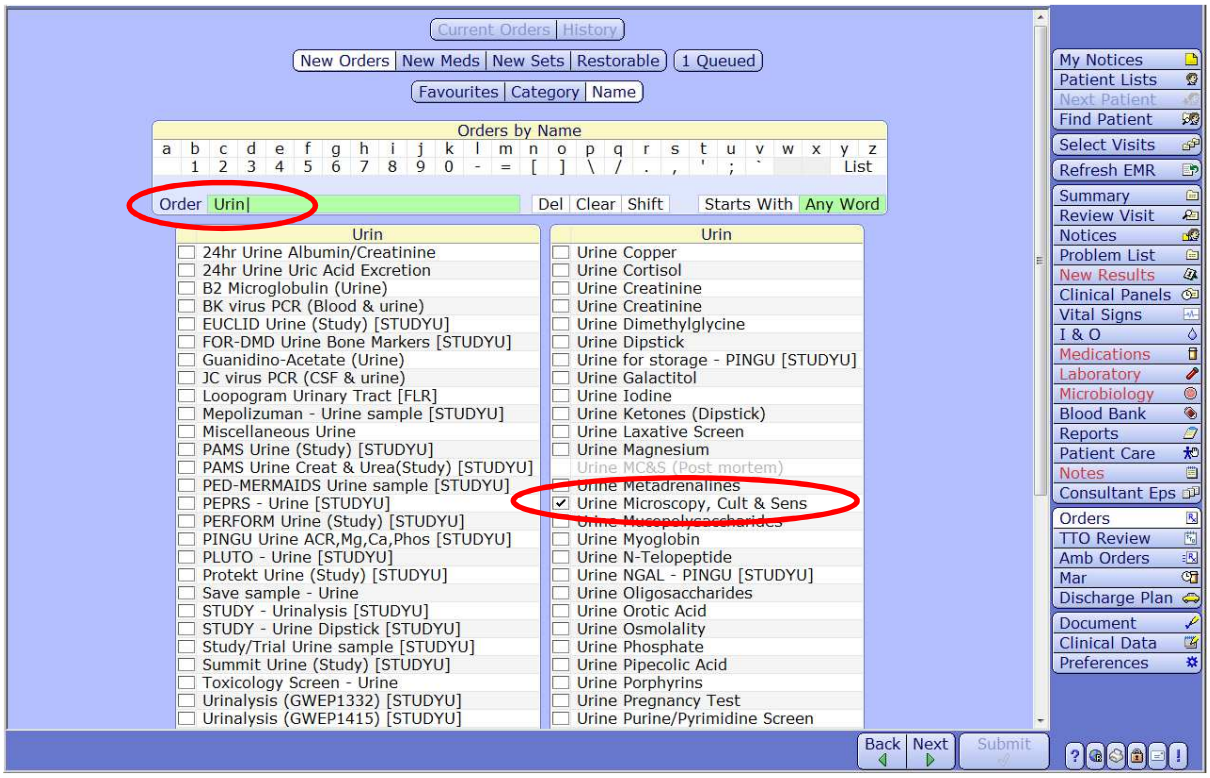


2. From the new screen select the “New Orders” (for individual tests) or the “New Sets” (for pre-defined order sets – see below) option from the top options list:

- Order sets are groups of tests requested by clinical teams for specific indications in order to ensure that all the relevant investigations are performed, and may include tests outside microbiology. For example, the order set “Oncology BAL” includes requests for: routine culture, cytology, TB culture, atypical respiratory, *Pneumocystis jirovecii*, *Aspergillus*, *Candida* and CMV PCRs, and galactomannan (*Aspergillus* antigen) testing.

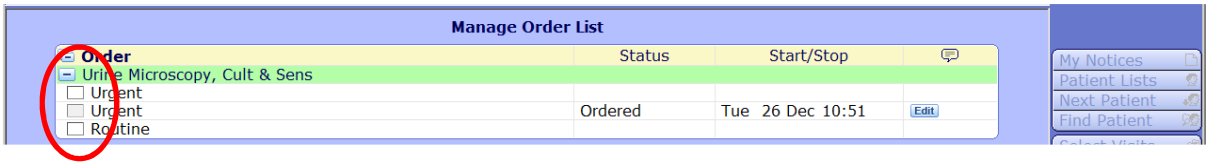
Please check with your clinical team which order sets are in routine use for your speciality.

3. Start to type the test you want to collect, e.g. a urine culture. A list of available options appears (which includes non-microbiological tests); select the correct test(s) from the list using the tick box(es):



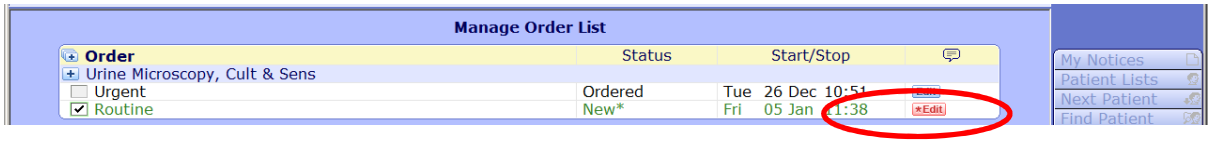
4. Once you have selected all the tests you require (e.g. once selecting the “Urine Microscopy, Cult & Sens” tests you can then go on to request blood cultures, wound cultures etc. on the same order) press the “Next” button at the bottom of the screen.

5. Select “Urgent” or “Routine” (given the nature of microbiology testing this option can be usually chosen as “Routine”. The Microbiology Department will automatically treat e.g. CSF, tissue samples etc. as an “Urgent” request regardless of the order, while most sample types are handled in a standard way regardless of their order status as “Urgent” or “Routine”).

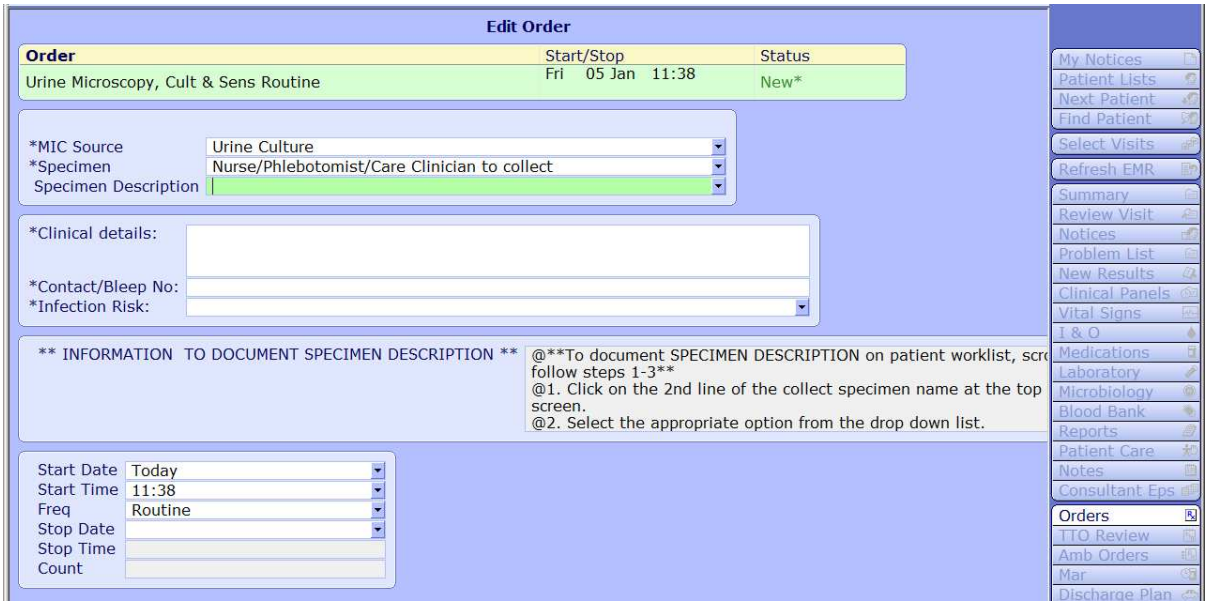


6. Once the “Routine” (or “Urgent”) option has been selected press the “*Edit” button to the

right.



7. On the following screen please enter the appropriate ‘Specimen Description’, ‘Clinical Details’ etc. as indicated.

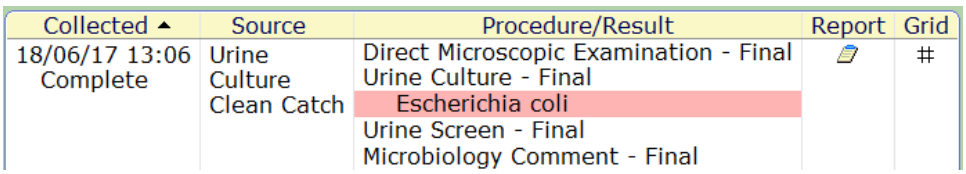


The order is placed once the “Submit” button in the bottom right hand corner is pressed (you will need your PIN in order to place an order. If an order is marked as “Uncollected” re-selecting the order and changing the “*Specimen” field to ‘Nurse/Phlebotomist/Care Clinician to collect’ before resubmitting the order will change to status to “Collected” and the specimen labels will be printed out on the appropriate label printer for use.

Please note that even when an order has been collected on Meditech the microbiology laboratory is still unaware of the request. The laboratory will only become aware of a request once the sample is received in the laboratory.

6.2. **EMR Microbiology Display**

Microbiology results are accessed using the “Microbiology” tab on the right hand side of the Meditech Physician Care Manager screen:



If a sample is not listed, please check the orders section on Meditech to confirm an order has been placed and the sample collected. Samples are formally received on Meditech by the

laboratory when they start to be processed; in many cases samples will therefore not be displayed on the EMR until the afternoon.

6.2.1. "Collected" column

This column shows the date and time when the sample was collected, together with the status of the sample in the laboratory. This status may be:

- Received: The sample has been received in the laboratory. No further results will be available at this point.
- Resulted: The sample is currently in process and partial results are available. For example, once a sterile site sample has a Gram stain result available, the status will change from "Received" to "Resulted".
- Complete: All appropriate sample processing has been completed and the final report is ready.
- Cancelled: The sample has received but not processed. A reason for cancellation should be available on the report.

6.2.2. "Source" column

This column shows the specimen type as selected when the order was placed on Meditech.

6.2.3. "Procedure/Result" column

Microbiology tests are built up from a number of separate procedures which can be completed and reported separately. Isolates are highlighted in pink as shown. Each procedure is followed by an indication of the current status:

- Pending: No results available at this time.
- Preliminary: Partial or interim results are available for this procedure.
- Complete: Final results are available for this procedure.
- Cancelled: This procedure has been stopped by the laboratory.

6.2.4. "Report" column

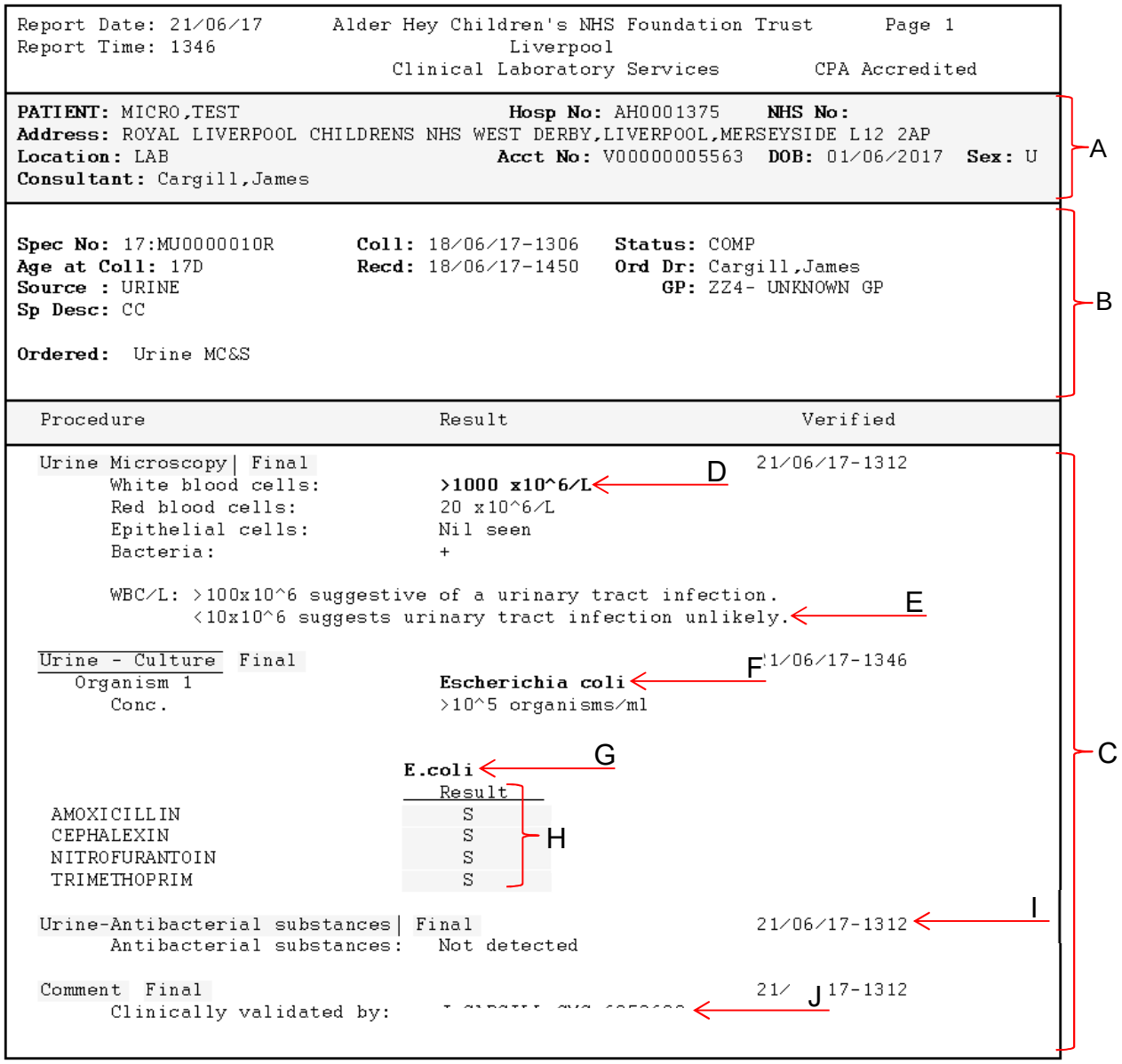
Select the clipboard icon to view the final report (discussed below).

6.2.5. "Grid" column

Please do not use this option.

6.4. **The Microbiology Report Explained**

A standard microbiology report is shown below.



Report Date: 21/06/17 Alder Hey Children's NHS Foundation Trust Page 1
Report Time: 1346 Liverpool
Clinical Laboratory Services CPA Accredited

PATIENT: MICRO,TEST **Hosp No:** AH0001375 **NHS No:**
Address: ROYAL LIVERPOOL CHILDRENS NHS WEST DERBY,LIVERPOOL,MERSEYSIDE L12 2AP
Location: LAB **Acct No:** V00000005563 **DOB:** 01/06/2017 **Sex:** U
Consultant: Cargill,James

A

Spec No: 17:MU0000010R **Coll:** 18/06/17-1306 **Status:** COMP
Age at Coll: 17D **Recd:** 18/06/17-1450 **Ord Dr:** Cargill,James
Source : URINE **GP:** ZZ4- UNKNOWN GP
Sp Desc: CC

Ordered: Urine MC&S

B

Procedure	Result	Verified
Urine Microscopy | Final		21/06/17-1312
White blood cells:	**>1000 x10^6/L**	D
Red blood cells:	20 x10^6/L	
Epithelial cells:	Nil seen	
Bacteria:	+	
WBC/L: >100x10^6 suggestive of a urinary tract infection.		E
<10x10^6 suggests urinary tract infection unlikely.		
Urine - Culture Final		21/06/17-1346
Organism 1	**Escherichia coli**	F
Conc.	>10^5 organisms/ml	

C

E.coli G

	Result
AMOXICILLIN	S
CEPHALEXIN	S
NITROFURANTOIN	S
TRIMETHOPRIM	S

H

Urine-Antibacterial substances \| Final 21/06/17-1312 I
Antibacterial substances: Not detected

Comment Final 21/ 17-1312
Clinically validated by: J

Key features are:

A. The patient details.
B. The sample details. This section includes the clinical details entered when the sample was ordered on Meditech.
C. The results for the Microbiology procedures performed in the laboratory.
D. Abnormal results are printed in bold text on the report.
E. Where available, normal ranges are displayed alongside the test results.
F. Bacterial and viral isolates are reported by name together with the concentration if applicable.
G. Where antibiotic susceptibility results are reported, a short form of the organism name is displayed.

H. Antibiotic susceptibility results are reported as:

S Clinically susceptible : level of antimicrobial susceptibility associated with a high likelihood of therapeutic success

H Susceptible with higher dosing. See below

R Resistant to the agent

Where additional interpretation for the susceptibilities is indicated (e.g. to highlight specific resistance mechanisms of concern) these comments are reported below the susceptibilities.

Some additional tests (e.g. carbapenemase expression) may be reported as:

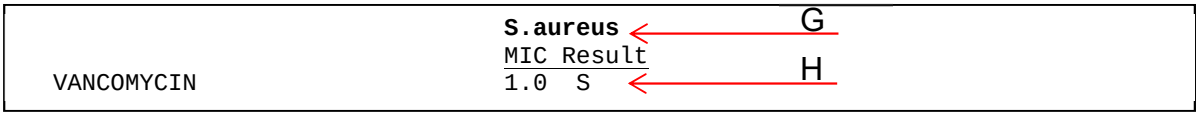
D Detected

N Absent (not detected)

Antimicrobial susceptibilities may also be reported with an MIC (Minimum Inhibitory Concentration) for that agent. These will be accompanied by an interpretation as above, and may include:

X No interpretation is possible

These MIC values are intended to be informative for the infectious diseases clinicians.



I. The date the procedure was validated is displayed for each procedure

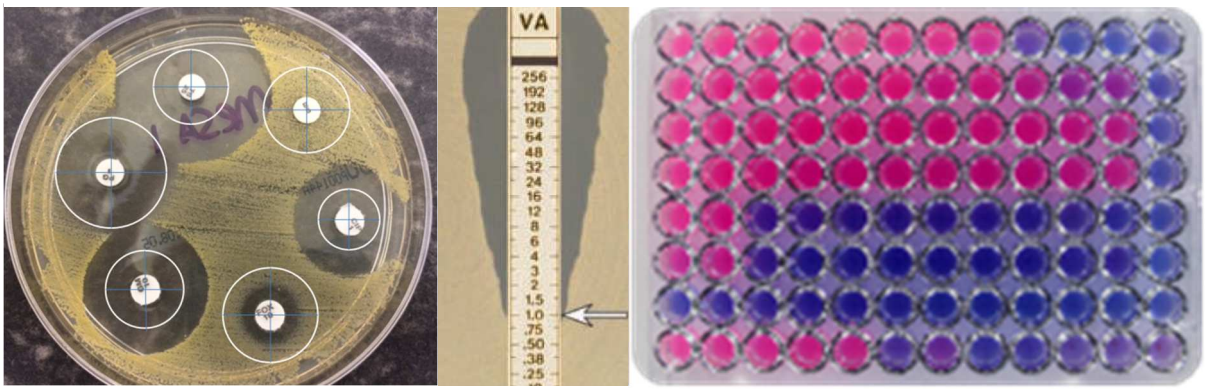
J. Positive results are clinically validated by one of the consultants; any additional interpretation is recorded here.

6.5. Antimicrobial Susceptibility Reporting

The department adheres to The European Committee on Antimicrobial Susceptibility Testing guidelines for the interpretation and reporting of antibiotic sensitivity results and antifungal sensitivity results. For some isolates susceptibility testing is referred to external laboratories (e.g. *Burkholderia* and similar species from cystic fibrosis patients, clinically significant moulds etc.).

The laboratory uses a combination of disc diffusion testing (where the zone of clearance around a disc containing a defined antibiotic concentration is used to determine susceptibility or resistance), and gradient strips and broth microdilution tests (where the specific MIC of an agent can be determined).

Disc diffusion Gradient strip Broth microdilution



The laboratory is making increasing use of susceptibility testing on an automated platform. Sensitivity results are reported as:

- **S – Clinically Susceptible:** level of antimicrobial susceptibility associated with a high likelihood of therapeutic success.
- **H - Susceptible with Higher dosing***

  *Please refer to the 'High Dose Antibiotic Table' in the Antimicrobial Prescribing Guidelines for optimal higher dose prescribing, when treating an infecting organism with a corresponding antimicrobial reported as “H”.

  A microorganism is categorised as "Susceptible with Higher dosing" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
  NB: EUCAST guidance calls this category: "Susceptible, Increased exposure*"
  *Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

- **R – Clinically Resistant:** level of antimicrobial susceptibility associated with a high likelihood of therapeutic failure.

The microbiology results are reported according to:

- Cheshire & Merseyside Antimicrobial Stewardship Group “Guidance for the Reporting of Sensitivity Results by Microbiology Laboratories” (November 2015)
- The Pan-Mersey “Antimicrobial Guide and Management of Common Infections in Primary Care” (July 2017) where appropriate
- The Alder Hey “Antimicrobial Prescribing Guidelines” (Version 1.87)
- The current antibiotic prescribing for the individual patient (if appropriate).

Additional susceptibility results can be discussed with either the Microbiology or Infectious

Diseases Consultants; please note however that amended reports can only be issued by the Microbiology Consultants.

The Infectious Diseases team maintain the Trust Antimicrobial and Infection Guidance on the Intranet (http://intranet/DocumentsPolicies/SitePages/Antimicrobials.aspx).

6.6. Antimicrobial assays

The biochemistry laboratory provides both the in-house and referral service for antibiotic, antifungal, and antiviral levels where required. These results will be found under the "Toxicology" tab on the "Laboratory" page of the Electronic Medical Record (EMR) on Meditech.

6.7. Referred tests

The laboratory refers a number of tests to external laboratories (details of these laboratories can be found at the end of the handbook). These results are recorded on Meditech on receipt of the results; any clinical interpretation from the external laboratory is also recorded. Where additional interpretation is made by the Consultant Microbiologists this will be recorded in a separate Comment at the end of the report.

6.8. Factors affecting the validity of results

- Microbiology tests can be affected by the concurrent use of antimicrobial agents; pathogens may be suppressed if samples are collected after antibiotics are started.
    - Please document clearly on the request form if samples are collected after antibiotics have been started; this is particularly important for blood cultures and CSF samples.
    - If taking samples after antibiotics have been started, please collect them immediately before an antibiotic dose where possible, so that the level of antibiotic is at its lowest.
    - In cases of suspected sepsis, prompt antimicrobials (within an hour) should be given according to Trust and National guidance; do not delay this for the collection of samples.
- The department undertakes duplicate testing of samples as a means of internal quality control and also participates in a number of external quality control schemes organised by Public Health England, Labquality, Finland and INSTAND, Germany.
- Whilst internal and external quality assurance programmes are in operation to ensure accuracy and precision of results, occasionally random errors may occur and escape detection.
- The clinician is often best placed to detect such errors, if you doubt the validity of any

result, it is vital that you contact the department at once so that we can investigate and re-test samples whenever possible.

- Certain factors may affect and possibly invalidate some test results, causing potential biological and analytical interference. For example, haemolysed blood samples, antibiotics and type of specimen tube used.
- Please give relevant clinical details at all times including details of recent travel abroad, current treatment etc. as this will ensure that samples are processed appropriately and reduce the need for additional test requests.

7. Specimen Containers

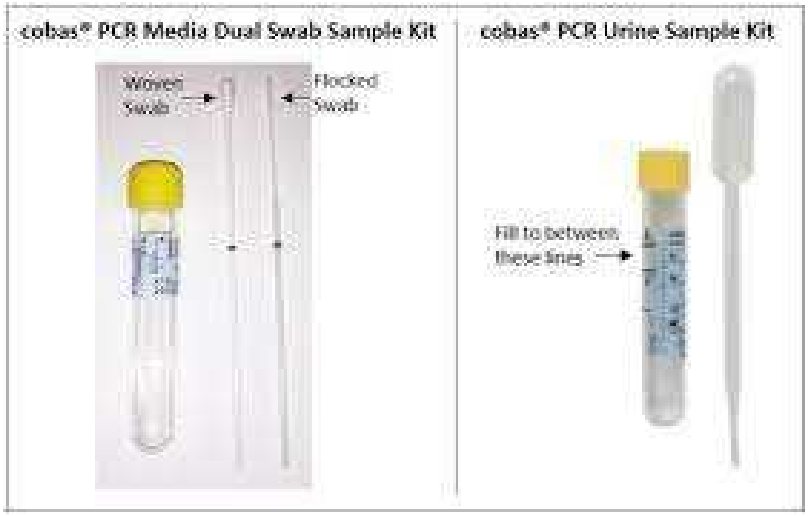
The following containers are available from Laboratory Medicine, with the exception of the containers for respiratory aspirates / BALs for PCR or bacterial culture (available from hospital stores directly to the ward).

A. Paediatric BacT/Alert Blood Culture Bottle (Yellow Top)
B. Adult BacT/Alert Blood Culture Bottle – Aerobic culture (Green Top)
C. Adult BacT/Alert Blood Culture Bottle – Anaerobic culture (Orange Top)
D. Amies Transport Media for routine bacterial investigations
E. Wire pernasal swab with charcoal media for pertussis isolation or PCR – this may also be used for ear swabs.
F. Amies Transport swab for Neonatal MRSA Nose swab screens
G. Sterile universal – For urines, CSFs, pus, hair and tissue samples
H. Fecon pot – for stool samples – C&S, Surveillance, Norovirus and Viral Gastroenteritis

I. Plain sterile tubes for clotted blood samples.
J. Cellotape slide for examination for Enterobius vermicularis (threadworms).
K. Dermapak collection kit for skin scrapings
L. EDTA tube for PCR samples
M. Viral Transport Media for Respiratory virus PCR testing from combined Nose/Throat swabs , SARS CoV-2 testing and Eye swabs and vesicles -viruses
N. Virocult swab - For viral PCR tests inc SARS CoV-2
O. Liquid media swab for Surveillance and CPE PCR tests
P. & Q. Traps for Respiratory secretions – Please replace the vented lid with the solid lid provided. These samples may leak in transit as shown, and the department request that these are NOT sent through the air tube system. Leaking samples may be discarded.
R. 10ml Lithium Heparin for IGRA tests
S. Cobas collection tube

Roche cobas collection tubes for Chlamydia /GC

Swabs (not eye swabs) Urine



- All specimen containers that contain transport media must be stored according to the manufacturer's instructions. The acceptable temperature range and expiry date are displayed on each swab or sample tube.
- Specimen containers must not be used if the seal is broken or after the given expiry date.

- Using an incorrectly stored or expired sample container may affect the quality of the results obtained.
- Once collected the samples must be sent to the laboratory as soon as possible to maximise the recovery of any pathogens present and prevent the overgrowth of any commensal (normal) organisms present.
- If there is a delay in transporting samples it is preferable (with the exception of blood cultures) that the samples are refrigerated, delays of >24hours are not acceptable.

8. Labelling Requirements for Request Forms

All requests from inpatients should be generated on the Meditech system, and will automatically state the required information. Hand written requests should only be used in Meditech down time, and must be filled in correctly.

Samples from General Practitioners and Walk-in-Centres must be accompanied by a hand written request form or a GP's letter. The form/letter should clearly state the following information for unequivocal identification of the patient and specimen:

- Patient name (in full – no abbreviations)
- GP name and address and Clinic name if applicable
- Date of Birth
- Sex
- Type of specimen
- Date and time specimen taken

NB It is **ESSENTIAL** that the laboratory knows the date on which a specimen is taken: processing delayed specimens can yield unhelpful or misleading results and they may be discarded (e.g. urine samples dated 2 days prior to day of receipt).

If patients are given a request form and asked to provide a specimen from home **they should be asked to write the date on which the specimen was collected on both the container and the form and to return the sample to the Alder Hey Out-Patients department and NOT the GP surgery.** The results of samples not returned to and processed at Alder Hey may not have interpretation from a paediatric microbiologist.

Also required:

- All relevant clinical details including any recent antibiotic treatment
- History of recent foreign travel, if applicable
- Risk status, if applicable

- Date of onset and duration of illness, particularly for serology
- Specify anatomical site from which "wound" specimens were taken.
- An indication of parental / legal guardian consent must be given for HIV testing, samples cannot be processed without consent in writing.

If the laboratory cannot unequivocally identify the sample and match it to a form, then it will be discarded.

8.1. Additional Test Requests

- Please telephone the laboratory before ordering additional tests to ensure that the sample is available and still suitable for examination.
- Requests for extra tests must be received within the sample storage period and must have an associated Meditech request. These requests must then be e-mailed to microbiology@alderhey.nhs.uk to comply with accreditation requirements.
- **Requests for additional tests on referred samples must be made to the microbiology department in the same manner, and not to the referral laboratory directly.**

The table below indicates how long samples are kept in the laboratory before they are discarded.

Sample	Time Kept
Faeces – C&S	2 weeks after primary culture
Faeces – *C. difficile*	2 weeks after primary test, positive samples are stored for 3 months.
Respiratory samples for C&S	2 weeks after primary culture
Swabs, fluids and aspirates	2 weeks after primary culture
Urines	1 week after primary culture
CSF Samples – routine	4 weeks after primary culture at 4-8ºC
Tissue	If there is any sample remaining after processing it will be stored for up to 2 months after primary culture.
Stained slides from positive Blood Cultures	A minimum of 1 week after bottle identified as positive.
Post mortem tissue	Any remaining sample will be stored for 4 weeks after primary culture or if requested can be returned to histopathology for humane disposal.

Sample	Time Kept
Respiratory Virus PCR	2 days
SARS Cov-2 PCR	2 days

Samples that are sent to referral laboratories are stored under local procedures and may not be available for additional tests.

Requests for additional tests will be accommodated if at all possible but in some instances samples are not suitable for additional analysis, these include:

Initial request	Reason
MRSA Screen swabs	Swabs are cultured on selective media that may compromise the recovery of additional pathogens
Stool samples for C&S	Additional bacterial culture will only be performed within 3 days of receiving the sample due to potential overgrowth of normal flora.
Serology samples	The ability to add additional tests is dependent upon the test required and the initial volume of blood received.
CSF Viral PCR	PCR tests may be added after discussion with the consultant Microbiologist if sufficient sample is received.

9. Labelling Requirements for Specimens

When using the computer generated labels the patient details on the specimen must match those on the request form. Please contact the laboratory on Ext 2268 if there are any problems in selecting the correct order.

Samples received with a hand written request form must be labelled in block capitals with:

- Full Name as registered on Meditech
- AH Number
- DOB
- Ward
- Specimen source
- Date and time of collection

Please note that unlabelled and mislabelled repeatable specimens cannot be processed and will be discarded.

If a staff member on the ward accepts responsibility for an unrepeatable specimen (e.g. CSF) the sample may be relabelled. The staff member must come down to the laboratory to identify the sample and sign the request form. The following disclaimer will be added to the report.

Specimen received incorrectly labelled.

No responsibility will be taken by the Microbiology Department for incorrectly labelled samples.

Please Note: If the laboratory cannot unequivocally identify the sample and match it to a form, then it will be discarded. The laboratory will inform senders by means of an electronic or printed report when a specimen has been discarded for the above reasons. Electronically produced requests may not be altered by hand; incorrect orders must be cancelled and re-ordered correctly.

10. Transport of Clinical Specimens

- The air tube system is available for delivering samples directly to the Pathology Specimen Reception Area.
- Samples may also be delivered by hand to the Specimen Reception Area.
    - To maintain patient confidentiality and Infection control procedures samples delivered this way must be transported in the red specimen boxes provided.
    - Please date stamp the samples when you drop them off, there is a machine next to the specimen box in the reception area.
- Please telephone the department (ext. 2268) if you are sending an **URGENT** specimen. Between 2300 and 0900 the on-call scientist can be contacted via the switchboard.
    - Samples of fresh tissue or frank pus collected during surgery should always be treated as urgent samples.
    - Following guidance from Public Health England (PHE) gastric biopsies for the detection of *Helicobacter pylori* must be received and processed as soon as possible after collection (preferably within 6 hours) to maximise the likelihood of detection.

10.1. Storage of Non-Urgent Samples

- Non-urgent samples, with the exception of blood cultures and surgical CSF's, should be refrigerated and sent to the department as soon as possible the next morning.
- Blood cultures and CSF's should never be refrigerated; the samples may either be kept at room temperature for transport to the laboratory the next morning or sent to the laboratory via the pod system on collection.

10.2. Packaging and Transport

- ALL specimens should be placed in a **separate** plastic bag and sealed.
- SARS CoV-2 specimens to be sent in red transport boxes
- The Meditech request label should be stuck to the attached card, NOT to the bag.
- Samples delivered to the laboratory by hand must be transported in the red boxes provided.
    - Please contact the department if your ward/department's box is damaged/missing

10.3. Leaking or damaged samples

- The plastic transport bags, if properly sealed, are designed to contain accidental specimen leakage from the container.
- Most incidents of specimen leakage are due to the fact that neither the container nor the

integral bag strips have been closed properly.

- If both container and transport bag are closed correctly, the practice of 'double-bagging', even when an infection with a Hazard Group 3 pathogen is suspected, does not confer any additional safety advantage and is, therefore, unnecessary. Using a separate bag for individual specimens reduces the risk of contamination if a leak does occur.
- The specimen bags supplied have a patented leak proof sealing method and the Red Transport boxes comply with the UN3373 standard for the transportation of biological material. In the event of a spillage in the box, please contact a member of laboratory staff.
- Upon receipt of a sample whose integrity was compromised or which could have jeopardized the safety of the carrier or the general public the sender is contacted immediately. The sender will be informed of any measures that should eliminate recurrence; all incidents will be recorded on the Trust's recording system, Ulysses.
- The laboratory will attempt to salvage any sample for processing from leaking non-repeatable specimens such as CSF, wherever possible repeat samples will be required from repeatable samples such as urine.

11. Standard Procedures for the Safe Collection of Specimens

These procedures concern all clinical staff who are qualified to collect diagnostic specimens from patients. **Staff must always follow aseptic techniques when handling blood, body fluids, excretions, or secretions,** even when these have not been specified as infectious.

11.1. Potential Hazards

All staff must be aware of the potential physical and infectious hazards, associated with the collection of samples for microbiological investigation.

- Follow all local procedures to protect personal safety, prevent injury and exposure to biological hazards.
- Follow all local procedures to reduce the risk to colleagues who are involved with the handling, transport and laboratory investigation of specimens.

11.2. Safety Precautions

- Staff collecting specimens must take care to prevent contaminating themselves, their environment, the external surfaces of the specimen containers, or the accompanying test request forms.
- If gross contamination of the hands with blood, faeces or other biological fluids is anticipated, then gloves should be worn. Hands should always be washed after taking specimens. If splashing into the eyes or onto mucous membranes is possible goggles should be worn.
- In addition, specimens should be collected aseptically, without allowing contamination by extraneous and, therefore, irrelevant micro-organisms.
- Staff should refer to COVID -19 hub for details on PPE when collecting specimens from patients suspected of having SARS CoV-2

Contaminated specimens can adversely affect the validity of many laboratory results. For example, the microbiological investigation of contaminated blood or other materials from sites, which are normally sterile, can commit patients to unwarranted courses of expensive and potentially toxic treatment.

All waste generated from obtaining a specimen should be disposed of according to the Trust's Waste Disposal Protocols available on the Intranet

- M42 - Waste management policy
- SOP – Waste management procedures
- C52 - Standard precautions policy

Please disinfect the outside of any specimen containers if they are contaminated during sample

collection.

12. Blood Cultures

Name of Test	Specimen Container	Turnaround time	Comments
Blood cultures	Paediatric blood culture bottle (PF Plus)  (0.5 to 4 ml of blood)	1-7 days* (organism dependant)	For the best chance of bacterial or fungal isolation, as close to the recommended maximum blood volume as possible should be inoculated. *If a fungal infection, brucellosis or endocarditis is suspected please inform the laboratory or clearly document on the request form as the culture will be extended to 21 days.
	Paired blood culture bottles (FA Plus and FN Plus)  (8 to 10 ml of blood per bottle)		

The following specimen descriptions are available on Meditech:

- Arterial
- Arterial line (not a recommended route of collection)
- Broviac line (blue, red, and white lumen options available)
- CVL (distal, medial, and proximal lumen options available)
- ECMO circuit
- Haemodialysis CVL (blue and red lumen options available)
- Heart (typically from post-mortem blood cultures)
- Long Line
- Bone Marrow
- Peripheral line
- PICC Line
- Portacath
- Peripheral stab
- Umbilical catheter (arterial and venous options available)
- Vas Cath (arterial and venous options available)

Please use the correct option for the blood cultures when ordering as the source details may affect the clinical interpretation of the results.

12.1. **Sepsis**

Please see the “Sepsis Guidance” and “Antimicrobial Prescribing Guidelines” on the Trust Intranet for empirical advice. The Trust also has guidelines on the collection of blood cultures: see “Guidelines for Taking Blood Cultures” and the “Guidelines for the Care and Maintenance of Intravenous Access Devices in Paediatric Patients” for details.

Wherever possible blood cultures should be taken in all cases of suspected sepsis and before antibiotics are started. This may not always be possible, and antibiotics should not be unnecessarily delayed in septic patients if there are difficulties collecting cultures.

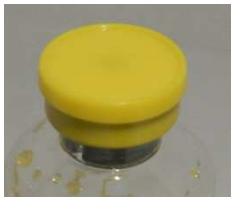
The NICE “Sepsis risk stratification tool: children and young people aged 12-17 in hospital”, “Sepsis risk stratification tool: children aged 5-11 years in hospital”, and “Sepsis risk stratification tool: children aged under 5 years in hospital” do not advise any additional routine microbiological investigations other than a venous blood culture (they do however recommend blood gases, full blood count, CRP, urea, creatinine, and electrolytes, and a clotting screen for patients at moderate or high risk of sepsis). Depending on the clinical presentation, consideration should be given to the need for additional investigations, e.g. CSF, urine, however these should not delay the administration of antibiotics. NICE advise that a lumbar

puncture should be performed in infants aged under 1 month and in infants aged between 1 and 3 months who appear unwell or who have a WBC count below $5x10^9$/litre or above $15x10^9$/litre (unless otherwise contraindicated – see the "Guidelines for the Management of Suspected Bacterial Meningitis and Septicaemia" on the Trust Intranet or NICE guideline NG51 "Sepsis: recognition, diagnosis and early management").

12.2. What blood culture bottle should I use?

The department provides three types of blood culture bottles:

- BacT/Alert PF Plus (yellow top) are designed for paediatric patients, one bottle is suitable for both aerobic and anaerobic culture.



- BacT/Alert FA Plus (green top) are for older patients, they are intended for aerobic culture only.
- BacT/Alert FN Plus (orange top) are for older patients, they are designed for anaerobic culture only.

- The FA Plus and FN Plus bottles should be collected as a pair.

These should be ordered on Meditech as "Blood culture" for the single paediatric PF Plus bottle, or as "Blood culture paired" when the FA Plus and FN Plus bottles have been collected

- Please do not mix and match the blood culture sets (i.e. do not send a paediatric bottle alongside either the aerobic or anaerobic bottles).
- If infection with *Mycobacterium tuberculosis* is suspected please contact the laboratory as special bottles will be requested from Liverpool Clinical Laboratories for this investigation. The Alder Hey laboratory does not hold a stock of these bottles as standard but they are usually available on the next working day after the laboratory has been informed.

12.3. How much blood do I need to collect?

The blood culture bottles are prefilled with culture media that is designed to provide optimal growth conditions when a particular volume of blood is inoculated into the bottle. There is no

advantage to overfilling the blood culture bottle; this may over-dilute the culture medium and affect the recovery of bacteria. Similarly, under-filling the bottle may also affect bacterial recovery; it is better to inoculate two or more PF Plus bottles than to under-fill the FA Plus and FN Plus bottles.

- The single paediatric PF Plus bottle is optimised for inoculum volumes of 0.5-4 ml of blood.
- The aerobic FA and anaerobic FN “adult” paired blood culture bottles are optimised for 8-10 ml of blood per bottle. As such, their routine use in all patients is not recommended. It is anticipated that they may be best used as routine in teenage patients, but may also be of benefit in the investigation of intra-abdominal sepsis or of pyrexia of unknown origin in other age groups.

When collecting central blood cultures it is important that the initial draw of blood (typically 3 to 5 ml) is not used for culture. Vascular access devices will become colonised with skin flora over time and this flora is gathered by this initial blood collection. Bacteria infecting the vascular devices develop a protective biofilm and are not as easily dislodged; they will therefore still be present when the line is re-sampled.

12.4. **How many blood cultures should I collect?**

Clinically significant bacteraemia may occur with very low numbers of circulating organisms per millilitre of blood. Evidence suggests that only 80% of bacteraemic patients will be identified from a single blood culture; this figure rises to 95% if three cultures are collected. The microbiology department therefore recommends that at least two cultures are collected, especially in cases of suspected meningitis, bone or joint infection, endocarditis or sepsis. If the patient is not acutely unwell, it is often possible to collect blood cultures over a period of time, e.g. for sub-acute endocarditis three blood cultures can be collected over a 24 hour period without the patient requiring antibiotics. In the acutely unwell where antibiotics need to be started, it is better to collect blood cultures from multiple sites immediately before starting treatment (collecting multiple cultures from the same access point, whether that is a central or a peripheral access, increases the chance of collecting the same contaminating organisms into multiple cultures and potentially leading to inappropriate management).

If there is the possibility of an infection associated with a central vascular access device, the microbiology department recommends collecting cultures from both the access device and from a peripheral stab. In these cases it is important to inoculate the same volume of blood into culture bottles for each site; the differences between the results of the central and peripheral cultures can assist in differentiating between line colonisation and systemic infection.

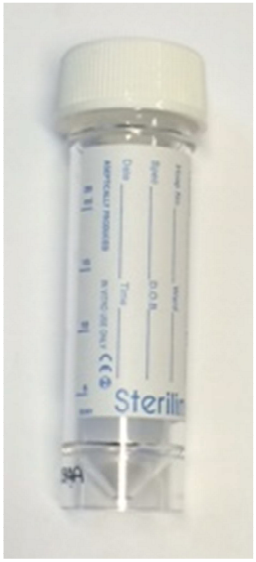
If blood cultures need to be collected from patients who are already receiving antibiotics the best time to collect is usually immediately before a dose of antibiotics when the circulating concentration of the agent is at its lowest.

12.5. Collecting blood cultures

- Inspect blood culture bottles prior to use
    - The plastic lid must be intact, check that the expiry date is valid and ensure that the culture broth is clear. The sensor in the base of the bottle should be a blue-green colour.

    - **DO NOT USE A BOTTLE IF THE SENSOR IS YELLOW.**
- Please return any out of date or potentially contaminated bottles to the laboratory for disposal.
- Please follow the Trust's Blood sampling and ANTT Policies when collecting blood for culture.
- To minimise the risk of contamination please inoculate Blood Culture bottles before any other sample tubes.
- Discard from flushing CV Lines is not a suitable sample for blood cultures.
- When collecting paired blood cultures, the manufacturer's recommendations are:
    - If collecting via a butterfly needle and adapter, inoculate the aerobic bottle first, to avoid adding the air from the tubing to the anaerobic bottle.
    - If collecting via a syringe draw, inoculate the anaerobic bottle first to avoid injecting air from the syringe into the anaerobic bottle.
- Plastic blood culture bottles may be sent to the laboratory using the air tube system. If glass bottles arrive with the patient when transferred from another hospital they cannot be transported using the air tube and must be delivered to the laboratory by hand.
- Please do not use the stickers on the side of the blood culture bottles in the clinical notes – these labels are used in the laboratory. Each blood culture bottle has a unique alpha-numeric code that is used to match bottles to request forms alongside the Meditech-generated sample number.

13. CSF

<table>
<tr><th>Name of Test</th><th>Specimen Container</th><th>Turnaround time</th><th>Comments</th></tr>
<tr><td>CSF culture and microscopy</td><td rowspan="3">Sterile universal
 
See text below for required sample volumes</td><td>Routine samples - 72 hrs
Surgical samples - 10days</td><td>The laboratory should be informed of all urgent CSFs and they should be hand delivered to the laboratory.
Surgical samples have extended anaerobic culture</td></tr>
<tr><td>Meningitis / Encephalitis PCR
Please not this procedure is not available to ward order;</td><td>Within 3 hours (during normal hours)</td><td>This test is available to PICU and general paediatric patients only and is added to a routine CSF C&S procedure at the request of a consultant microbiologist or infectious disease clinician.
Blood stained samples are not suitable for testing.</td></tr>
<tr><td>Common Referred Tests</td><td colspan="2">Referral Lab</td></tr>
<tr><td>CSF PCR
(HSV, VZV, Enterovirus, Parechovirus, CMV)</td><td colspan="2">LCL</td></tr>
<tr><td>Meningococcal/Pneumococcal PCR</td><td colspan="2">MRI</td></tr>
</table>

The following specimen descriptions are available on Meditech:

- EVD
- Lumbar Puncture
- Ventricular Tap (Left and Right options)
- See Clin det *NEURO USE ONLY* (“See Clinical Details” – for the neurosurgical team to provide detailed information regarding sampling)
- Shunt
- VA – Shunt Reservoir
- VP Shunt – Distal End

- VP Shunt – Reservoir

CSF samples may require processing in multiple laboratories depending on the clinical findings, e.g. meningococcal/pneumococcal PCR sent to the Public Health England laboratories in Manchester. Each test has an optimal sample volume to obtain the best quality results, and this should be considered when collecting the sample.

A blood sample taken at the same time as the CSF can be helpful when interpreting the results of molecular tests for bloodstained CSF samples. An EDTA blood should always be sent for Meningococcal/Pneumococcal PCR if bacterial meningitis is suspected (see the section on "Molecular (PCR) Tests" below).

13.1. **Meningitis and Encephalitis**

Please see the "Guidelines for the Management of Suspected Bacterial Meningitis and Septicaemia" and "Antimicrobial Prescribing Guidelines" on the Trust Intranet for empirical advice.

Meningitis is defined as inflammation of the meninges. Meningitis can be acute or chronic and can have both infective and non-infective causes. Encephalitis is an inflammatory process involving brain parenchyma. Over 100 causes have been associated with encephalitis; the majority are viral infections, but other infection and immune-mediated conditions (including post-infectious inflammatory processes) are possible. Over one third of cases have no identified aetiology. Viral encephalitis is usually acute and is often associated with some elements of meningitis (i.e. meningoencephalitis), although neck stiffness occurs in less than one in three cases.

Typical bacterial causes of meningitis include:

- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Escherichia coli*
- *Listeria monocytogenes*
- Group B *Streptococcus*

Typical viral agents of meningitis and encephalitis include:

- Herpes Simplex Virus
- Varicella Zoster Virus
- Enteroviruses (Throat swabs and faeces are additional appropriate sample types for

consideration; detection of virus in these samples is suggestive, but not diagnostic, of the cause of illness. Blood for Enterovirus detection is unhelpful.)

- Parechovirus (Typically seen under the age of 3 in the immunocompetent; testing outside of this age is not recommended. In the immunocompromised Parechovirus should be considered at all ages. Parechovirus PCR is included as standard in the CSF viral PCR tests used by the laboratory.)

HIV testing is recommended for all adults with meningoencephalitis; discussion with the infectious diseases team is strongly recommended if HIV infection is considered a possibility.

Clinical features, season and travel history provide important information and may affect the need for additional testing. Patients who are immunocompromised may have atypical clinical features due to an altered immune response or disseminated infection.

13.2. CSF shunt infection

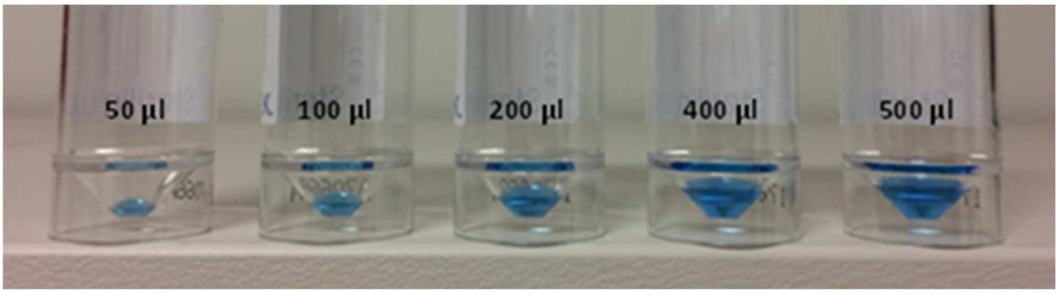
Suspected infection of CSF shunts should be referred to the neurosurgical team as a surgical emergency. The prompt acquisition of a CSF sample by the neurosurgeons helps in the immediate management of cases, although the cell counts may be difficult to interpret. Shunt taps should be performed under the guidance of the neurosurgical team.

13.3. CSF sample aliquots

It is usual to collect LP samples in consecutively numbered sterile universal tubes. The first tube is used to prepare the Gram stain for bacteria, while the last (typically third) tube is used for molecular testing where required. (The second tube is typically used for protein measurement in blood sciences.)

13.4. How much CSF should I collect?

Test	Sample volume (optimal)
Microscopy and culture	As much as available
Referred viral PCR	400 µl (Liverpool Clinical Laboratories)
Meningococcal/Pneumococcal PCR	400 µl (Meningococcal Reference Laboratory)
In-house PCR	200 µl
Mycobacteria culture	>10 ml (Liverpool Clinical Laboratories)If TB meningitis is suspected then discussion with the infectious diseases team is strongly recommended.



13.5. **How much CSF can be safely collected?**

One drop of CSF is approximately 60 µl in volume; 15 drops is therefore around 1 ml of CSF and should be adequate for the majority of molecular microbiology tests (i.e. please collect at least 15 drops in the final universal tube wherever possible).

Age	Mean CSF Production (ml/h)	CSF volume (ml)	Safe CSF volume to take at LP (ml)
Term neonate	1	20-40	2-4
Infant	10	60-90	6-9
Young child	12	100-150	10-15
Adolescent	18	120-170	12-17
Adult	22	150-170	15-17

- Thwaites *et al*. *Journal of Infection* **59**: 167 (2009); data based on studies of
    - 100 Infants & children with external ventricular drains (2002)
    - 11 adults with CNS neoplasms undergoing CSF perfusion chemo (1966)
    - 23 healthy adults undergoing 2D cine-phase contrast MRI (2004)

With thanks to Dr Rachel Kneen.

14. Respiratory Samples

Name of Test	Specimen Container	Turnaround time	Comments
Respiratory Culture	Sterile universal (Culture, Viral PCR)  Swab (Cough swab – culture only) 	72 -96 hrs (routine culture. For CF samples some culture plates are incubated for 10 days)	Samples of sputum (either induced or non-induced), tracheal or endo-tracheal aspirates, BAL (in BAL traps), and cough swabs (Transwabs) will be processed for routine culture. Naso-pharyngeal aspirates are not suitable for routine culture and will be discarded.
Respiratory Virus PCR SARS CoV-2 (COVID -19)	Aspirates (Culture, including pertussis, Viral PCR, RSV Clearance)   Nose / throat swab in virus transport media (Viral PCR only) 	< 3 hours (for samples received between 0900 and 2100) This is dependent on a machine being free when the sample is received. Up to two hours for urgent specimens	Samples of sputum (either induced or non-induced), BAL, tracheal or endo-tracheal aspirates or naso-pharyngeal aspirates sent in sterile universal containers, or combined nose and throat swabs sent in viral transport media, will be processed for respiratory PCR. See COVID-19 hub Clinical guidance and quick ref section for details on collection of nose/throat specimens See text for restrictions on testing
Pertussis Culture	Wire Charcoal Swab (pertussis culture) 	Up to 10 days	If sending a pernasal swab and the child is <12mths of age and is admitted, please send a second sample for PCR

RSV Clearance	NPA samples only	Same day	Available to critical care (ward 1B HDU and PICU) only for patients with known RSV infection.
Referred Tests		**Referral Lab**	**Sample type**
Aspergillus PCR (outside of oncology please discuss the appropriateness of this test)		MRI	BAL
Atypical respiratory pathogens PCR NB. Includes *Chlamydia* and *Legionella* – not usually indicated if in-house respiratory PCR has been performed unless *Legionella* infection thought likely		LCL	BAL
Bordetella PCR		MRI	Pernasal swab, naso-pharyngeal aspirate
Bordetella pertussis serology		RVPBRU, Colindale	Clotted blood
Candida PCR (outside of oncology please discuss the appropriateness of this test)		MRI	BAL
CMV and HSV PCRs (outside of oncology please discuss the appropriateness of this test)		LCL	BAL
Galactomannan test (*Aspergillus* antigen; outside of oncology please discuss the appropriateness of this test)		Wythenshawe	BAL, Clotted blood
Pneumocystis jirovecii PCR		LCL	BAL
Pneumococcal and *Legionella* urinary antigen (not typically indicated, please discuss the appropriateness of this test)		LCL	Urine
SARS CoV-2 –PCR SIREN study swabs and non-urgent swabs that are unable to be processed at Alder Hey		LCL	Combined nose/throat
For suspected Mycobacterial infection (e.g. TB) see the separate section “Mycobacterial investigations” below			

The following sample types are available on Meditech:

- Bronchoalveolar lavage (Lingular, Left Lower Lobe, Left Upper Lobe, Right Lower Lobe, Right Middle Lobe, Right Upper Lobe, No specific

lobe)
- Cough swab
- ET aspirate
- Nasopharyngeal aspirate
- Nose / throat (swabs)
- Sputum
- Tracheal aspirate

14.1. What should I send if I suspect Bronchiolitis?

Bronchiolitis is a common presentation, particularly during the winter months, for respiratory tract infections in young children. See the “In-Patient Care of Babies and Children with Bronchiolitis”, “Bronchiolitis Initial Assessment Flowchart” and “Bronchiolitis: Ward Management Summary” on the Trust Intranet for details of management. This is typically a viral infection, classically respiratory syncytial virus (RSV), and as such many of the pathogens can be diagnosed using the in-house respiratory PCR. For this reason, during the winter respiratory virus season there is no restriction on testing of new patients.

14.2. What should I send if I suspect Pneumonia?

Patients diagnosed with pneumonia should have as a minimum a blood culture and a respiratory sample for culture collected. During the winter patients with respiratory will often receive PCR testing as described for bronchiolitis, which includes the ‘atypical pneumoniae’ pathogens *Mycoplasma pneumoniae* and *Chlamydophila* (*Chlamydia*) *pneumoniae*. It is therefore unnecessary to request the “Atypical pneumonia PCR” unless *Legionella* infection is suspected.

- Urinary antigen testing is not commonly used at Alder Hey for either *Streptococcus pneumoniae* or *Legionella* infection. If it is thought relevant please discuss with the consultant microbiologists; other tests may be considered more appropriate.
- If Mycobacterial infection is suspected (e.g. TB) samples will be referred to Liverpool Clinical Laboratories, however the microbiology department strongly recommends discussing such patients with the Paediatric Infectious Diseases team.

14.3. What should I send if I suspect pertussis?

Bordetella pertussis may be detected by culture, PCR, or serology. For sample sent from outside Alder Hey, serology is the preferred sample type.

14.3.1. Culture

- Pernasal swabs: Gently insert the fine, flexible per-nasal swabs (charcoal media) swab horizontally to the back of the nose. If an obstruction is encountered, withdraw and re-insert through the other nostril.
- Nasopharyngeal aspirates are also suitable for pertussis culture

14.3.2. PCR

Nasopharyngeal aspirates are the preferred sample for pertussis PCR

- Pertussis PCR is routinely available from PHE for acutely ill children less than 12 months of age who are admitted with respiratory illness compatible with pertussis.
    - Any PCR sample on an older child not sanctioned by a consultant microbiologist will be discarded.
- If sending swabs for pertussis PCR, two separate samples are required as swabs used for culture will not be accepted by the reference laboratory.
- Nasopharyngeal aspirates are also suitable specimens for pertussis PCR and culture; in this case a single specimen is sufficient.
- *Bordetella pertussis* and *B. parapertussis* are detected by the FilmArray Respiratory PCR.

14.3.3. Serology

- For older children a clotted blood sample for pertussis antibodies may be sent or if PCR is required please contact the Consultant Microbiologist.

14.4. **What should I send if my patient has Cystic Fibrosis?**

The same sample types are processed from Cystic Fibrosis (CF) patients as for any other respiratory infection, however there are additional cultures for specific CF-associated pathogens which as routinely performed (e.g. specific culture for *Burkholderia* and other non-fermenting Gram-negative bacilli, rapid-growing mycobacterial species such as *Mycobacterium abscessus*, and extended fungal culture to detect *Exophiala* and similar species).

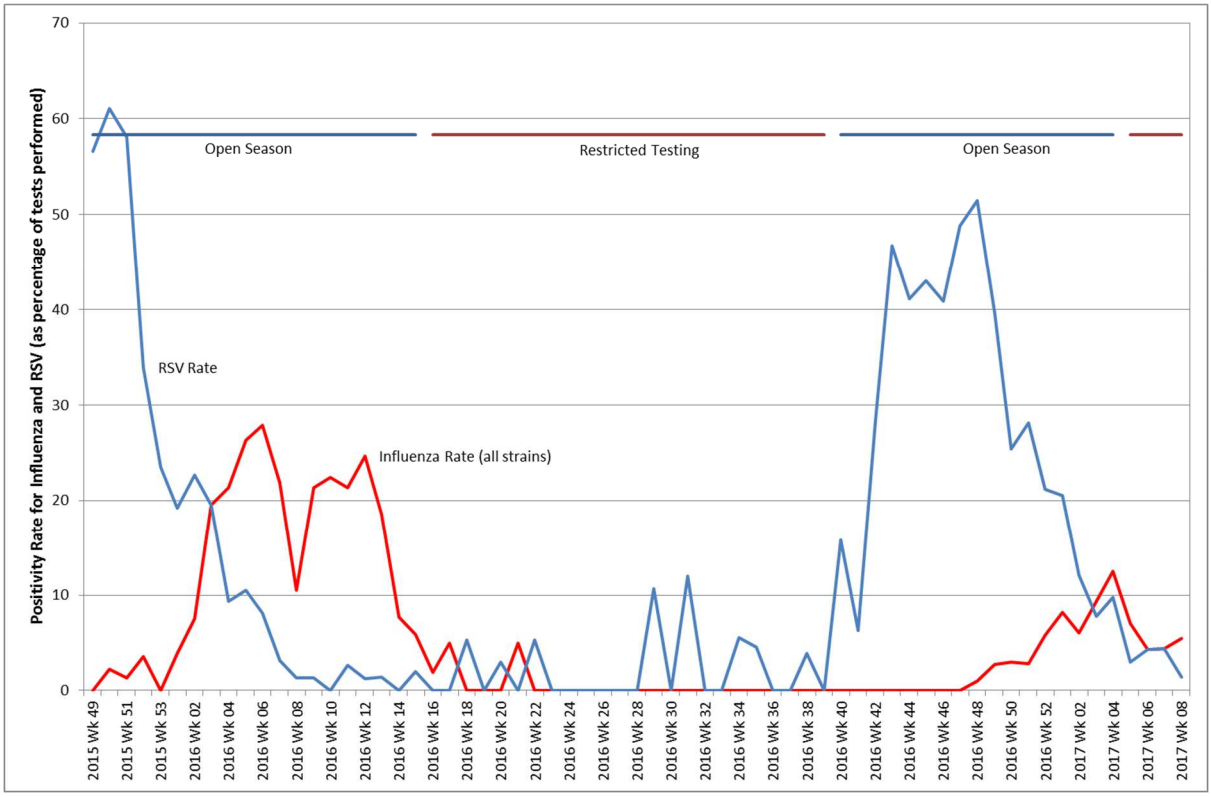
Please include in the clinical details that the patient has cystic fibrosis.

For patients seen in the community or in the Emergency Department, the cystic fibrosis team recommend that a respiratory sample is collected before starting antibiotics if possible. They also appreciate early contact regarding these patients as management can be complex.

14.5. **Respiratory viral PCR**

The laboratory offers an in-house respiratory viral PCR which also includes SARS CoV-2 using the Biofire FilmArray platform. During the winter season (starting when PHE recommend Palivizumab for RSV prophylaxis and ending when Influenza detection rates drop below 10% of submitted samples for two consecutive weeks) there is open access to testing for new patients. Outside of this period tests must be discussed with the Consultant Microbiologists or one of the Infectious Disease clinicians prior to sending (unless from a previously agreed high-risk area –

PICU, HDU or Oncology).

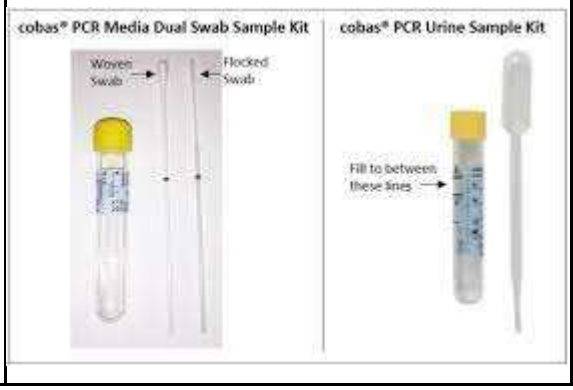


No repeat testing will be performed within a two week period unless agreed by a Consultant Microbiologist or an Infectious Diseases clinician. For PICU patients, a rapid RSV antigen test is available to identify patients who are no longer infectious (for other patient groups clearance is identified by either a minimum time period defined by the Trust policy C17 “Isolation of Patient Policy” on the Intranet, or by the resolution of clinical symptoms).

- It is not appropriate to test patients without respiratory symptoms using the respiratory PCR during periods of open access.
- Testing should not be requested for patients who are not being considered for admission.
- It is also inappropriate to contact the laboratory to chase the results of the PCR; samples are processed in order of receipt unless otherwise decided by the consultant medical microbiologists and results are released onto Meditech as soon as they are available.
- The results of PCR tests should not be required in order to transfer patients from admission areas to the wards unless cohorting of respiratory infections has been introduced.

For further information on COVID-19 (SARS CoV-2) please see COVID -19 hub on intranet

15. Urine Samples

Name of Test	Specimen Container		Turnaround time	Comments
Urine Microscopy, Cult & Sens	Sterile universal (2-3mls) Also Boric acid or Vacuum tubes from outside Alder Hey	 Boric acid container Universal container Vacuum tube	48 hrs	The best routine sample is a clean catch specimen. Bag samples may be contaminated with skin flora.
Schistosoma detection			Same day for in house microscopy, 48hrs if referred	Please contact the laboratory with appropriate clinical and travel history; samples may be referred to LSTM.
Referred Tests		**Container**	**Referral Lab**	**Comments**
Chlamydia and gonorrhoea detection (typically from Rainbow patients)			LCL	Sample obtained after not having passed urine for one hour

The following specimen descriptions are available on Meditech:

- Bag Urine (not recommended)
- Clean Catch
- Catheter (intermittent)
- Catheter (long term)
- Catheter (new insertion)
- Mid Stream Urine
- Nephrostomy Urine (Left and Right options)
- Pad Urine
- Surgically obtained urine
- Suprapubic catheter
- Ureteric Stent (Left and Right options)

15.1. **Urinary tract infections**

See the “Antibiotic Prescribing Guidelines” for empirical advice and the “Urinary Tract Infection In-Patient Pathway” and “Urinary Tract Infection Out-Patient Pathway” for additional information. Each document is available on the Trust Intranet.

The significance of culture results and their associated microscopy is based on Hoberman *et al.* (*J Pediatr*. 1994; **124**: 513-519): “For urine specimens obtained by catheter, we believe that urinary tract infection is best defined by both a leukocyte count ≥10/mm^3 and a CFU count ≥50,000/ml”. Colony counts at Alder Hey are reported as 10^4-10^5 cfu/ml (10,000 to 100,000 cfu/ml) or >10^5 cfu/ml (counts <10^4 cfu/ml are typically reported as ‘No significant growth). A pure growth of between 10^4-10^5 cfu/mL is consistent with UTI in a carefully taken specimen from a child (a lower threshold than in adults, where ≥10^5 cfu/ml is usually thought of as suggestive of infection and lower levels as contamination). The probability of UTI is increased by the isolation of the same organism from two specimens.

UTIs may present with nonspecific symptoms and signs, particularly in infants and young children.

15.1.1. Preterm infants

Clinical signs of UTI in preterm infants include:

- Feeding intolerance
- Apnea and bradycardia
- Lethargy
- Tachypnea
- Abdominal distension

- Hypoxia with documented oxygen desaturation

15.1.2. Term infants

Signs and symptoms of UTI in neonates are typically nonspecific and can include lethargy, irritability, tachypnea, or cyanosis; neonates may appear acutely ill. Clinical signs include:

- Fever
- Failure to thrive
- Jaundice (typically conjugated hyperbilirubinemia related to cholestasis)
- Vomiting
- Loose stools
- Poor feeding

UTI may be the presenting manifestation that identifies a neonate with an underlying congenital anomaly of the kidney and urinary tract.

15.1.3. Younger children (up to 2 years of age)

Signs and symptoms UTI in children younger than two years include:

- History of previous UTI
- Temperature >40°C
- Suprapubic tenderness
- Fever >24 hours

Absence of another source for fever is not always helpful in diagnosing a UTI; likewise an identified alternate source of fever does not exclude UTI. Reports of foul-smelling urine or gastrointestinal symptoms are not typically reliable in diagnosis.

15.1.4. Older children

Symptoms of UTI in older children include:

- Fever
- Urinary symptoms (dysuria, urgency, frequency, incontinence, macroscopic haematuria)
- Abdominal and/or back pain
- New-onset urinary incontinence
- Suprapubic tenderness and costovertebral angle tenderness

The combination of fever, chills, and flank pain may suggest pyelonephritis.

15.2. **Complicated versus uncomplicated (simple) infection**

- Uncomplicated UTIs are limited to the lower urinary tract and may be typically seen in children older than two years with no underlying medical problems or anatomic or physiologic abnormalities.

    - Although uncomplicated cystitis may occur in children younger than two years, it is difficult to differentiate upper from lower UTI in such children and they are usually assumed to have upper UTI.
- Complicated UTIs involve coexisting upper renal tract infection, multiple-drug resistant pathogens, or hosts with specific indication (e.g. anatomic or physiologic abnormality of the urinary tract.

It is important to indicate to the laboratory if a complicated infection is suspected; the antibiotics that may be appropriate for an uncomplicated infection are not always suitable for a complicated infection. For example routine susceptibility testing of common urinary pathogens such as Escherichia coli against nitrofurantoin and trimethoprim are only valid for uncomplicated infections but not for an upper renal tract infection, while co-amoxiclav has different interpretations for susceptibility versus resistance for uncomplicated UTI compared to other infections. Where such susceptibility testing is limited in its interpretation it is noted on the report (e.g. “Ciprofloxacin (simple UTI)”).

Neonatal urinary tract infection is associated with bacteraemia and congenital anomalies of the kidney and urinary tract. Upper renal tract infections may result in renal parenchymal scarring and chronic kidney disease. Neonates with UTI should be evaluated for associated systemic infection, and anatomic or functional abnormalities of the kidneys and urinary tract.

Asymptomatic bacteriuria may occur in 1-3% of infants and preschool-age children, and ~1% of older children; the organisms are typically of low virulence and easily eliminated by antibiotics, however, in most case the asymptomatic bacteriuria resolves spontaneously without complication.

15.3. **What urine sample should I collect?**

The microbiology department recommend wherever possible that mid-stream or clean-catch urine be collected for the diagnosis of UTI. Alternately, samples from intermittent or newly introduced catheters are suggested.

National standards for the processing of urine samples state that catheters, bag urines, catheter tips and ureteric stents are not acceptable sample types; at Alder Hey we accept but strongly discourage bag urine samples.

15.3.1. Mid-stream or clean catch urine

If a mid-stream urine can be obtained, the first part of the voided urine is not collected and,

without interrupting the flow, collect the urine into an appropriate container. For a clean-catch specimen, the whole flow is collected and an aliquot sent to the laboratory.

- **Please ensure that the patient's peritoneal / genital area is physically clean before collecting a urine sample for culture.**
    - **For Girls**

      Clean from the front to the back and gently pat dry with clean dry sterile gauze.
    - **For Boys**

      Gently retract the foreskin and clean the entire surface with gauze soaked in the sterile water. Replace the foreskin and dry with clean sterile gauze.
- Catch the middle portion of the urine in a clean wide-mouth receptacle. Such a receptacle need not be sterile: any container, previously washed thoroughly with detergent and hot water and stored dry, is suitable.
- A sample of the middle portion of the urine must be poured into a 20ml universal container (white top). The pot should be labelled with the patient's details.

15.3.2. CSU (catheter sample of urine) collection

Catheter samples may be obtained either from transient catheterisation (and requested as "Catheter (intermittent)" samples) or from indwelling catheters (and requested as "Catheter (new insertion)" for urine collected at the time of catheter insertion) or "Catheter (long term)" for indwelling catheters).

- The specimen should be collected aseptically from a sample port in the catheter tubing or by aseptic aspiration of the tubing.
- For indwelling catheters, the distal end, or preferably the sampling port if present, must be disinfected with 70% isopropyl alcohol and urine aspirated with a sterile syringe.
- The urine must then be transferred to an appropriate container.
- The specimen should not be obtained from the collection bag.

Growth from indwelling catheters may not identify the genuine pathogen as bacterial biofilms develop on the catheter surface. Interpretation of specimens from long term-catheterised patients may be extremely difficult to impossible.

15.3.3. Ileal conduit or urostomy specimen collection

Urine is obtained via a catheter passed aseptically into the stomal opening after removal of the external appliance. Results may be difficult to interpret.

15.3.4. Pad collection

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, or alternately the wet fibres may be inserted into the syringe barrel and the urine squeezed directly into the container with the syringe plunger. Clean-catch samples are considered to be more accurate than pad samples for diagnosis.

15.3.5. Surgically-obtained urines (e.g. suprapubic aspirate, cystoscopic urine collection)

Please indicate these samples clearly as additional laboratory work may be indicated for invasively-obtained urine samples.

Public Health England. (2017). National user manual worked example for urine tests. UK Standards for Microbiology Investigations. U 3 Issue 1.

https://www.uptodate.com/

16. Eye, Ear, Nose, and Throat Samples

Name of Test	Specimen Container	Turnaround time	Comments
ENT routine culture & sens	Amies Transport Swab 	48 - 72 hrs	
Eye – Routine C&S	Sterile universal (e.g. corneal scrape) 	Corneal scrape 1-7 days (organism dependant)	
Eye – Surgical sample			Used for e.g. vitreous biopsies for culture
Referred Tests	**Specimen Container**	**Reference Lab**	**Sample Type**
Chlamydia / gonococcal PCR	Viral transport media (virus  detection)	LCL	Eye swab
Viral eye PCR – HSV, VZ, Adeno			

The following sources are available on Meditech for ENT cultures:

- Antral washout (Left and Right options)
- Cornea (Left and Right options)
- Ear (Left and Right options)
- Eye (Left and Right options)

- Nose
- Throat

For the eye-specific requests left and right options are available.

16.1. What samples should I send for eye infections?

16.1.1. Conjunctivitis

Infectious conjunctivitis is one of the causes of red or sticky eyes. Conjunctivitis may occur in association with infection of the eyelid (blepharoconjunctivitis), inflammation of the eye lid (blepharitis) or of the cornea (keratoconjunctivitis).

- An eye swab will typically be sufficient for conjunctival infections
- For neonatal infection consider sending a sample for gonococcal and Chlamydia PCR (*Neisseria gonorrhoeae* is a very sensitive organism and may not survive the culture process).
    - Please ensure that suspected cases of ophthalmia neonatorum are clearly indicated on the request so that specific culture plates can be set up.

16.1.2. Keratitis

Keratitis is infection of the cornea, and may progress to endophthalmitis if inappropriately treated.

- Ideally send a corneal scrape.
- Consider viral PCR for HSV infection.
- Keratitis may be caused by *Acanthamoebae*; suspected parasitic infection of this kind are referred to Liverpool Clinical Laboratories for *Acanthamoebae* culture if required (please indicate on the request form).

16.1.3. Endophthalmitis

Infectious endophthalmitis is a relatively uncommon but potentially sight-threatening infection of intraocular fluids and tissue. Endophthalmitis may be acute post-operative (usually within 1 to 7 days of intraocular surgery), chronic (months to years after intraocular surgery), post-traumatic (after penetrating or perforating ocular injuries, worse if organic material is associated with a penetrating injury), or endogenous (rare and occurs in patients with bacteraemia or fungaemia)

- Ideally send intraocular fluids (aqueous and vitreous humours).
- Intraocular samples are typically aspirated using a needle and syringe and are of small volume; if sending the syringe if possible protect it by placing it inside a sterile universal container to prevent the sample being accidentally discharged from the syringe during transport (please removed the needle first and dispose of safely)
- Eye swabs may be taken but may not show the infecting organism.

- For chronic infection consider Mycobacterial culture.
- Blood cultures are advised if endogenous endophthalmitis is possible.

16.1.4. Orbital cellulitis

Orbital cellulitis is the infection of orbital tissue resulting from trauma, surgery, or an extension of paranasal sinus infections. It is a serious infection and may cause blindness, septic thrombosis of the cavernous sinus or intracranial infections.

- Eye swabs are of limited value.
- Ideally send aspirates from the affected tissues if possible
- Blood cultures should be taken.

16.1.5. Canaliculitis

Canaliculitis is a rare, usually chronic, condition often caused by anaerobic organisms.

- Canalicular pus samples are preferred to eye swabs.

16.1.6. Collecting eye swabs

Eye swabs for routine culture will be processed from in-patients, Rainbow and ophthalmology clinics only. All swabs from suspected ophthalmia neonatorum will be processed. A separate swab is required for each eye.

- Collect before antimicrobial therapy, where possible, and preferably before application of local anaesthetic or dye.
- Prior to collecting any samples for processing remove the exudate from the eye.
- Moisten the swab tip with normal saline to provide optimum collection for bacterial/viral/chlamydial detection.

From infants:

- Lay the child flat (on a bed or a parent's knee)
- Gently fold down lower eyelid and run swab across the inner surface rotating swab to ensure optimum specimen collection.

From older children:

- Sit or lay the patient with the head well supported, ask the patient to look up and gently pull down the lower lid exposing the conjunctiva.
- Gently sweep the swab stick along the lower eye from the inside out taking care not to touch the eyelids. Place swab immediately into the transport tube.

Unless otherwise stated, blue Transwabs should be used for bacterial culture, any available pus should be sampled as well as the lesion of interest.

16.1.6.1. Chlamydia detection

- For chlamydial examination the cells from the inner canthus must be sampled.
- Separate samples must be collected into appropriate transport media for detection of viruses or chlamydiae.

16.2. What samples should I send for ear infections?

16.2.1. Otitis Externa

Infection of the external ear canal resembles skin and soft tissue infection elsewhere, however, as the canal is narrow foreign materials and fluid that enter can become trapped and cause irritation and maceration of the superficial tissue.

- A swab of the external ear canal is usually sufficient.

16.2.2. Otitis Media

Middle ear infections may be caused by a number of bacterial species, often those found in the upper respiratory tract. Respiratory viruses may also play a role in the development of acute infection. Pseudomonads and methicillin resistant *Staphylococcus aureus* (MRSA) may be isolated from chronic suppurative infections, with anaerobic bacteria found in 25% of patients.

- An external ear swab is not useful unless there is perforation of the eardrum.
- Tympanocentesis to sample middle ear effusions is rarely justified, but if performed samples should be sent in a sterile universal container.
- Respiratory PCR should be considered for acute infections.

16.3. What samples should I send for nasal infections?

16.3.1. Sinusitis

The sinus cavities are usually sterile or may contain small numbers of bacteria that are continuously removed by the mucociliary system. Acute community acquired sinusitis is typically caused by upper respiratory tract bacteria, however viral infections are also an important cause. Complications of sinusitis include orbital infection, and less commonly intracranial infection and osteomyelitis. Acute nosocomial sinusitis is often a complication of endotracheal intubation and mechanical ventilation, and often shows no clinical signs of infection. Nosocomial sinusitis is often polymicrobial.

- Superficial swabs are likely to be inadequate; scrapings or biopsy material are most likely to yield the diagnosis.
- Nose swabs are not a suitable sample type for sinusitis and should only be used for carriage detection (see “Surveillance and screening for resistant organisms”).
- Specimens should be obtained by careful aspiration of the sinus (avoiding contamination by upper respiratory tract flora), and sent in a sterile universal container.
- Respiratory PCR should be considered in acute infection.

16.3.2. Fungal Infection

Fungal infections are usually due to filamentous fungi, including *Aspergillus*, *Rhizopus* and *Mucor* species. In the immunocompromised, sinusitis caused by filamentous fungi may cause life-threatening infections and is often locally invasive. *Candida* and *Cryptococcus* species are also causes of infection in patients who are immunocompromised.

- Aggressive surgical debridement is often required in addition to systematic antifungal therapy and treatment of the underlying cause.
- Examination of tissue rather than pus is important in fungal sinusitis.
- Community-acquired chronic fungal sinusitis is a relatively common problem in some tropical and subtropical countries, e.g. in Africa and India, and imported cases may be encountered, therefore please provide an appropriate travel history where relevant (this is also important for laboratory safety as some of the potential fungal species need to be handled under higher safety precautions).

16.4. What samples should I send for throat infections?

16.4.1. Pharyngitis

Inflammation of the pharynx may be acute or chronic. Most cases are viral but it is difficult to differentiate between bacterial and viral pharyngitis on symptoms alone. The most common cause of bacterial pharyngitis is *Streptococcus pyogenes* (Group A *Streptococcus*). In children *S. pyogenes* carriage (not infection) rates have been reported as 20% - 30%. Groups C and G streptococci may also cause pharyngitis. Fungal infections may be seen in the immunocompromised, or in patients receiving antibiotics.

- A throat swab should be considered for culture.

Please indicate in the clinical details if any of the following are present to suggest diphtheria:

- Membranous or pseudomembranous pharyngitis/tonsillitis
- Contact with a confirmed case of diphtheria within the last 10 days
- Travel to a diphtheria high risk area (Africa, South East Asia, South America) within the last 10 days
- Contact with someone who has been to a high risk area within the last 10 days
- Contact with any animals (e.g. household pets, farm, petting zoo) within the last 10 days
- Recent consumption of any type of unpasteurised milk or dairy products

For these patients a naso-pharyngeal swab may be considered if a throat swab is unobtainable for any reason.

16.4.2. Epiglottitis

Inflammation of the epiglottis commonly affects children and is associated with fever, hoarseness of voice, stridor and difficulty in swallowing. Acute epiglottitis in young children is a rapidly progressive inflammation of the epiglottis and surrounding tissues and may result in complete airways obstruction.

- Because trauma from the swab may precipitate obstruction, throat swabs are contraindicated in cases of suspected acute epiglottitis.
- Blood cultures should be taken in all cases of suspected epiglottitis.

16.4.3. Tonsillitis

Inflammation of the tonsils is usually due to a viral infection but may also be bacterial. It is a common type of infection in children, and symptoms include a sore throat that can feel worse when swallowing, fever, coughing and headache.

- A throat swab can be sent for culture.

16.4.4. Quinsy

Quinsy (peritonsillar abscess) is a rare acute infection usually on one side of the throat only, with the swelling behind the tonsil near the back of the roof of the mouth. Symptoms are similar to that of tonsillitis, including dribbling, generally feeling unwell and neck swelling because of the abscess.

- Pus may be aspirated from the abscess and if so, should be sent in a sterile universal container.
- If pus is unobtainable send a throat swab for culture.
- Blood culture should be considered for severe infection.

16.4.5. Laryngitis

Acute inflammation of the larynx may be caused by a viral infection (such as a cold), or voice strain or occasionally by bacterial infection. This eases without treatment within a week. This is known as acute laryngitis.

- A throat swab should be considered for culture.

Chronic laryngitis can occasionally have other causes, such as smoking, alcohol misuse, voice overuse, gastroesophageal reflux disease, rare infections or allergies, or inhalation of irritants or chemicals.

17. Swabs

Name of Test	Specimen Container	Turnaround time	Comments
Wound – Culture & sensitivity	Amies Transport Swab 	72 -96 hrs	Pus in a universal is always the preferred sample to a wound swab.

The following sources are available on Meditech:

- Abdomen
- Abscess
- Ankle (Left and Right options)
- Appendix
- Arm (Left and Right options)
- Arterial line
- Axilla (Left and Right options)
- Back
- Bone
- Brain
- Broviac line
- Buttock (Left and Right options)
- Cervix
- Cheek (Left and Right options)
- Chest
- Chin
- Colostomy
- Drain
- Elbow (Left and Right options)
- Endocervix
- Eye (Left and Right options)
- Face
- Finger
- Flank (Left and Right options)
- Foot (Left and Right options)
- Gastrostomy
- Groin (Left and Right options)
- Haemodialysis CVL
- Hand (Left and Right options)
- Head
- Heart
- Heel (Left and Right options)
- Hip (Left and Right options)
- Homograft (Pre and Post options)
- High vaginal swab
- Kidney
- Knee (Left and Right options)
- Leg (Left and Right options)
- Line site
- Lip (Upper and Lower options)
- Liver
- Lung
- Lymph node
- Maxillary process (Left and Right options)
- Mediastinum
- Mouth
- Neck
- Pin site (numbers 1 to 50)
- Pericardial fluid
- Peritoneal dialysis site
- PEG site

- Pelvis
- Penis
- Pericardium
- Peritoneum
- Portacath site
- Prosthesis
- Rectum
- Scalp
- Shoulder (Left and Right options)
- Shunt
- Skin
- Spleen
- Stump (Left and Right, Arm and Leg options)
- Testis (Left and Right options)
- Thigh (Left and Right options)
- Thorax
- Thrombus
- Thumb (Left and Right options)
- Tissue
- Toe
- Tongue
- Tracheostomy site
- Umbilicus
- Urethra
- Vegetation
- Vulva
- Wrist (Left and Right options)

17.1. **Choosing between swabs, tissues and fluids**

There are multiple options on Meditech to send clinical samples. In general, the more sample that can be sent to microbiology, the more the laboratory can do. Therefore, where possible, send pus/fluid samples or tissue samples in preference to swabs.

Sending tissue samples or pus/fluid samples is considered in more depth in the “Tissue samples” and “Pus and Fluid samples” section later.

17.1.1. Collecting wound swabs

- Decontaminate the skin to remove as much of the superficial flora.
- If taking a swab from another surface (e.g. abscess cavity at incision and drainage) superficial decontamination may not be indicated.
- Taking a Transwab (blue top), remove the swab and gently but firmly rotate it on the surface directly where infection is suspected.
- Do not take swabs from slough or necrotic tissue.
- Place the swab into the transport medium.
- If pus is present send as much as possible in a sterile universal container.

17.2. **Superficial skin infections**

Skin infections can be categorised typically as:

- Cellulitis – a diffuse spreading infection involving the deeper layers of the skin and subcutaneous tissues

- Erysipelas – a diffuse spreading infection involving the upper dermis and superficial lymphatic system.
- Impetigo – a superficial, intra-epidermal infection producing erythematous lesions that may be bullous or nonbullous[6].
- Paronychia – a superficial infection of the nail fold occurring as either an acute or chronic condition.
- Folliculitis – infection and inflammation of a hair follicle.
- Ecthyma gangrenosum – a focal skin lesion characterised by haemorrhage, necrosis and surrounding erythema.

Superficial swabs of intact skin are often unrewarding; consider sending swabs for suspected surgical site infections or where the skin has broken down. Skin biopsies may produce better results but are not frequently done. It may also be appropriate to consider superficial fungal infection (see 'Mycology Investigations' below). Consider sending blood cultures for severe infection.

17.2.1. Necrotising skin and soft tissue infections

Necrotising skin and soft tissue infections are typically described as gangrene (e.g. gas gangrene, Fournier's gangrene) or necrotising fasciitis, depending on the clinical features and the tissue planes involved. In cases of necrotising infection swabs are not the appropriate samples to send; these conditions require urgent surgical intervention, as well as antimicrobial therapy.

- Appropriate specimens are blood cultures, fluid from bullae, and tissue samples.

17.3. **Ulcers**

A skin ulcer is a lesion of the skin with loss of the skin integrity, which can extend from the epidermis down to deeper layers. All ulcers are invariably colonised by a polymicrobial flora and microbiology samples should be taken only if a clinical diagnosis of infection has been made.

- When swabs are taken from infected ulcers, they should be taken after cleansing and debridement to reduce the presence of superficial colonising flora.
- Chronic ulcer swabs taken to identify the cause of underlying bone infections need careful interpretation.
    - Bone biopsy specimens would be preferable

17.3.1. Burns

Patients suffering from severe burns are at a higher risk of both local and systemic infection. Gram negative organisms cause the most severe infections; fungal infections on the other hand can spread quickly, but are more easily treated. A definitive diagnosis can be difficult to obtain.

- Skin swabs should be collected as from ulcers (see above).
- Consider sending blood cultures and tissue samples for severe infection.

17.4. **Bite wounds and contact with animals**

Bite wounds can become contaminated by oral flora and normal human skin flora. Most bites are due to cats and dogs, but some are due to other pets (including reptiles, rodents and birds), domesticated animals (including horses, sheep etc.) wild animals or other humans.

- Skin swabs should be collected from broken skin.
- If pus is present a sample of pus in a sterile universal is preferred.
- Some organisms associated with animals may not grow from wound swabs – consider blood cultures and joint aspirates as appropriate, e.g.:
    - *Capnocytophaga canimorsus* is associated with dog bites and causes septicaemia. This organism is usually isolated only from blood cultures.
    - *Streptobacillus moniliformis* is associated with rat bites and diagnosis is confirmed by culturing the organism from blood or joint fluid.
- Please note some pets carry Salmonella (snakes & turtles).
- Mycobacterium marinum is associated with fish tanks.

18. Tissue Samples

Name of Test	Specimen Container	Turnaround time	Comments
Wound – Culture & sensitivity	Sterile universal 	72 -96 hrs	Pus in a universal is always the preferred sample to a wound swab.
Biopsy – Helicobacter pylori		See text	May be referred to PHE for molecular testing and susceptibility testing if successful culture

Where “Tissue” is selected as the source, the following sites are available on Meditech:

- Abdomen
- Ankle (Left and Right options)
- Appendix
- Arm (Left and Right options)
- Arterial line
- Axilla (Left and Right options)
- Back
- Brain
- Buttock (Left and Right options)
- Cheek (Left and Right options)
- Chest
- Chin
- Colostomy
- Elbow (Left and Right options)
- Face
- Finger
- Flank (Left and Right options)
- Foot (Left and Right options)
- Groin (Left and Right options)
- Hand (Left and Right options)
- Head
- Heart
- Heel (Left and Right options)
- Hip (Left and Right options)
- Kidney
- Knee (Left and Right options)
- Leg (Left and Right options)
- Liver
- Lung
- Lymph node
- Maxillary process (Left and Right options)
- Mediastinum
- Neck
- Pericardium
- Peritoneum
- Scalp
- Shoulder (Left and Right options)
- Shunt
- Skin
- Spleen
- Stump (Left and Right, Arm and Leg options)
- Thigh (Left and Right options)
- Thorax
- Thumb (Left and Right options)

- Tracheostomy site
- Wrist (Left and Right options)

18.1. **Sending tissue samples**

Ideally tissue specimens should be discussed with the laboratory prior to sampling to ensure that transport and processing are timely and appropriate tests are performed. Tissue samples are considered to be non-repeatable samples by the laboratory, therefore clinicians will be contacted if samples are mislabelled in order that corrections can be made. These corrections must be made in person.

Tissue samples collected at surgery are typically larger than percutaneous samples, and so allow for additional work if required; percutaneous samples are often used up by the initial culture procedures.

Samples for histopathology should be considered when taking tissue samples. Histological investigation can influence microbiological investigations, for example the presence of caseating granulomata raises the suspicion of mycobacterial infection; similar appearances may also be caused by deep fungal infections.

Samples from chronically infected tissues may also require investigation for fungi, *Mycobacterium* species and parasites; it is important to provide appropriate clinical information for these tests to be performed.

18.1.1. Necrotising skin and soft tissue infections

Necrotising skin and soft tissue infections are typically described as gangrene (e.g. gas gangrene, Fournier's gangrene) or necrotising fasciitis, depending on the clinical features and the tissue planes involved. In cases of necrotising infection swabs are not the appropriate samples to send; these conditions require urgent surgical intervention, as well as antimicrobial therapy.

- Appropriate specimens are blood cultures, fluid from bullae, and tissue samples.

18.1.2. Heart valves

Where patients with infective endocarditis undergo valve replacement the valve tissues (native or prosthetic) can be submitted for culture. Ideally the culture results should be correlated with blood culture or serology results. In culture-negative endocarditis additional molecular investigations may be discussed with the consultant medical microbiologists.

18.1.3. Lymph nodes

Excised lymph nodes may be sent for the investigation of lymphadenitis, particularly if mycobacterial lymphadenitis is suspected. The most common cause in under 15-year olds are non-tuberculous *Mycobacterium* species, however *M. tuberculosis* may also be isolated. Other causes of lymphadenitis, including toxoplasmosis and cat scratch disease are considered best diagnosed by a combination of histological and serological investigations; additional molecular testing may be discussed with the consultant medical microbiologists.

18.1.4. Skin biopsies

Skin biopsies may be sent for:

- The diagnosis of bacterial and fungal skin and soft tissue infection
    - Samples of abscess tissues can be sent for culture alongside pus.
- Tissue parasites such as *Leishmania* species.
- Mycobacterial infection, e.g. swimming pool or fish tank granuloma

18.1.5. Helicobacter pylori culture

If culture of a gastric biopsy for H. pylori is required, please notify the laboratory in advance; cultures must be set up promptly on receipt and not all appropriate media is kept as routine laboratory stock.

18.1.6. Prosthetic materials

Artificial materials may also be sent to the laboratory for investigation. Such materials include prosthetic cardiac valves, pacemakers, grafts, artificial joints and tissue implants.

19. Fluid Samples

Name of Test	Specimen Container	Turnaround time	Comments
Wound fluid – Count, C&S	Sterile universal 	48 -72 hrs	

The following sources are available on Meditech:

- Ankles aspirate (Left and Right options)
- Ascitic fluid
- Chest drain fluid
- Elbow aspirate (Left and Right options)
- Hip aspirate (Left and Right options)
- Knee aspirate (Left and Right options)
- Pericardial fluid
- Peritoneal dialysis fluid
- Pleural fluid
- Synovial fluid
- Shoulder aspirate (Left and Right options)
- Wrist aspirate (Left and Right options)

19.1. Sending pus and fluid samples

When sending fluid samples please ensure the sample container tops are securely fastened; samples may be non-repeatable and leaking samples may not be suitable for processing.

The detection of organisms in fluids that are normally sterile indicates significant infection, which can be life-threatening. Blood cultures may also be positive with the same organism, and may be positive when culture of the fluid fails to reveal the organism.

Where there are clinical signs of infection i.e. inflammation, oedema, pyrexia, pain or purulent exudates, it is preferable to obtain a specimen of pus rather than to take a swab.

- Pus or exudates can be drawn up in a syringe and transferred to a universal container.
- Samples may also be inoculated into blood culture bottles. This may increase the yield of fastidious or hard-to-culture organisms but limits the additional investigations that can be performed.

19.2. Necrotising skin and soft tissue infections

Necrotising skin and soft tissue infections are typically described as gangrene (e.g. gas gangrene, Fournier's gangrene) or necrotising fasciitis, depending on the clinical features and the tissue planes involved. In cases of necrotising infection swabs are not the appropriate samples to send; these conditions require urgent surgical intervention, as well as antimicrobial therapy.

- Appropriate specimens are blood cultures, fluid from bullae, and tissue samples.

19.3. Orthopaedic infections

19.3.1. Septic arthritis

Septic arthritis occurs either via haematogenous spread or directly from contiguous lesions. Infected synovial fluid is usually turbid or purulent with >75% of cells being polymorphonuclear leucocytes, although this is not specific for septic arthritis.

- The ideal sample to send is a joint aspirate in a sterile universal container.
- Send blood cultures if systemic signs of infection.
- Swabs of the joint space are not ideal samples as they cannot be sent for PCR or molecular detection.
- Inoculation of a blood culture bottle will increase yield, particularly of fastidious organisms, but again limits the ability to perform additional tests. Blood culture bottles should only be inoculated if at least 5 ml of aspirate is already collected in a sterile universal.

Purulent arthritis and synovitis may also be caused by sodium urate crystals (gout) and calcium pyrophosphate crystals (pseudo-gout). If required, samples can be referred for microscopic examination.

19.3.2. Bursitis

Inflammation of a bursa is often accompanied with prominent overlying cellulitis. The olecranon and prepatellar bursae are the most commonly affected sites. They are often subjected to repeated trauma.

- The ideal sample is an aspirate in a sterile universal container.
- Superficial swabs of intact skin are often unrewarding; consider sending swabs where the skin has broken down.

19.4. Intra-abdominal sepsis

Intra-abdominal sepsis is infection occurring in the normally sterile peritoneal cavity, and

covers primary and secondary peritonitis, as well as intra-abdominal abscesses.

19.4.1. Peritonitis

Spontaneous primary bacterial peritonitis accounts for <1% of bacterial peritonitis and is seen most frequently seen in children, and particularly those with nephrotic syndrome. Spontaneous bacterial peritonitis is the infection of pre-existing ascites in the absence of known intra-abdominal infection, and is almost always mono-microbial, usually resulting from haematogenous spread. Secondary bacterial peritonitis usually arises following leakage within the peritoneal cavity; the commonest cause in western countries is acute appendicitis.

- The ideal sample to send is an aspirate in a sterile universal container.
- Swabs of the peritoneum are not recommended if free fluid can be sent instead.
- As most samples will be sent from surgical procedures, appropriate tissue samples are also encouraged (e.g. from the appendix at appendicectomy)
- Send blood cultures if systemic signs of infection.

Chronic peritonitis may develop as a result of abscess formation and persist for weeks or months unless drained. Chronic infection may also be caused by *M. tuberculosis*. Tuberculous peritonitis is a rare disease in the UK but is more common elsewhere. If TB is suspected then discussion with the infectious diseases team is strongly recommended.

19.4.2. CAPD Peritonitis

Diagnosis is usually based on the presence of at least two of: cloudy dialysate effluent, symptoms of peritonitis, positive culture and/or Gram stain of peritoneal fluid. Cloudiness generally represents a white blood cell (WBC) count of >100 x 10^6 per litre, however chyle, fibrin or blood may also cause turbidity. The presence of >100 WBC x 10^6 per litre correlates closely with infection, although false negative culture results are possible. Low WBC counts of <100 x10^6 per litre may be associated with the early stages of infection. There is no correlation between the WBC count and the number of bacteria present; the sensitivity of a Gram stain is low (about 50%) unless there are large numbers of organisms present.

- The ideal sample to send is an aspirate in a sterile universal container.
- Swabs of the peritoneum are not recommended if free fluid can be sent instead.
- Send blood cultures if systemic signs of infection.
- Inoculation of a blood culture bottle will increase yield, particularly of fastidious organisms, but limits the ability to perform additional tests. Blood culture bottles should only be inoculated if at least 5 ml of fluid is already collected in a sterile universal.

If routine cultures are negative and abnormal dialysate findings persist after treatment of presumed or documented bacterial peritonitis, consider sending samples for mycobacterial culture. If TB is suspected then discussion with the infectious diseases team is strongly recommended.

19.4.3. Solid organ abscess (liver, spleen, pancreas)

Pyogenic liver abscesses often present as multiple abscesses, and can be a due to, biliary tract disease, haematogenous spread from another focus, surgery, or trauma. Pancreatic abscesses are potential complications of acute pancreatitis, while splenic abscesses are typically due to haematogenous spread from another focus or trauma.

- The ideal sample to send is an aspirate in a sterile universal container.
- Blood cultures are recommended.

19.4.3.1. Parasitic liver abscesses

These arise typically as a result of the spread of *Entamoeba histolytica* via the portal vein from the large bowel which is the primary site of infection. Hydatid (tapeworm) cysts may also occur as fluid-filled lesions in the liver, and cysts may become super-infected with gut flora and progress to abscess formation. If parasitic infection is suspected then discussion with the infectious diseases team is strongly recommended.

19.4.4. Subphrenic abscess

Subphrenic abscesses occur immediately below the diaphragm, often as a complication of gastrointestinal pathology (e.g. perforation) or trauma. Subphrenic abscesses are typically caused by mixed infections from the normal gastrointestinal flora.

- The ideal sample to send is an aspirate in a sterile universal container.
- Send blood cultures if systemic signs of infection.

19.5. **Retroperitoneal collections**

19.5.1. Psoas abscess

Psoas abscesses may be seen as complications to gastrointestinal pathology (e.g. appendicitis), spinal infections (e.g. osteomyelitis, discitis), bacteraemia or perinephric abscesses.

- The ideal sample to send is an aspirate in a sterile universal container.
- Blood cultures are recommended.
- If TB is suspected then discussion with the infectious diseases team is strongly

recommended.

19.5.2. Renal abscess

Renal abscesses are typically caused by Gram negative bacilli and result from ascending urinary tract infection, pyelonephritis, renal calculi or bacteraemia. Pyuria may be present, but urine culture is usually negative.

- The ideal sample to send is an aspirate in a sterile universal container.
- Blood cultures are recommended.

19.5.3. Perinephric abscess

Perinephric abscesses are an uncommon complication of UTI, which usually affects patients with one or more anatomical or physiological abnormalities. The abscess may be confined to the perinephric space or extend into adjacent structures. Pyuria, with or without positive culture, is normally seen on examination of urine.

- The ideal sample to send is an aspirate in a sterile universal container.
- Blood cultures are recommended.

19.6. **Pericarditis**

Most pericardial effusions are small in volume and are sterile. Infectious pericarditis can be separated into three groups:

1. purulent, which are caused by bacteria and is fatal if untreated.
2. benign, either due to viruses or post pericardiotomy syndrome
3. hypersensitivity or post-infectious

- If bacterial infection is suspected, blood cultures should be sent.
- The best sample for diagnosis is a pericardial aspirate if possible.
- Viral testing on other sample types (e.g. blood) may not identify the pathogen even if positive.

19.7. **Respiratory Tract infections**

19.7.1. Pleural effusion

Pleural effusions may be infective, e.g. as the result of pneumonia or the direct spread of infection into the pleural cavity from tuberculosis, or sterile, e.g. secondary to chronic heart failure, malignancy, or uraemia.

- Only small numbers of organisms are found in the effusion, and as a result microscopy is rarely positive.
- Other diagnostic samples are preferred e.g. sputum.

- Consider sending a sample for pneumococcal PCR.
- If TB is suspected then discussion with the infectious diseases team is strongly recommended.

19.7.2. Empyema

Empyema thoracis most often occurs as a complication of either pneumonia or lung abscess. The most common cause is *S. pneumoniae*, however any organism may be isolated. In the immunocompromised there are a number or unusual infections that may present with empyema; please ensure that any immune compromise is noted in the clinical details.

- Send empyema samples in a sterile universal container.
- Consider sending a sample for pneumococcal PCR.
- If TB is suspected then discussion with the infectious diseases team is strongly recommended.
- Please see the Trust "Empyema Management Guidelines": http://intranet/DocumentsPolicies/Documents/Empyema%20Management%20Guidelines.pdf

19.7.3. Lung abscess

Lung abscesses involve the destruction of lung parenchyma and present on chest radiographs as large cavities often exhibiting air-fluid levels.

- Send aspirates samples in a sterile universal container.
- Send blood cultures if systemic signs of infection.
- Consider sending a sample for pneumococcal PCR.
- If TB is suspected then discussion with the infectious diseases team is strongly recommended.
- If there is a history of foreign travel or immunocompromised/suppression please provide full details. Such patients may have unusual causes of lung abscess which may require additional cultures to be performed.

19.8. **Head and neck abscesses**

19.8.1. Brain abscess

Brain abscesses are serious and life-threatening, and as such should be referred to the neurosurgical team as soon as possible. Typical sources of infection include:

- Direct contiguous spread, e.g. from sinus infection
- Haematogenous spread

- Penetrating wounds
- Surgery
- Cryptogenic (i.e. source unknown)

Treatment of brain abscesses involves the drainage of pus and appropriate antimicrobial therapy.

- Send samples of pus from surgical procedures.
- Send blood cultures.
- If TB is suspected then discussion with the infectious diseases team is strongly recommended.
- If there is a history of foreign travel or immunocompromised/suppression please provide full details. Such patients may have unusual causes of lung abscess which may require additional cultures to be performed.
- Molecular testing will be considered by the Consultant Microbiologists after initial cultures have been performed.

19.8.2. Throat/neck abscess

- Send samples of pus from surgical procedures.
- Send blood cultures if systemic signs of infection.

19.8.3. Dental abscess

- Aspiration of dental abscesses may be taken, where possible, to identify the infecting organism(s).
    - Swabs may be contaminated with superficial commensal flora.
- In cases of intraosseal abscess, swabs can be useful, but only if taken from a disinfected site.

20. Line Tips

Name of Test	Specimen Container	Turnaround time	Comments
Wound – Line tip C&S	Sterile universal 	72 -96 hrs	Send the final 4-5 cm of the catheter for culture

The following sources are available on Meditech:

- Broviac line tip
- Chest drain tip
- CVL tip
- Drain tip
- Epidural tip
- Femoral line tip
- Line tip
- Mitrofanoff stoma
- Shunt

20.1. When should intravascular catheter tips be sent for culture?

Culture of intravascular catheter tips is recommended only if there is a suspected catheter-related bloodstream infection, where the catheter is considered to be the source of bacteraemia or fungaemia. This is typically if the catheter is infected with the same organism as isolated from blood culture(s), usually in the absence of an alternative focus of infection. Catheter-related sepsis is defined as the presence of clinical sepsis when two or more of the following occur; such as fever, leucocytosis, or hypotension, positive culture from the catheter, and negative blood cultures obtained within 48hr before and 24hr after catheter removal. There is usually no other source of sepsis demonstrated, and symptoms resolve following catheter removal.

- Send the final 4 centimetres of the catheter in a sterile universal container.

Localised infections can occur at either the insertion site or along the subcutaneous track of intravascular catheters. When the same organism is isolated from skin and blood culture, removal of the catheter may be recommended Routine investigation of catheter site swabs from asymptomatic patients is not recommended.

- Send a skin swab if local infection is present.

20.2. When should CSF shunt tips be sent for culture?

Suspected infection of CSF shunts should be referred to the neurosurgical team as a surgical emergency. CSF shunts may become infected by the following routes:

- Organisms directly colonise the shunt, usually at the time of surgery
- Organisms travel along the shunt by retrograde spread
- Organisms reach the CSF and the shunt via haematogenous spread

If a CSF shunt is removed because of suspected infection:

- Send CSF samples at the time of removal (CSF microscopy may be unremarkable in shunt infection).
- Send the final 5 centimetres from both the proximal and distal ends of the shunt itself in a sterile universal container.
- Send any associated valves or reservoirs in a sterile universal container.
- For ventriculo-peritoneal shunts, if the distal end is associated with an intra-abdominal abscess, send a pus sample.
- For ventriculo-peritoneal shunts, send peritoneal fluid if there is evidence of peritoneal inflammation.
- If there are signs of infection at either the insertion site or along the subcutaneous track send a skin swab from the affected area.
- Consider blood cultures.

Bacterial biofilms have been shown to be a problem when dealing with shunt infections and can cause delays in the effect of treatment. CSF results are of questionable value when biofilm infections are involved.

20.3. When should urinary catheter tips be sent for culture?

Never.

21. Faeces Samples

Name of Test	Specimen Container	Turnaround time	Comments
Faeces for C&S and virology	Fecon™ container (1-2ml) 	24 hours	Direct microscopy
		96 hours	Please notify the laboratory if there is a history of foreign travel as additional cultures may be required. Virology tests are performed less frequently out of season – samples are not tested for clearance purposes
Clostridium difficile Toxin Detection		24 hours	Samples will only be accepted for Clostridium difficile toxin (CDT) detection from Children over two years of age and if the sample takes the shape of the container. Samples from CDT positive patients will not be re-tested within 28 days unless approved by the consultant microbiologist.
Helicobacter pylori antigen		4 days	
Cellotape slide for Threadworm detection (available from Microbiology)	Cellotape kit 	24hr	N.B. Stool samples are NOT suitable for examination for threadworms and will be discarded.

21.1. **Community-associated gastroenteritis**

Routine faeces culture detects the typical causes of food poisoning; for this reason bacterial culture will not be undertaken if the child has been in hospital for three days or more. If bacterial culture is required after this period the laboratory must be notified (stool samples are routinely stored for 14 days); cases can either be discussed with one of the Consultants or with Infection Control. The culture procedure is in line with Public Health England's protocol. Samples cancelled as a result of this three-day rule will be displayed with the following comment:

> Sample does not meet the required testing criteria as the patient has been an inpatient for >3 days.
> Faecal culture is suitable for community-associated episodes of diarrhoea.
> Please discuss with the Infection Prevention and Control Team who will contact the laboratory if the sample requires processing.

When collecting a specimen of faeces it should be obtained in a convenient container (potty, bedpan or nappy) and transferred into a blue Fecon™ container with a plastic spoon attached. The laboratory requires a "grape sized" sample for each procedure requested.

All faecal samples submitted from children with gastroenteritis will be subject to the following investigations:

- Routine microscopy for parasites*
- Routine bacterial culture for *Salmonella*, *Shigella*, *Campylobacter* and *E. coli* O157**
- Enteric virus PCR will be restricted to children under 5 years of age or Oncology patients

* If parasitic infection is seriously considered three stool samples are required on alternate days. (Please note stored samples are not suitable for some parasitic investigations.)

Additional bacterial culture for *Vibrio cholerae* and *Yersinia enterocolitica* will be undertaken if clinical details suggestive e.g. for *Vibrio* sp. recent travel to an endemic area (Asia, Africa or Latin America); and mesenteric adenitis for *Yersinia* sp.

21.2. **Viral gastroenteritis**

Suspected cases of norovirus infection should be notified to the Infection Prevention and Control Team who will arrange appropriate testing with the laboratory. Enteric virus is routinely tested on faecal specimens from oncology patients and under 5 year olds

Limitations of in house Norovirus PCR:

- With raw or unpreserved unformed stool specimens, assay interference may be observed in the presence of Barium sulfate (found in contrast medium)(≥ 1% w/w) and Benzalkonium chloride (found in antiseptic towelettes) at all concentrations tested (1% w/v, 0.2%, w/v, and 0.04% w/v).
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown norovirus variants resulting in a false negative result.

21.3. **Clostridium difficile**

C. difficile testing will be undertaken on request only on children over two years of age.

- Samples will only be accepted for *Clostridium difficile* toxin (CDT) detection from Children over two years of age **and** if the sample takes the shape of the container.
- Samples from CDT positive patients will not be re-tested within 28 days unless approved by the consultant microbiologist.
- Rectal swabs are not accepted as a substitute for faeces for the above tests.

21.4. **Threadworm detection**

Cellotape collection kits are available from the department for the detection of *Enterobius vermicularis* (threadworm).

- The female worm emerges from the anus at night to lay its eggs so diagnosis of threadworm infection is by anal sampling in the morning BEFORE the patient has bathed and in girls before urination if possible.
- Ova are seldom seen in faeces therefore stool samples are not suitable for the detection of threadworm.
- The worm can sometimes be persuaded to emerge to lay eggs by wrapping the child in a blanket and keeping still for around 30 minutes. Negative results obtained by this method are not totally reliable.

Using the slide:

- Peel back the cellotape
- Spread the anal folds apart and firmly press the sticky side of the tape against the anus.
- Return the tape to its original position on the slide, keeping the tape as flat as possible.
- Return the slide to the petri dish provided.

- WASH YOUR HANDS
- Label the slide with a Meditech request label and send to the laboratory with the appropriate request form

21.5. *Helicobacter* antigen detection

- It is recommended that the initial diagnosis of paediatric *H. pylori* infection is based on either a positive histopathology plus a positive rapid urease test or a positive culture.
- The detection of *H. pylori* antigen in stool is a test of eradication.
- A 'test and treat' strategy is NOT recommended in children.
- See *Journal of Pediatric Gastroenterology and Nutrition* 2011;53:230-43

22. Mycobacterial investigations

Test	Sample required	Comments and expected turnaround times
TB culture & sensitivity	Appropriate specimen containers are the same as those for routine cultures.	Samples are transported daily (Mon-Fri) to Liverpool Clinical Laboratories. Positive microscopy will be reported on day of receipt by the referral laboratory. Mycobacterial culture can take up to 6 weeks to be reported. Any positive results will be notified as received.

Specimens collected for the diagnosis of mycobacterial infection should be taken (whenever possible) before anti-tubercular treatment is started. Please note 'Other' antimicrobials may also have significant anti-mycobacterial activity, notably fluoroquinolones and macrolides. Specimens other than blood or bone marrow should be refrigerated if transport to the laboratory or specimen processing is delayed for >1hr.

22.1. **Mycobacterial culture**

22.1.1. Respiratory specimens

Sputum specimens should be relatively fresh (less than 1 day old) to minimise contamination. Purulent specimens are best but induced samples may be helpful if the cough is dry. Three samples (including one early morning specimen) should be collected approximately 8-24 hours apart. Bronchoalveolar lavage or bronchial washings may be sent if spontaneous or induced sputum is unavailable, or if such specimens are AFB smear negative.

22.1.2. 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.

22.1.3. Blood and bone marrow cultures

Blood and bone marrow aspirate cultures should be pre-arranged with the microbiology department

22.1.4. Sterile site body fluids

Please provide as much sample as possible. CSF, pleural fluid etc. should be collected into a sterile universal container. If only a small volume of CSF is available from the 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 the chances of achieving a positive culture.

Please note that pleural or pericardial fluids are not very sensitive samples for the detection of *M. tuberculosis,* and a pleural or pericardial biopsy should where possible be taken with the fluid.

22.1.5. Urine specimens

Urine specimens should be collected in the early morning on three consecutive days into a universal container (that does not contain boric acid). 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.

22.1.6. Pus or pus swabs

Pus should be collected aseptically, and the largest practical sample submitted for testing. Swabs are not suitable as mycobacteria, if present, may adhere to the swab rather than be transferred successfully to the culture media.

22.2. **Interferon Gamma Release Assays (IGRA)**

Name of Test	Sample required	Comments and expected turnaround times
IGRA T-spot (age<5)	Heparin Blood Sample 	T-Spot TB test; initial processing performed at Liverpool Clinical Laboratories and then sent to a third-party laboratory
IGRA Quantiferon (age>5)		Quantiferon Gold Test performed at Liverpool Clinical Laboratories. Results usually available in 7 days.
IGRA T-spot (Immunocompromised)		T-Spot TB test; initial processing performed at Liverpool Clinical Laboratories and then sent to a third-party laboratory. Consider requesting the Quantiferon test as a first line test before the T-spot.

The T-Spot TB test and QuantiFERON TB test detect both active tuberculosis (TB) in

patients who are not yet showing symptoms and latent TB in contacts during an outbreak situation or immunocompromised patients before reactivation occurs.

- Please inform the microbiology laboratory when sending the samples to ensure they can be delivered to the Immunology Department at Liverpool Clinical Laboratories in time.
- **The sample must be received in the laboratory before 11am on the agreed date and must have the correct day & time of collection on the specimen label.**

- To be dispatched on the normal transport to Liverpool Clinical Laboratories the sample must be received in the laboratory before 11am on the agreed date.
- IGRA tests are not accepted on Bank Holiday Mondays or any day before a bank holiday.

22.2.1. <u>The T-Spot TB Test</u>

- Children under 5 years of age and immunocompromised patients
- Available on a Monday to Thursday, or on Friday if pre-arranged.
- A minimum 10 ml of blood is required for this test; in children under 2 years of age a minimum volume 4 ml is accepted.
    - Lithium heparin, sodium heparin or sodium citrate samples can be used.
- **Samples must not be refrigerated.**

22.2.2. <u>Quantiferon Test</u>

- Children over 5 years of age and Occupational Health samples.
- Available Monday to Friday.
- 6ml of heparinised blood is required for this test.
- Samples are not suitable for testing the following day and will be discarded if they miss the transport.

23. Surveillance and screening for resistant organisms

The patient groups from whom surveillance and screening cultures should be sent to the laboratory can be found in the Trust policy C20 “MRSA & Surveillance Screening Policy”, available on the Intranet. If the policy is unclear, enquiries regarding which patients to test and how frequently should be directed to the Infection Prevention and Control Team rather than the laboratory. Surveillance may be occasionally extended to additional areas of the hospital under the guidance of Infection Control – in such circumstance the Infection Control team will inform the laboratory to ensure samples are processed.

Name of Test	Specimen Container	Turnaround time	Comments
Surveillance, (multiple options for specific patient groups)	Liquid media swabs  or Fecon for stool samples 	3-5 days	Faeces samples are preferred to Rectal swabs. Throat swabs are accepted from specific patient groups
Rapid CPE PCR screens	Liquid media swabs  or Fecon for stool samples 	24 hours	Please note: All CPE Screening samples will also be processed as surveillance swabs.
Screen – MRSA (multiple options for	Amies Transport swab (orange top from Neonatal Unit, blue top elsewhere)	48 hours	

Name of Test	Specimen Container	Turnaround time	Comments
specific patient groups)			
Screen – Staph aureus			For cardiac surgery /Neurosurgical patients. Must be send IN ADDITION to MRSA screen.

23.1. **Screening for MRSA**

Using Transwabs (orange top for NNU nose swabs, blue top for all other areas) moisten each swab by inserting the swab into the agar in the bottom of the transport tube prior to sampling the required area. It is important to moisten swabs before use – this improves the isolation rate of MRSA.

Groin	Rotate the moistened swab gently but firmly over each area. One swab can be used for both groins.
Nasal	Rotate the moistened swab gently but firmly around the anterior nares of each nostril. One swab can be used for both nostrils
Other samples	Refer to the MRSA Screening Policy for the types of samples accepted.

23.2. **Surveillance for multi-resistant Gram-negative bacteria**

Screening samples are received from patients in critical care and high dependency areas. Samples include stool specimens, colostomy / ileostomy outputs and rectal swabs that show visible faecal matter. When collecting a rectal swab rather than faeces, the swab should be passed beyond the anus.

Rectal swabs that show no faecal staining will be discarded.
Please note that a stool sample is the preferred specimen for this test.

These cultures are to detect the carriage of multi-resistant bacteria in the gastrointestinal tract, in line with national guidance. To this end, the preferred specimen type is faeces itself

(or the equivalent, e.g. colostomy output). Where patients are unable to provide a faeces sample directly, it is acceptable to take a swab from e.g. a nappy, making sure that the swab collects some faecal material. Samples should be collected as soon as possible after admission, but it is preferable to wait in order to collect a good specimen if faeces is not immediately available from the patient.

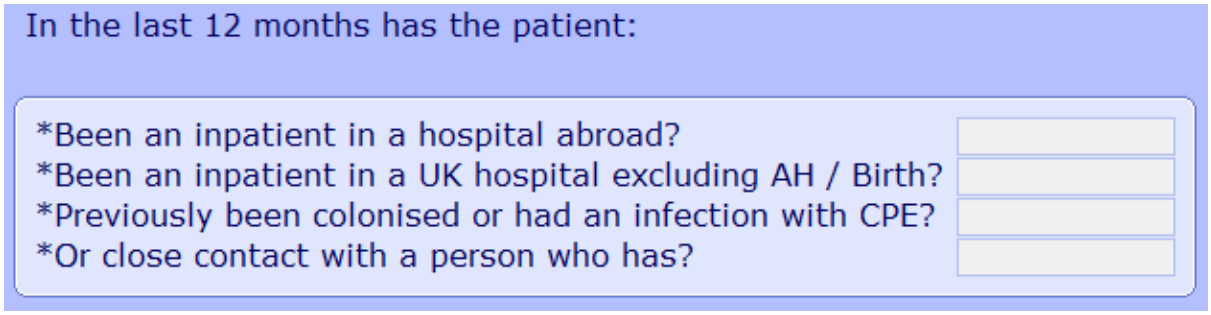
All patients are also screened for the carriage of Vancomycin resistant Enterococci

Throat swabs are processed from ventilated patients, and are screened for staphylococcal and beta-haemolytic streptococcal carriage as well.

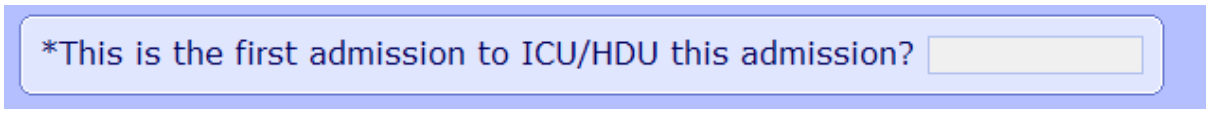
23.3. **CPE PCR Screens**

Rapid CPE screening tests are available but are restricted to patient groups as defined by PHE.

To ensure the correct patients are being screened the following questions are compulsory when creating a Meditech order.



There is an additional question when a child is being admitted to the intensive care ward from within the hospital, this answer is also compulsory.



Limitations of the test:

- The Xpert Carba-R Assay detects blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP detection of these gene sequences does not indicate the presence of viable organisms.
- Co-colonization with two or more carbapenemase-producing organisms has been reported with Xpert Carba-R Assay but competition may occur and low level colonisation by a second organism may not be detected.
- Interference with the Xpert Carba-R Assay may be observed with barium sulfate (imaging compound) at > 0.1% w/v and Pepto-Bismol at >0.025% w/v.

- The detection of other OXA-carbapenemase genes, besides blaOXA-48 and blaOXA-181, has not been evaluated.

23.4. **What do all these abbreviations mean?**

When reporting resistance mechanisms detected in Gram-negative bacteria, the following terms and abbreviations are used. Where a resistance mechanism has been identified, the comments include brief details of the appropriate infection control procedures to be followed.

23.4.1. AmpC

AmpC enzymes affect penicillin and first and second generation cephalosporins, and lead to resistance to agents such as amoxicillin and cefalexin. They are found on the chromosome in a number of species (e.g. *Enterobacter* and *Citrobacter* species), but can also be found on mobile pieces of DNA called plasmids which can be passed between species. Third generation cephalosporins (e.g. cefotaxime) are weak inducers and poor targets for AmpC enzymes, however the development of resistance is a possibility; these agents should therefore be used with caution in infection caused by AmpC-expressing isolates.

23.4.2. ESBL

Extended-spectrum beta-lactamase (ESBL) enzymes have evolved from a number of bacterial beta-lactamase enzymes. Different enzymes affect different antibiotics, however the microbiology department has opted to report ESBL-expressing isolates at resistant to cephalosporins as an antibiotic class on the reports. ESBL enzymes are commonly carried on plasmids and are therefore transferrable between species; these plasmids now often carry resistance to other agents such as aminoglycosides and quinolones. ESBL-expressing isolates are identified in the organism name on Meditech (e.g. "*Escherichia coli* ESBL").

23.4.3. CRE

Carbapenem-resistant Enterobacteriaceae (CRE) are species such as *Enterobacter*, *Klebsiella*, and *Citrobacter* that have developed resistance to at least one carbapenem antibiotic (typically ertapenem). This resistance is usually due to the expression of either an AmpC or ESBL enzyme together with mutations in the cell wall. A CRE is always resistant to a carbapenem but does not carry an enzyme that specifically targets carbapenem agents (compare with CPE isolates below). CRE isolates are identified in the organism name on Meditech (e.g. "*Escherichia coli* CRE")

23.4.4. CPE

Carbapenemase-producing Enterobacteriaceae (CRE) are species such as *Enterobacter*,

Klebsiella, and *Citrobacter* that are expressing an enzyme that breaks down carbapenem antibiotics. Carbapenemase enzymes may be carried on plasmids and be both transferrable and associated with additional resistances. Because different carbapenemase enzymes show different degrees of activity, a CPE is not always resistant to a carbapenem (compare with CRE isolates above). CPE isolates are identified in the organism name on Meditech (e.g. "*Escherichia coli* CPE").

23.4.5. MBL

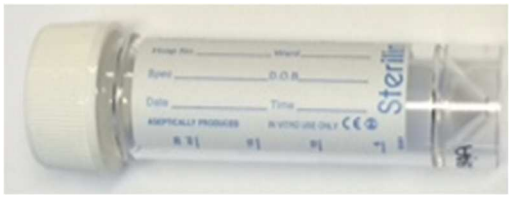
Metallo-beta-lactamase (MBL) enzymes are reported by name when detected in *Pseudomonas* species (e.g. "*Pseudomonas aeruginosa* MBL"). MBL expression leads to resistance to anti-pseudomonal penicillins (e.g. piperacillin) and cephalosporins (e.g. ceftazidime) as well as carbapenems in *Pseudomonas* and *Acinetobacter* species. MBL enzymes may be carried on plasmids and be transferrable between species.

23.4.6. OXA

Oxacillinase (OXA) enzymes are reported by name when detected in *Acinetobacter* species (e.g. "*Acinetobacter baumanii* OXA"). OXA expression is typically due to chromosomal genes in *Acinetobacter* species (unlike in *Enterobacteriaceae*) and lead to resistance to carbapenems.

24. Mycology Investigations

Name of Test	Specimen Container	Turnaround time	Comments
Fungal direct micro and culture	Dermapak™ type 3 envelopes For skin flakes (5-10 flakes)  Universal container for hairs or nail clippings (4-5 clippings)	1 - 4 weeks	Larger samples may be sent in sterile universals. All specimens are referred to the mycology reference laboratory at UKSM

Name of Test	Specimen Container	Turnaround time	Comments
			

24.1. **Dermatophyte infection**

The “Fungal direct micro and culture” is the correct test to order for superficial infections of the skin, hair and nails by fungi. If an invasive infection is suspected then the specimen types to send are as for bacterial infection, e.g. if a fungal bloodstream infection is thought possible blood cultures should be sent, if a fungal respiratory tract infection is considered, respiratory samples should be collected (and consideration to BAL if infection from a mould such as Aspergillus is considered).

Dermatophyte infections include:

- Tinea barbae – a mild to severe pustular folliculitis of the beard which can be misidentified as a Staphylococcus aureus infection
- Tinea capitis – infection of the scalp which can range from mild scaling lesions to a highly inflammatory reaction with folliculitis, scarring and alopecia. A history of animal contact or a travel history can be helpful in identifying the causative agent.
- Tinea corporis – “ringworm” of the body and may involve the trunk, shoulders and limbs. Infection may range from mild to severe, commonly presenting as annular scaly lesions with sharply defined, raised, erythematous vesicular edges.
- Tinea cruris – infections of groin, perianal and perineal sites are the most common in adult males. Lesions are erythematous and covered with thin, dry scales and may have a raised, defined border and small vesicles.
- Tinea manuum – usually presents as a diffuse hyperkeratosis affecting the palms and interdigital areas of the hands are affected. Hands are also a likely site for infection with zoophilic or geophilic dermatophytes particularly if the lesions are inflammatory, and involvement can spread to other body sites by contiguous spread and scratching.
- Tinea pedis (athlete’s foot) – toe webs and soles of the feet are most commonly affected; particularly the spaces between the fourth and fifth toes. Alternately a chronic, squamous, hyperkeratotic infection covering the pink areas of the soles,

heels and side of feet may be seen.

- Tinea unguium / onychomycosis – fungal infection of the nail.
- Pityriasis versicolor (tinea versicolor) – infection of the stratum corneum by lipophilic yeasts of the Malassezia genus.

24.2. **How should I collect samples from superficial fungal infections?**

24.2.1. 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. Dermapak™ collection kits are available from the department or a sterile universal is also suitable.

24.2.2. Nails

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. 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. Send samples to the department in a sterile universal container.

24.2.3. Hair

Samples from the scalp should include skin scales and plucked hairs or hair stumps. Cut hairs are not suitable for direct examination as the infected area is usually close to the scalp surface. Plastic hairbrushes, scalp massage pads or plastic toothbrushes may be used to sample scalps for culture where there is little obvious scaling but such samples do not replace a scraping for direct examination. Send samples to the department in a sterile universal container.

23.3 Referral of skin, hair and nail specimens for microscopy and culture

Currently specimens of skin, hair and nail are referred for microscopy and culture to the Mycology reference laboratory at UHSM –Manchester Foundation Trust

25. Virology

The majority of our virology testing is performed by Liverpool Clinical Laboratories; a small number of tests are sent to the Manchester Medical Microbiology Partnership or to Public Health England laboratories. The external laboratory where a test is performed is shown on the Microbiology report.

Samples are transported by local courier twice daily (approximately 0800 and 1300) to Liverpool Clinical Laboratories, or by national courier once daily (at approximately 1600) to other laboratories, Monday to Friday (excluding bank holidays). No routine courier service is available at weekends. A courier service is provided on a weekend/bank holiday for the transport of specimens for SARS CoV-2 as this service is provided 7 days a week

Please contact the laboratory on extension 2268 if you require any further information or if any result is required urgently; we will notify the referral laboratory in advance of any urgent request.

25.1. **Choosing serology or PCR tests**

Serology tests are usually required for immunocompetent individuals. The combination of IgM (for acute infection) and IgG (for immunity), can be used to determine if the infection was acquired recently or in the more distant past.

PCR testing can demonstrate the presence of genomic material (DNA or RNA) from a pathogen, but this does not necessarily indicate an acute (primary) infection. Viral genomic material can be detected in immunocompetent individuals with no associated disease as a consequence of prolonged virus replication after acute infection, virus reactivation from latency (secondary infection) or reinfection.

In immunocompetent non-neonates, it is not appropriate to request both viral serology and PCR at the same time. For example, if screening for acute hepatitis B virus infection, by the time of presentation with hepatitis the infection is best detected by virus specific serology tests for antigens and antibodies (HBsAg and HBc antibodies). PCR tests may be appropriate following confirmation of infection to demonstrate (and quantify) ongoing viral replication (viral load tests) in chronic infections.

If PCR tests are requested it is also important to ensure that the correct samples are sent, e.g. if adenovirus infection is suspected, respiratory or faecal samples are more appropriate

to test than blood for the presence of the virus, if congenital CMV infection is suspected then urine or saliva are the most appropriate samples.

Serology tests are usually poor tests for the immunocompromised (both iatrogenic or disease-related) and neonates (where maternal IgG will be present) because these individuals do not develop good antibody responses. Therefore PCR tests are generally required to both diagnose virus infection and to follow the virological response of any antiviral treatment given.

25.2. **Viral loads**

There are a number of “Viral Load” tests available; these should not be ordered unless the patient is known to be infected and you are monitoring the response to treatment. They are not appropriate tests to request for diagnosis.

Liverpool Clinical Laboratories advise that “the integrity of samples may be compromised if they are not received in the laboratory on the same day as they are taken. If transport is delayed then store at +4°C”. Therefore for best results sample should be taken to arrive in the Alder Hey laboratory before midday. Samples received after this time will be transported to Liverpool Clinical Laboratories the next morning and will be routinely refrigerated until collection.

25.3. **Immunoglobulins**

Human normal immunoglobulin is available from the Alder Hey pharmacy for a number of clinical indications; these can be found in the Trust “Immunoglobulin Guidelines”, available on the Trust intranet. Tetanus immunoglobulin is also available from the Alder Hey pharmacy for tetanus-prone wounds (see the Immunisation Green Book chapter 30 for full details; https://www.gov.uk/government/publications/tetanus-the-green-book-chapter-30).

Immunoglobulin for chickenpox (VZIG), hepatitis B virus (HBIG) and rabies virus (RIG) requests should be discussed directly with the virologists at Liverpool Clinical Laboratories. The medical virologists can be contacted on 0151 706 4410, or out-of-hours via the Royal Liverpool Hospital switchboard on 0151 706 2000. (Please note that the Trust “Disease Specific Immunoglobulin And Antitoxins Guidelines For Access And Use” available on the Trust intranet has not been updated to reflect the merger of the Royal Liverpool and Aintree laboratories; there are no laboratory facilities at Aintree Hospital.)

25.3.1. Varicella zoster virus IgG and immunoglobulin

Public Health guidance for issuing VZ immunoglobulin is dependent on the level of VZ IgG

detected. From July 2017 Liverpool Clinical Laboratories, and therefore Alder Hey, have reported VZ IgG results in line with the PHE guidance:

- VZV IgG antibodies DETECTED
  Past VZV infection or vaccination: indicates immunity.
- VZV IgG antibodies DETECTED (100-150 IU/ml)
  Past VZV infection or vaccination: for immunocompetent individuals (including pregnant women) indicates protective immunity.
  If immunosuppressed or neonate AND exposed to chickenpox or shingles contact medical virologist urgently.
- VZV IgG antibodies NOT detected
  Susceptible to VZV infection (chickenpox).
  If immunosuppressed, pregnant or neonate AND exposed to chickenpox or shingles contact medical virologist urgently.

25.4. **Viral infections in pregnancy**

Although Alder Hey does not provide antenatal or maternal care, it is possible that pregnant patients or staff may come into contact with viral infections that may affect the unborn child. Such cases should be managed by the team providing antenatal care. Samples from staff members will only be processed if submitted via occupational health or by arrangement with the IPCT during outbreaks.

25.4.1. Hepatitis B, Hepatitis C and HIV exposure

Exposure to Hepatitis B, Hepatitis C and HIV via needlestick injury or mucous-membrane contamination should be managed as per the Trust policy RM21 “Exposure To Blood Borne Virus (BBV) And Safe Sharp Management Policy”, available on the Trust intranet

25.4.2. Chickenpox, CMV, Parvovirus and Rubella exposure

Where patients are diagnosed with chickenpox, CMV, parvovirus or rubella infection as an inpatient, the IPCT will undertake contact tracing to identify patients or staff at risk. Staff members who recognise they have been in contact with any of these infections are advised to contact their antenatal provider for advice.

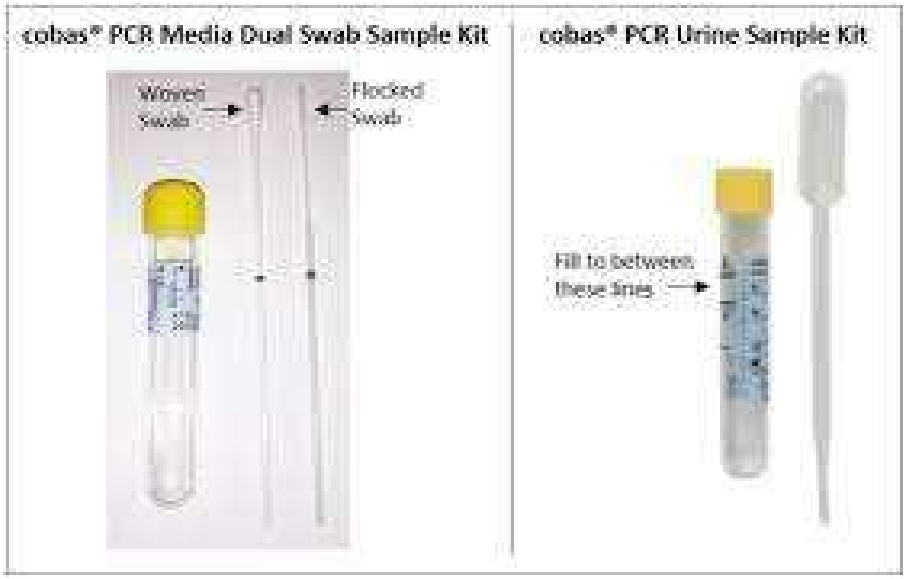
https://www.nhs.uk/conditions/pregnancy-and-baby/pages/pregnancy-infections.aspx

26. Molecular (PCR) Tests

Test	Sample required	Comments and expected turnaround times
PCR Tests	Blood sample – pink EDTA tube  CSF samples, Respiratory – Sterile container  Tissue / eye swabs for Chlamydia – Virus Transport media  Rapid CPE Screen 	Samples are transported daily (Mon-Fri) at: • ~0800 and 1200 (local courier to LCL) • ~1700 (DX courier to laboratories excluding LCL) Results are usually available within 7 days but may take longer. Please contact the laboratory if any test result is required urgently. Please note –Rapid CPE screens are restricted tests – See Surveillance section above.

There are many molecular tests that can be performed on appropriate samples. Listed below are the more commonly requested tests (either as individual requests, e.g. Hepatitis PCR, or as part of specific order sets, e.g. *Aspergillus* and *Candida* PCR on the oncology BAL order set).

Chlamydia/GC swabs – PCR testing



Instructions are provided with the collection packs

Specimens are collected into a sterile urine container and transferred into the PCR collection tube within 24 hours. Urine specimens must fill the tube between the two black lines (see above)

Genital /rectal swab (not eye swabs)

Instruction sheet provided with the swab packs. Swabs can be collected by an appropriate health care professional as per current practise

Please ensure that the swab is snapped off at the line marked on the shaft, this ensures that the swab fits correctly into the tube. If the swab is snapped off at a higher point it can pierce the cap and cause the specimen to leak

Chlamydia and viruses – eye swabs continue to use green capped viral transport media

Please note the advice regarding the appropriate selection of serology versus molecular testing given in the “Virology” section above. Inappropriate tests may be stopped either at the Alder Hey laboratory or at the receiving laboratory.

The molecular testing listed is not exhaustive and individual test requests can be discussed with the consultant medical microbiologists. Please note that some tests are only appropriate on specific sample types, e.g. VZ PCR is appropriate on vesicle fluids (for the diagnosis of chickenpox or shingles) or on CSF, but is not an appropriate diagnostic test on blood, and hence there is no VZ PCR test option on EDTA blood samples.

Name in Order Entry	Referral Lab	Sample type
Aspergillus PCR	MRI	BAL, Tissue
Atypical respiratory pathogens PCR NB. Includes *Chlamydia* and *Legionella* – not usually indicated if in-house respiratory PCR has been performed or *Legionella* infection thought likely	LCL	BAL, ET aspirate, Naso-paryngeal aspirate, Nose/throat swab (NB. Legionella PCR will not be performed on upper respiratory tract samples)
BK virus PCR	LCL	EDTA Blood, Urine
BK/JC virus PCR	LCL	CSF
Bordetella PCR	MRI	PNS, NPA
Candida PCR	MRI	BAL
Chlamydia/GC PCR (GC; *Neisseria gonorrhoea*)	LCL	Urine, Eye, Genital samples, Rectal swabs
CMV PCR (NOT BLOOD) (Cytomegalovirus PCR)	LCL	BAL, CSF, Urine, ET aspirates
CPE PCR Screen	In House	Faeces / Rectal swab
CSF PCR - HSV,VZ,Enterovirus	LCL	CSF
EBV PCR (NOT BLOOD) (Epstein-Barr virus PCR)	LCL	CSF, Bone marrow
Enterovirus PCR	LCL	Faeces, Throat swab
Faecal virus PCR (Norovirus, Rotavirus, Adenovirus, Sapovirus, Astrovirus	MRI	Faeces
Hepatitis B PCR	LCL	EDTA Blood
Hepatitis C PCR	LCL	EDTA Blood
HHV 6 PCR (Human herpesvirus 6 PCR)	MRI	EDTA Blood
HHV 6/7 PCR (Human herpesvirus 6 & 7 PCR)	MRI	EDTA Blood
HHV 6 PCR (Human herpesvirus 7 PCR)	MRI	EDTA Blood
HIV PCR – Proviral DNA	VRD, Colindale	EDTA Blood
HIV PCR – Viral load	LCL	EDTA Blood
HIV Resistance testing	LCL	EDTA Blood

Name in Order Entry	Referral Lab	Sample type
HIV tropism determination	LCL	EDTA Blood
JC virus PCR	LCL	CSF
Measles PCR	MRI	CSF, Throat swab, Urine
Mumps PCR	MRI	CSF, Throat swab, Urine, saliva NB. Best with massage of inflamed salivary gland (parotid massage
Meningococcal/Pneumococcal PCR	MRI	CSF, EDTA Blood
Parvovirus PCR NB. Specific for Parvovirus B19	LCL	EDTA Blood
Pneumocystis jirovecii PCR	LCL	BAL
Renal PCR – Adenovirus, EBV, CMV, BK	LCL	EDTA Blood
Toxoplasma PCR	MRI	EDTA Blood, CSF
Viral blood PCR- Adenovirus, EBV, CMV	LCL	EDTA Blood
Viral eye PCR – HSV, VZ, Adenovirus	LCL	Viral swab
Vesicle fluid – HSV, VZ, Enterovirus	LCL	Viral swab

Please note Enterovirus PCR requests include Parechovirus as standard and there is no separate request for Parechovirus testing.

27. Serological Tests

27.1. Collecting blood samples

Blood samples should only be taken by trained staff, there are Trust guidelines for collecting capillary samples, please contact Sara Melville the lead nurse for vascular access if advice is required.

There are WHO guidelines on best practice for drawing blood: http://www.euro.who.int/__data/assets/pdf_file/0005/268790/WHO-guidelines-on-drawing-blood-best-practices-in-phlebotomy-Eng.pdf?ua-1

Please note the advice regarding the appropriate selection of serology versus molecular testing given in the "Virology" section above. Inappropriate tests may be stopped either at the Alder Hey laboratory or at the receiving laboratory.

Test	Sample required	Comments and expected turnaround times
Serology Tests	1-2ml Clotted blood in a plain white tube  (Needle-stick testing 2-3 ml Clotted blood) Where other sample types are required this is indicated in the tables below.	Samples are transported daily (Mon-Fri) at: • ~0800 and 1200 (local courier to LCL) • ~1600 (DX courier to laboratories excluding LCL) Results are usually available in 3-21 days depending on the test. Please contact the laboratory if any test result is required urgently.

Clinical details are important when requesting any serology tests, including date of exposure and onset of symptoms. This is particularly true for zoonotic and parasitic infection; without these details the samples may be cancelled. Paired samples may be required to assess change in antibody titre.

27.2. Viral Serology

There are many serological tests that can be performed; listed below are the more commonly requested tests (either as individual requests, e.g. EBV, CMV etc. immunity

status, or as part of specific groups, e.g. the acute chronic hepatitis panel).

Please Note: For any request that includes HIV testing written / electronic documentation of patient consent is required.

Name in Order Entry	Test(s) Included	Referral Lab
Acute hepatitis diagnosis	HBsAg, HBc, HAV IgG & IgM, HCV Ab	LCL
Adoption screen	**HIV Ag & Ab**, HBsAg, HCV Ab, Syphilis serology	LCL
BMT donor screen	HBsAg, anti-HBc, HCV Ab, **HIV Ag & Ab**, CMV IgG, EBV VCA IgG, HTLV 1&2, Syphilis, Toxoplasma IgM & IgG, HSV IgG, VZV IgG	LCL
Cardiomyopathy screen	Parvovirus IgG & IgM, Toxoplasma IgG & IgM	LCL
Chronic hepatitis diagnosis	HBsAg, HBc, HCV Ab	LCL
CMV diagnosis	CMV IgG & IgM	LCL
CMV immunity	CMV IgG	LCL
Congenital infection screen	Toxoplasma IgM & IgG, CMV IgG & IgM	LCL
Dialysis screen	HBsAg, HCV Ab, **HIV Ag & Ab**	LCL
EBV diagnosis (symptomatic)	EBV VCA IgM & IgG	LCL
EBV immunity	EBV VCA IgG	LCL
Hepatitis A - immunity status	HAV IgG	LCL
Hepatitis B surface antigen	HBsAg	LCL
Hepatitis B post vaccine status	anti-HBs	LCL
Hep B core Ab - Gastro team only	HBc	LCL
Hepatitis C antibody	HCV Ab	LCL
HIV serology	HIV Ab & Ag	LCL
HIV serology– Rainbow	HIV Ab & Ag	LCL
Hodgkins – hepatitis screen	HBcAb, HBsAg, HCV Ab	LCL
LAC serology	**HIV Ag & Ab**, HBsAg, HCV Ab, Syphilis serology	LCL
Measles diagnosis	Measles IgG & IgM	MRI

Name in Order Entry	Test(s) Included	Referral Lab
Measles immunity	Measles IgG	LCL
Mumps diagnosis	Mumps IgG & IgM	MRI
Mumps immunity	Mumps IgG	MRI
Needlestick – donor	**HIV Ag & Ab**, HBsAg, HCV Ab	LCL
Needlestick – recipient	anti-HBs, serum to store	LCL
See Trust Policy RM21 “Exposure to BBV and Safe Sharp Management Policy”		
Oncology –pre sperm collection	**HIV Ag & Ab**, HBsAg, HBcAb, HCV Ab	LCL
Organ donor screen	HBsAg, anti-HBc, HCV Ab, **HIV Ag & Ab**, CMV IgG, EBV VCA IgG, HTLV 1&2, Syphilis, Toxoplasma IgM & IgG	LCL
Parvovirus serology	Parvovirus IgM and IgG	LCL
Rainbow viral serology	**HIV Ag & Ab**, HBsAg, HBcAb, HCV Ab, Syphilis serology	LCL
Renal dialysis blood borne virus	HBsAg, HCV Ab, **HIV Ag & Ab**	LCL
Renal nephrotic	HBsAg, HCV Ab, CMV IgG, EBV IgG	LCL
Renal pre transplant baseline	HBsAg, anti-HBc, HCV Ab, CMV IgG, EBV IgG, Measles IgG, VZ IgG, **HIV Ag & Ab**	LCL
Renal transplant A/R	HBsAg, anti-HBc, HCV Ab, Measles IgG, VZ IgG	LCL
Renal transplant base returned	HBsAg, anti-HBc, HCV Ab, CMV IgG, EBV IgG	LCL
Rubella diagnosis	Rubella IgG & IgM	MRI
Rubella immunity	Rubella IgG	LCL
Store sample	None	LCL
Transplant recipient screen	HBsAg, anti-HBc, HCV Ab, **HIV Ag & Ab**, CMV IgG, VZV IgG, EBV VCA IgG	LCL
VZ diagnosis NB. VZ PCR on vesicle fluid is preferable to serology	Varicella IgG & IgM	MRI
VZ immunity	Varicella IgG	LCL

27.3. **Bacterial serology**

Name in Order Entry	Referral Lab
Anti DNase b & ASOT For interpretation purposes an ASOT is always performed with this test	In-house
ASOT A screening test will be performed initially; positive results will be further investigated.	In-house
ASOT screening test reported same/next working day, titres will be performed within 7 days	
Bartonella serology	VITROME, Marseille
Bordetella pertussis serology	RVPBRU, Colindale
Brucella serology	LCL
E.coli 0157 serology	GBRU, Colindale
Func. Ab. (Hib & Pneumo only) (*Haemophilus* and pneumococcal functional antibody screen)	SNGH
Functional Abs (ID team only) (*Haemophilus,* tetanus and pneumococcal functional antibody screen)	SNGH
Used to measure the response to vaccination. Please request only the tests required and when available please include any relevant vaccination details.	
Leptospira serology	LHTD
Lyme serology	PHE, Porton
Meningococcal serology *	MRI
* Use reference laboratory form for convalescent serum tests	
Pneumococcal specific antibodies	MRI
For testing post Prevenar vaccination. Results are reported within one month.	
Pneumococcal antigen	LCL
Q fever serology	PHE, Porton
Rickettsia serology	PHE, Porton
Syphilis serology	LCL
Toxocara screen	LHTD
Toxoplasma serology	LCL or

Name in Order Entry	Referral Lab
	PHE, Swansea**
** Dependent upon clinical information – refer all samples to Consultant Microbiologist	
Urine – Legionella antigen	LCL

Two bacterial antigen tests can be performed on urine but are not considered to be routine:

Name in Order Entry	Referral Lab
Pneumococcal antigen	LCL
Urine – Legionella antigen	LCL

27.4. Fungal serology

Name in Order Entry	Sample type	Referral Lab
Aspergillus precipitans	Clotted blood	LCL
Cryptococcal antigen	CSF	LCL
Galactomannan test (*Aspergillus* antigen)	Clotted blood, BAL	LCL

27.5. Zoonotic infection (e.g. Lyme disease) and imported diseases

Requests for zoonotic infection or imported pathogens are referred to the PHE Rare and Imported Diseases Laboratory at Porton. Referral forms for such tests require clinical information that is often not present on the Meditech request; for this reason some requests have a statement on the Meditech order referring the requester to the laboratory, e.g. for Lyme serology requests:

Lyme (Borrelia burgdorferi) serology is often not appropriate for diagnosis of an acute infection. Serology requests will not be processed without prior approval; please contact one of the Medical Microbiology consultants to discuss this request. Samples will be stored in the laboratory for one week from receipt before discarding if no approval is received.

28. Quality Control

28.1. External assessment

The microbiology department is accredited to United Kingdom Accreditation Service (UKAS) ISO 10189 standards.

28.2. External quality assurance

The microbiology laboratory is registered with the following external quality assurance schemes:

Scheme	Clinical Applications Covered	Provider
General Bacteriology	Isolation of pathogens from simulated clinical material	UKNEQAS
Antimicrobial Susceptibility Testing	Determination of susceptibility to antibacterial agents	UKNEQAS
Clostridium difficile detection	Detection of *C. difficile* toxin	UKNEQAS
MRSA	Detection of MRSA	UKNEQAS
Viruses in CSF	Detection of virus	Labquality
Faecal parasitology and teaching	Examination for the presence of parasites	UKNEQAS
Respiratory rapid: RSV	Detection of RSV	UKNEQAS
Molecular detection of respiratory viruses inc SARS CoV-2	Detection of virus	Labquality
Antifungal Susceptibility Testing	Determination of susceptibility to antifungal agents	UKNEQAS
Viral Gastroenteritis	Detection of viruses in a simulated faeces specimen	UKNEQAS
SARS CoV-2 PCR	Detection of SARS CoV-2 in nose /throat swabs	UKNEQAS
Endoscopic Rinse Waters	Detection of *Pseudomonas* in a simulated water sample	FEPTU
Recreational and Surface Water	Swimming pool water contamination	FEPTU
Helicobacter pylori antigen detection	*H. pylori* antigen detection in faeces	Labquality
Gram stain – blood culture	Gram stain of simulated blood cultures	Labquality
Resistant Gram-negative bacilli and VRE	Detection of multi-drug resistance mechanisms	Labquality
Streptococcal antibodies	Streptococcal ASOT and anti-DNase B detection	INSTAND eV

- UKNEQAS is the UK National External Quality Assurance Scheme for microbiology operated by Public Health England.
- FEPTU are the Food and Environment Proficiency Testing Unit operated by Public Health England.
- Labquality are a Finnish provider of laboratory quality control materials.
- INSTAND eV is a German provider of laboratory quality control materials.
- QCMD is an international provider of quality control materials.

28.3. Internal quality assurance

- All test kits are tested before being used clinically to ensure they are working correctly.
- Antimicrobial susceptibility testing is subject to weekly control testing.
- There is an ongoing internal quality assurance scheme involving the duplicate testing of clinical samples, performed monthly.
- CSF and tissue samples are excluded from duplicate testing as the sample volumes are typically too small and may be required for further testing.

28.4. Document Control

- All bench guides and standard operating procedures used in Microbiology are controlled and managed electronically using iPassport (Genial Genetics Ltd).
- Laboratory standard operating procedures are based on the Public Health England Standards for Microbiological Investigations.

28.5. Patient Confidentiality

- All Staff are aware of the importance of patient confidentiality; they are all required to complete the following Statuary and Mandatory training:
    - Information Governance
    - Safeguarding Level 1
    - Safeguarding Level 2
- All access to Meditech and email is controlled by secure passwords which must not be shared, staff must log off shared PC's when they leave or lock a personal PC if it is left unattended.

28.6. Requests for Results from External Agencies:

- Staff must ensure that the caller is genuine; they must take a number and telephone the caller back to confirm identity. If the caller refuses no result may be given.
- Any faxed results must be sent to a confidential secure fax to a named individual.
- Patient data may be sent by email internally between @alderhey.nhs.uk email addresses or externally between secure @nhs.net accounts.
- Personal information is not sent from Alder Hey accounts to external email accounts.

28.7. Complaints Procedure

- Please contact a consultant microbiologist or the Lead Biomedical Scientist if you have any concerns or cause for complaint regarding any aspect of the service

provided by this department.

- We will endeavour to act on any concerns raised and will inform you of any actions taken.
- If appropriate, an incident will be logged on the Trust Inphase system.

29. List of Referral Laboratories

The Microbiology Department refers work to the following UKAS accredited laboratories. Our primary referral laboratory for virology (molecular and serology) is Liverpool Clinical Laboratories (based at the Royal Liverpool Hospital); this was selected after an open tendering process. Other referral laboratories have been selected on the basis of:

- Providing a recognised national reference service (e.g. Public Health England (PHE) laboratories)
- The most local accredited provider of an appropriate test (e.g. Manchester Medical Microbiology Partnership)
- Single provider of the test (e.g. VITROME, Marseilles)

Abbreviation	Laboratory	Address
GOSH	Department of Microbiology, Virology and Infection Control	Level 4 Camelia Botnar Laboratories Great Ormond Street Hospital NHS Foundation Trust Great Ormond Street London WC1N 3JH
LCL	Liverpool Clinical Laboratories • Microbiology Department • Immunology Department • Virology Department	CSSB Liverpool University Hospitals NHS Foundation Trust–Royal Liverpool Site Mount Vernon Street Liverpool L7 8YE
LHTD	Department of Parasitology	The Hospital for Tropical Diseases Mortimer Market LONDON WC15 6AU
LSTM	The Diagnostic Laboratory	Liverpool School of Tropical Medicine Pembroke Place Liverpool L3 5QA
UHSM/MRC	University Hospital of South Manchester Mycology Reference Centre	Regional Mycology Laboratory 2nd Floor Laboratory, Education and Research Centre Wythenshawe Hospital Southmoor Road Manchester. M23 9LT
MRI	Molecular Diagnostic Laboratory Meningococcal Reference Unit Vaccine Evaluation Unit	Manchester Medical Microbiology Partnership PO Box 209 Clinical Sciences Building Manchester Royal Infirmary Oxford Road Manchester M13 9WZ

Abbreviation	Laboratory	Address
PHE, Birmingham	National Mycobacteria Reference Service	Public Health Laboratory Birmingham Heart of England NHS Foundation Trust Bordesley Green East Birmingham B9 5SS
PHE, Colindale	Antibiotic Resistance and Healthcare Associated Infections (AMRHAI) Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) Gastrointestinal Bacteria Reference Unit (GBRU) Sexual Transmitted Bacteria Reference Unit (STBRU) Virus Reference Department (VRD)	Centre for Infection Public Health England 61 Colindale Avenue London NW9 5EQ
PHE, Porton	Rare and Imported Pathogens Laboratory	Rare and Imported Pathogens Laboratory Porton Down Salisbury Wiltshire SP4 0JG
SNGH	Department of Immunology	Sheffield Northern General Hospital PO Box 894, SHEFFIELD, S5 7YT
PHE, Swansea	Toxoplasma Reference Laboratory	Toxoplasma reference laboratory (TRL) Department of Microbiology Singleton Hospital Sgeti Swansea SA2 8QA
VITROME	Pr Pierre-Edouard Fournier VITROME, Aix-Marseille Université, IRD	IHU - Méditerranée Infection 19-21 Bd Jean Moulin 13005 Marseille France Tel: +33 (0)413 732 401 Fax: +33 (0)413 732 402