Pathology Services

Microbiology Handbook

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Microbiology Laboratory Hours

The microbiology laboratory is based at Stoke Mandeville Hospital

Monday – Friday	08.00 hrs – 20.00 hrs	Normal Laboratory Service
Saturday, Sunday	08.00 hrs – 16.00 hrs	Restricted service

At all other times an on-call service is available.
On-call Biomedical Scientists can be contacted via the hospital switchboard.
Consultant Microbiologists are also available via the hospital switchboard for advice.

Telephone contacts (prefix with 110 if phoning from sites other than Stoke Mandeville)

	Ext. numbers
Dr. Ruby Devi, Consultant Microbiologist	5481
Dr. Jean O'Driscoll, Consultant Microbiologist	5329
Dr. Karthiga Sithamparanathan, Consultant Microbiologist	5593
Dr. Nick Wong, Consultant Microbiologist	5327
Department Secretaries	5330/5322/8162
Clinical Advice Hotline (09:00hrs – 17:00hrs) or email **buc-tr.micro@nhs.net**	5322
Mr D. Pritchard Head Biomedical Scientist	6392
Microbiology Reception/Results	5321
Microbiology Laboratory	5328/5334
Virology Laboratory	5323
Anand Pancholi Head Biomedical Scientist Blood sciences	5355
Mehwish Khalid Lead Biomedical Scientist Blood sciences	5355
Pathology Reception	5591
Infection Prevention & Control	5337 (Bleep 762)
Ms Gladys Lawson, Pathology Service Manager	5346
Ms Jules Hicken, General Manager Radiology and Pathology	6913
Mr D. Green, Pathology IT Manager	5013

(The majority of extension can be accessed directly 01296 31<ext.>)

For **microbiology supplies** (i.e. forms, containers and swabs) please telephone the appropriate extension and leave a message on the answer phone for Wycombe and send an email with an attached supplies form to Stoke Mandeville.

Stoke Mandeville	bht.pathologysupplies@nhs.net
Wycombe	5248

Microbiology Forms

A completed microbiology form **must** accompany or be available to view for every request. The form can be created either via the computer "ICE" system (as a request form or sticker) or by using the pre-printed microbiology or serology request form (It **should** contain the following legible information:

- Patient's full name, sex, hospital number (if hospital based) or NHS number (if GP based), date of birth.
- Location i.e. Ward name, Out-patient dept, GP practice code.
- Full Consultant or General Practitioner name (not just their initials).
- Requesting doctor's (or nurse's) name with bleep number (if hospital based).
- Where relevant, details of GP/other clinician to who copy of report to be sent.
- NHS or Private Patient.
- Type of specimen and site from which it was collected e.g. wound swab from abdomen.
- Date of specimen collection. In some cases, time of specimen collection also required i.e. antibiotic assay, semen analysis.
- All relevant clinical details – this is vital for the laboratory to decide on appropriate processing and subsequent interpretation of results. Any previous or current antibiotic therapy should be stated.
- For virology/serology investigations in particular, date of onset of the suspected illness or date of exposure in a viral contact case is very important.

Please note:

a) Locum GPs are asked to ensure they fill in the correct GP practice number on forms.
b) The out of hours GP services must ensure they quote the patients' usual GP name and location code when sending a specimen.
c) For GP requests, if the NHS number is not available, this must be clearly stated on the form i.e. if patient is temporary resident.
d) Add the patient's address, as this will help if any out of hour's action is required or where follow up by the local Public Health Department is needed.
e) Sexual Health Clinic patients will be identified by a code only.
f) Occupational Health requests may not include an NHS or hospital number.
g) Once Request forms are sent to Microbiology and receipted on LIMS, it is classed as consent from patient and requester to share clinical information, family history (if provided) to relevant healthcare professional and referral laboratory.
h) Prior to labelling a sample, ensure that you have asked the patient to confirm their full name and date of birth (positive identification of patient).

Specimens and Containers

Use only containers/swabs/bottles provided by the laboratory to collect specimens. They must be securely closed and fully labelled with the patients' full name, date of birth, hospital or NHS number, type of specimen, date (and time) of collection. All specimens must be placed in a plastic bag with the accompanying completed form. If both a routine vaginal swab and an endocervical swab for Chlamydia investigation is taken, send the routine vaginal swab with a microbiology request form and the Chlamydia swab with a serology/immunology request form.

Please note:

a) Inadequately labelled specimens will be rejected unless the specimen is unrepeatable e.g. CSF. In such a case, the requesting doctor will be asked to attend the laboratory to verify the identity of the sample before it is processed if practically possible.

b) Any specimen received without a completed form will also be rejected. However the laboratory will complete their own microbiology form using details available on the specimen itself. The sample will then be entered onto the computer system and an attempt will be made to establish the most likely source from previous pathology results so that a rejection report can be sent out.

See sections entitled "Guidelines for specimen collection" and "Guidelines for microbiology tests". These provide further information on how to obtain appropriate samples for microbiological testing and the average time for results to be available. Samples should be taken whenever possible before antibiotics/antiseptics are started.

High Risk Patients

High-risk patients refers to those individuals known or suspected of being infected with certain pathogens such as TB, Hepatitis B, Hepatitis C, HIV, Enteric fever group Salmonella species and Brucella, or any other organism that may pose a risk to staff. Any pathology specimens and accompanying forms sent from such patients should be labelled as 'High-Risk'. To maintain patient confidentiality, the nature of the risk (e.g. HIV positive) must **not** be written on the form. A 'High-Risk' label can be used and will be all that is required (contact microbiologists if further advice is needed).

Specimen Transport and Storage

Successful laboratory diagnosis depends on the collection of specimens at the appropriate time using the correct technique and equipment, followed by safe transport to the laboratory.

The transport of specimens is under the control of the Portering Services Department. For samples originating from General Practitioners, a daily delivery and collection service is provided for each surgery. All microbiological samples should be processed as soon as possible after collection but if taken in the community late in the day, after the last collection service, the sample may be kept overnight in a refrigerator at 4^{o}C until collection the next morning. Patients may deliver their own specimens directly to the Pathology reception areas at either Wycombe or Stoke Mandeville Hospitals during normal working hours. If specimens are delivered out of normal hours at Stoke Mandeville Hospital, they should be taken to the Porters Lodge next to the A&E department.

All samples collected from in-patients at Wycombe or Stoke Mandeville Hospitals will be delivered by porters to the respective Pathology reception areas. The Wycombe based specimens together with specimens originating from Amersham Hospital will be transported several times each day to the Microbiology department at Stoke Mandeville. For samples taken out of normal laboratory hours, blood culture bottles must be stored in the incubator whilst all other specimens except CSF must be placed in the refrigerator at 4^{o}C, available in the Pathology reception areas. CSF specimens must be kept at room temperature before being processed as low (refrigeration) temperatures may adversely affect the quality of the sample. When

urgent microbiological specimens are being sent during the day e.g. CSF or joint aspirate, the Microbiology laboratory should first be informed by telephone.

Emergency Microbiology Specimens Out-of-Hours

Outside normal laboratory hours, there is a Biomedical Scientist (BMS) on-call for processing urgent specimens who must be contacted via the hospital switchboard before any specimen is sent. All on-call specimens must be clearly marked on the accompanying form as 'URGENT'.

At Stoke Mandeville Hospital, on-call specimens (except CSF which is kept at room temperature) must be left in the refrigerator in the "out of hours" reception area to await pick-up by the BMS unless other individual arrangements are made between the doctor and BMS. At Wycombe Hospital, on-call specimens must be delivered to the Cardiac & Stroke Recovery Unit (CSRU), normally by a porter. The clinician must then contact the Helpdesk (Ext. 5010) to request transport of the specimen to Stoke Mandeville "out of hours" pathology reception area via a courier. At Amersham hospital, on-call specimens must first be delivered to the porter's office at the hospital's main entrance from where courier transport to Stoke Mandeville will be arranged.

Please state requester's name and bleep number on all accompanying forms, so that results can be quickly relayed back. The types of specimen which may be processed as an emergency include the following:

a) CSF.
b) Joint aspirates in cases of suspected septic arthritis.
c) Operative samples of pus/tissue.
d) Urine samples in children.
e) Specimens from ITU patients where clinically indicated.
f) Acute ophthalmology specimens.

Issuing of Results/Advice and Telephone Enquiries

To ensure confidentiality **under no circumstances** will information or results be given to patients making telephone enquiries. GPs and hospital clinicians are therefore advised **not** to instruct patients to contact the laboratory for their own results.

Under the restrictions/requirements of their Professional Body, please note that Biomedical Scientists are **not** permitted to give treatment advice. Any such enquiries should be addressed to the Consultant Microbiologists within the Department, who can be contacted via details given earlier in this Handbook.

When telephoning the laboratory, callers must identify themselves (GP surgery number is a useful identifier). If the member of staff is in any doubt concerning the identification of the caller, they will arrange to ring the caller back. This is done purely to protect the confidentiality of patient results. For any HIV enquiries, these must be directed to the Consultant Microbiologists. Telephone requests for results should wait till after 11.00 a.m. as laboratory work is often ongoing before that time. Urgent enquiries only should be made between 9.00 a.m. and 11.00 a.m. In the hospitals, before telephoning the laboratory, check on "ICE" computer system to see if results are available. There is a specific clinical advice hotline (Ext. 5322) for when microbiological advice is required during normal working hours.

Please remember that for the majority of specimens sent for microbiological testing, meaningful results may take a few days to finalise. Guidance on average turn-around times for most specimens sent to the laboratory is provided in this handbook.

Quality, Feedback, Compliments and Complaints

It is essential to have confidence in the data and results produced by the laboratory. The laboratory runs a comprehensive quality management system, operating a schedule of Internal Quality Assurance (IQA), corrective action and quality improvement alongside External Quality Assurance (EQA) programmes. These programmes are designed to give an estimation of the laboratories performance against other users and also to highlight the performance of particular tests or equipment against rival manufacturers. Performance data is available on request to the laboratory. Clinically relevant changes to test performance are communicated to clinicians as they occur.

Information about service users and patients is treated confidentially and with respect. All laboratory premises are secure and all computer systems are password protected as per the Trust's guidelines and Caldicott principles. Accuracy of data is audited by random sampling of records by our Quality Management programme. Confidential waste is disposed of securely.

If you have any comments, suggestions, questions, or compliments please contact the Lead BMS's or Consultants who will record your contact and discuss with laboratory management or investigate your concerns in accordance with Trust policies. Your views are important to us as we strive to improve the service we provide. Please also let us know about new services you would wish to see developed.

In particular, let us know of any untoward delay in receipt of reports, any discrepancies between results and clinical picture, and any errors in patient or clinician name or location on the report. When samples have been referred to other laboratories for testing, this will be indicated on the report form.

If a result needs to be amended due to new information or a correction, depending on the severity of the issue the requesting clinician or representative may be contacted. A new report will also be produced both hardcopy and electronic. A comment will be added to the report saying that it is an amended report and to disregard the previous report associated with that specimen, identified by the unique laboratory number. If any further clarification is needed please contact the laboratory immediately.

You can also contact the Trust:
Email your complaint to bht.complaints@nhs.net
Telephone the complaints team on 01494 734958
The Patient Advice and Liaison Service (PALS) provide support and advice to patients, their families and friends. Contact PALS on 01296 316042 or send an email to bht.pals@nhs.net

General Points for Bacteriology Specimens

- Specimens should be as fresh as possible for the optimal isolation of bacteria. Some organisms do not survive long outside the body e.g. gonococcus, anaerobes. Delay in specimens reaching the Microbiology department can result in either death of significant bacteria before isolation or overgrowth of contaminating organisms i.e. in urine samples.

- Remember that actual pus is always superior to a pus swab for microbiological examination. Pus should be sent in a sterile container whilst all swabs must be sent in charcoal transport medium to help organism survival.

- Always provide as much clinical information as possible. It will help the laboratory to provide a more helpful interpretation of bacteriological results.

- Specimens should ideally be collected before the start of antibiotics. Even one dose may damage organisms enough to prevent their growth and isolation in the laboratory.

- Make sure that the container used to hold the specimen does not contain formalin.

Virology/Serology Specimens

1. General Points

- Many infectious diseases are only diagnosed using serological techniques to detect antigen, antibody or nucleic acid in blood. For most antigen/antibody tests, 7-10 mls of clotted blood is required. For nucleic acid detection (PCR methods etc.), including viral load measurements, 3 mls of EDTA blood is usually needed.

- For serology tests, it is essential to give full clinical details including date of onset of any illness or date of exposure if a potential contact situation is being investigated.

- Each ward, hospital clinical area and GP surgery should have a small supply of chlamydia/viral collection packs which can be stored at room temperature.

- For the viral investigation of a vesicular rash, remove roof of vesicle with a sterile needle then using a swab from the viral collection pack, rub over vesicle base and snap swab off into the plastic bottle containing viral transport medium (VTM).

- Excluding swabs sent for PCR tests, all other specimens such as nasopharyngeal secretions should be sent in sterile containers without any additive.

- Diagnosis of influenza: send a nasopharyngeal swab or nose and throat swabs in VTMswab

- To demonstrate a recent infection, two blood samples (acute and convalescent) may sometimes be required, taken 10-14 days apart, to look for any significant change in antibody titre. In some clinical situations e.g. suspected acute Hepatitis A or Ebstein Barr Virus, recent infection may be diagnosed from one sample by detecting IgM antibody.

- If in doubt regarding the investigation of a patient or requesting the most appropriate tests, contact the Microbiologists for further advice.

- For the immunological investigation of latent tuberculosis, the laboratory should be contacted for advice on appropriate blood tests i.e. Quantiferon, Elispot. These tests should not be performed before discussion with the serology laboratory.

Below is a table of common BD Vacutainer™ tube types supplied by the laboratory.

BD Vacutainer Colour	Tube Name	Sample Produced	Routine Application
	SST II Advance	Serum	All routine Virology Investigations.
	Potassium EDTA	Plasma or Whole Blood - EDTA	Lymphocyte markers, Molecular assays, detection of pathogens by PCR and viral loads

2. Known High Risk Samples

The laboratory operates a policy of universal precautions applied to all samples received for testing. This means that all samples are treated with the degree of care necessary to minimise infection risks to staff. When a patient is known to be “high risk” or has a confirmed infection with a blood borne virus this should be documented on the request form and “high risk” stickers used. This allows laboratory staff to ensure that relevant samples are handled in the correct way to minimise the risk of any laboratory acquired infection.

3. Storage and Retention of Blood Samples

All initial laboratory testing, whenever possible, is undertaken from the primary sample tube which remains stored in the laboratory for a minimum of 14 days.

The laboratory has a responsibility to store aliquots of antenatal booking bloods for 2 years to ensure that the samples remain available for additional testing if required during the pregnancy or post-natal period.

The laboratory can be contacted on 01296 315323 to request that additional tests are added to stored samples. Laboratory staff will need to confirm that a sample is available and then the additional requests can be performed.

4. Urgent Samples

The laboratory staff will make every effort to respond to requests for urgent results. Urgent requests are considered to be those which may impact directly on patient care or treatment, or when patient specific requirements need to be met.

If you have a requirement for an urgent result then please follow the instructions below:

- Contact the laboratory on 01296 315323 (normal laboratory hours Mon-Fri) to inform them of the urgent request
- Provide details of the patient to be tested and clinical indications for testing
- Provide details of the patient location
- Expected time of arrival of the sample
- Contact details for telephoning the result

The laboratory will confirm whether the sample can be processed in the time frame requested and provide a guide as to when the result will be available.

5. Key Factors Which May Adversely Affect Test Results

It is well accepted that the best possible results can only be achieved if the samples received are of the highest quality. The quality of the samples received remains a shared responsibility for both the laboratory and the requester.

Acute Infections	It is not advisable to request auto-antibody testing on patients who have significant acute infections, bacterial or viral, as these patients will give weakly false positive reactions in many assays. This can lead to confusion and delay in diagnosis and treatment.
Drips & Infusions	Do not collect samples from the same arm as any intravenous infusion as this will dilute the peripheral circulation and lead to a poor quality result.
EDTA	EDTA samples are not suitable for many viral antibody tests and clotted samples should be sent whenever possible.
Haemolysed Samples	Grossly haemolysed samples can adversely affect some antibody tests, and results should be interpreted with caution.
Heterophile Antibodies	These are antibodies with non-specific binding properties which can cause confusion when looking for viral antibodies. They are particularly common in patients who have received multiple blood transfusions or in women with a history of multiple pregnancies.

Immune Deficiency	Patients receiving replacement IV immunoglobulin cannot be tested for IgG, IgA or IgM responses, as they do not have a normal humeral immune response. All results will be falsely negative.
Immunocompromised	Patients who are significantly immunocompromised do not demonstrate normal antibody responses, and all of their results must be interpreted with caution. Please discuss the testing requirements for these patients with the laboratory before undertaking any testing.
Lipaemic Samples	Grossly lipaemic samples can interfere with antibody binding and either reduce the sensitivity of an assay or lead to weakly false positive results.
Lithium heparin	We strongly discourage the sending of samples which have lithium heparin anti-coagulant in them. This can lead to alterations in specific protein measurements and these samples are not suitable for agglutination assays where the antibody binding is inhibited.
Neonatal Samples	Sample volumes in the handbook are quoted for adult patients. Please contact the laboratory to discuss the sample requirements for neonatal or paediatric patients.
Post Transfusion	Results are unreliable on all patients who have received significant blood transfusions as the laboratory investigations will detect antibodies and antigens present in the transfused units.
Request Review	In order to maintain the confidence in the sensitivity and specificity of the laboratory results, all requests are reviewed by experienced members of the laboratory staff. If the investigation is not indicated by the clinical information provided, then the request may be withdrawn and additional information requested. Samples are stored within the laboratory awaiting the information and are not discarded.
Results	All laboratory results are issued electronically either onto the hospital Review system, ICE electronic requesting or via GP links. Every effort should be made to obtain the required results from these locations before contacting the laboratory for a verbal result. This reduces the likelihood of a verbal transcription error.
Sample Type	If in any doubt, please contact the laboratory to confirm the specific sample type required for any specific investigations.
Tipped Samples	If a sample has been collected into the wrong tube, DO NOT tip it into the correct one. The sample will already have been adversely affected and then cause added complications as it appears to be in the correct sample tube.

Transport	All samples are transported to the laboratory as quickly as possible and in a way appropriate for the test request. There may be occasions when extreme or adverse conditions may affect sample integrity and this will be documented on any reports.

Guidelines for Specimen Collection of Samples for Microbiological Investigation

Successful laboratory diagnosis depends on specimen collection at the right time, ideally before starting antimicrobial therapy and using the correct technique to avoid problems such as contamination.

This section provides guidance and instruction on the optimal collection of the most commonly requested microbiological specimens.

i) Mid-Stream Urine (MSU)

Explain to the patient what is required.

Female patients – Label up the sterile container provided beforehand. Sit comfortably on the toilet seat with legs spread. Part the labia and start passing urine. After a few seconds, stop and then use the container to collect the next (middle) portion of urine flow, usually 10-15 mls. Stop once more then pass the remaining urine into the toilet again. Try to avoid the container touching the genital area, legs or clothing. When finished make sure the container lid is securely tightened.

If the patient is particularly obese, has restricted mobility or has already produced a 'contaminated' sample, the same procedure should be followed but with an additional 'vulval cleansing' step before any urine is passed. Swabs or cotton balls dampened with sterile saline/water are used to clean the urethral opening by wiping the area once only from front to back. This is repeated 3-4 times with a new swab/cotton ball each time.

Male patients – The same principles are followed except that the man should be advised to pull back his foreskin before urine flow begins.

ii) Clean Catch Urine (CCU)

If the patient is bed bound or has some other reason for not being able to provide an MSU sample, a clean catch urine may be collected. Before starting, if the patient's vulva or penis is heavily soiled, wash the area with sterile saline or water. The patient then passes urine into a clean bedpan and 10-15 mls is transferred into a labelled sterile container.

iii) Catheter Sample Urine (CSU)

Catheter drainage systems have a port through which urine specimens are taken without opening the system. The tubing is clamped below the level of the port and urine allowed to accumulate. The port is wiped with an alcohol swab and allowed to dry. The urine is then collected via a sterile needle and syringe, taking care not to go through the tubing, and transferred into a labelled sterile container. CSU's should never be taken from the drainage bag as this produces misleading culture results due to bacterial growth in stagnant urine.

Bacterial colonisation is extremely common once a patient is catheterised. CSU specimens should only be sent if a patient has new urinary tract symptoms e.g. acute haematuria, suprapubic pain or is systemically septic.

iv) Faeces

Formed stools will not normally be examined.
In hospital, only send type 5-7 stool (Bristol Stool Chart).

In hospital, the patient passes faeces into a clean bedpan. The bedpan and contents are taken to the sluice and wearing disposable gloves, a labelled sterile laboratory approved container is one third filled with faeces using some form of spatula or swab. In young children, faecal matter removed from nappies in a similar way is adequate.

In the community, patients should be advised to open their bowels into a suitable receptacle such as a potty or disposable foil tray. From here a specimen can be transferred into the sterile container again using either the blue spoon or another implement. If no suitable receptacle is available the patient should lay several sheets of toilet paper (at least 6 sheets thick) above the surface of the water in the toilet and after opening their bowels, transfer some of the specimen into the container as previously described.

v) Blood Cultures

Taking blood cultures is one of the most important investigations for aiding in the diagnosis of patients with infections and their subsequent treatment. Blood culture contamination is a common occurrence (up to 10%) if the correct technique is not followed. This will result in a significant level of false positive readings which may negatively impact patient care. Contamination can originate from the patient's skin, the equipment used, and the hands of the clinicians taking the sample or the general environment.

Blood cultures should only be taken after identification of possible bacteraemia or sepsis and before the administration of antibiotics. Signs of sepsis may be minimal or absent in the very young and the elderly but the following clinical features should be considered in potential bacteraemia or sepsis:

Core temperature out of normal range
Tachycardia/hypotension/tachypnoea
Chills/rigors
New or worsening confusion
High or very low white blood cell count

Do not use existing peripheral lines/cannula or sites immediately above an in-situ IV device to obtain the blood culture sample. If a central line is present, blood may be taken from each lumen of the catheter **and** from a separate peripheral vein. Femoral vein sampling should only be performed as a last resort because of the difficulty in adequate skin cleansing and disinfection.

Procedure to follow for taking blood cultures – please refer to Trust Guideline 706 for full details

vi) Cerebrospinal Fluid (CSF)

The examination of cerebrospinal fluid (CSF) is an important and potentially unrepeatable investigation. On most occasions, CSF is obtained to exclude the diagnosis of meningitis or meningo-encephalitis and therefore ideally the sample should be obtained before antimicrobials are given. This may not always be possible nor appropriate in cases of suspected life-threatening infection, but pathogen detection is now possible by methods such as PCR in addition to direct culture.

A CSF sample taken via lumbar puncture must be collected using strict aseptic technique (see Section 2.11 in Trust Infection Prevention and Control Manual). For the majority of cases (excluding neonates/young paediatric patients and in CSF examination for non-infective investigations) CSF should be collected into 3 sterile conical bottom universal containers. The first and third aliquots are sent to microbiology whilst the second container is sent to biochemistry for glucose, protein, lactate and any other relevant biochemical tests. A minimum of 1ml of CSF is required for microbiological testing. However 2-3mls in each bottle is a more ideal volume. For neonates or small infants, as much CSF as can be safely obtained should be collected in two containers, one each for microbiology and biochemistry. For any patient requiring mycobacterial investigations, 10mls of fluid will be needed for optimal examination and for this potential diagnosis, accompanying forms and containers themselves should be labelled with “High Risk” stickers.

CSF samples need to be examined and processed as soon as possible and certainly within 2 hours of collection (see Specimen Transport and Storage, Emergency Microbiology Specimens out-of-hours for details on making sure the specimen reaches the laboratory promptly). Please note: CSF samples should <u>not</u> be refrigerated before the initial laboratory examination/processing as this is likely to affect the quality of the specimen including the survival of any leucocytes, erythrocytes and organisms present.

Normal CSF Values

Leucocytes	Neonates	0-30 cells x 10^6/L (few polymorphs allowed)
	1-4 yr old	0-20 cells x 10^6/L (few polymorphs allowed)
	5 yr - puberty	0-10 cells x 10^6/L (all lymphocytes)
	Adults	0-5 cells x 10^6/L (all lymphocytes)
Erythrocytes	Newborn	0-675 cells x 10^6/L
	Others	0-10 cells x 10^6/L

Protein	Neonates (up to 6 days old)	Up to 0.7g/L
	Others	0.15 – 0.45g/L
Glucose		>60% of simultaneously examined blood glucose concentration.

vii) Sputum

Sputum samples are often best obtained early in the morning. For in-patients, help from a physiotherapist may be necessary. The patient should be asked to expectorate sputum into a sterile container. It is essential to ensure that the specimen is not just saliva and does contain purulent material. Sputum samples should be sent to the laboratory immediately as most respiratory pathogens will not survive for prolonged periods externally. Only purulent specimens received a maximum of 24 hours after collection will be processed.

viii) Nose, Throat and Ear Swabs

Nose - insert into both nostrils, one after the other and gently rotate. The swab is then placed in the transport medium tube and sent to the laboratory.
Throat - the use of a tongue depressor is often helpful. The swab should be quickly but gently rubbed over the pharyngeal wall and/or tonsillar area, then placed in the transport medium and sent to the laboratory.
Ear - slowly introduce the swab into the ear canal and gently rotate. Place swab in transport medium and send to the laboratory. If a deep ear swab is required, this should only be performed by experienced medical staff using a small speculum.

ix) Eye Swab

If a purulent conjunctivitis is present, pus may best be collected on a swab from the inner canthus of the eye. If chlamydial eye infection is also suspected, any swab for routine bacterial culture should be taken first, then the special thin chlamydial swab found in the chlamydia/viral collection pack must be rubbed along the conjunctival lining inside the lower eyelid. This will pick up potentially infected epithelial cells. The swab is then broken off into chlamydia transport medium and sent to the laboratory.

x) Wound and Pus Swabs

These should be obtained at the beginning of any dressing procedure prior to wound cleansing. The swab should be taken from the suspected infective site, avoiding contact with surrounding normal skin or mucosa. If a significant amount of pus is present, this should be aspirated wherever possible with a sterile syringe and transferred into a universal container, as actual pus will be superior to a swab for optimal laboratory examination.

xi) Fluids

For all types of fluids e.g. joint, pleural, ascitic, collect good representative amount of specimen using an aseptic collection method (usually sterile

needle and syringe). Send fluid to the laboratory in a sterile conical bottom universal container.
If infected ascites is suspected e.g. subacute bacterial peritonitis, 30-40mls of ascitic fluid should be obtained. Using a sterile needle and syringe, inject 10mls of fluid into each bottle of a blood culture set and the remaining fluid into the universal container, then send to the laboratory. Microscopy including a cell count and Gram stain will be performed on the neat fluid whilst the inoculated blood culture bottles will act as an enrichment culture particularly for fastidious or damaged organisms.

xii) Genital Tract Swabs including Chlamydia Swabs and Urine Samples for Chlamydia

Female genital tract swabs should be taken through a vaginal speculum. If investigating for possible pelvic infection or cervicitis, a clear view of the cervical is required. Two swabs will be needed. Firstly using an ordinary swab, remove any pus and/or mucus from the cervical and send to the laboratory in charcoal medium for routine bacteriological (including gonococcal) culture. Then using the thin swab supplied in the chlamydia/viral collection pack, insert into the cervical canal and rotate for 15-20 seconds. Withdraw the swab, insert it in the plastic tube of C/VTM and break off the shaft at the obvious breaking point. Do **not** use the C/VTM to moisten the swab first as it is potentially toxic. Tightly recap the tube and fully label. Refrigerate the specimen if there is a delay in sending it to the laboratory.

A self-taken vaginal swab, sampling the lower end of the vagina with the same thin chlamydia swab then breaking off into the C/VTM, is an acceptable alternative specimen. For male patients in particular, a 'first pass' urine (i.e. the first 10-15 mls of the stream) can be collected in a tall narrow 30 ml sterile container and sent for chlamydial PCR test. The patient should not have passed urine for at least 2-3 hours before providing this sample.

xiii) Skin, nail and hair samples for mycology

Patients' skin and nails can be swabbed with 70% alcohol prior to specimen collection if creams, lotions or powders have previously been applied. For suspected skin lesions, the edges of lesions yield the greatest quantity of viable fungus. Lesions should be scraped with a blunt scalpel blade.

Good nail samples can be difficult to obtain. Take material from any discoloured, dystrophic or brittle parts of the nail. Using nail clippers, cut as far back as possible through the entire thickness of the affected nail and include any crumbly material especially from under the nail. When there is purely superficial nail involvement, scrapings may be taken with a curette.

In suspected fungal scalp infection, samples should include skin scales and either plucked hairs or hair stumps removed with forceps. Cut hairs are not suitable as the infected area is usually close to the scalp surface.

Scrapings, clippings and plucked hair samples should be plentiful and representative. Specific "Dermopak" black card envelope packs or sterile containers should be used to collect samples and send to the laboratory. If

more than one site is being investigated use a separate pack or container for each site.

To help with interpretation of subsequent results, the following points may be helpful:

1. A positive microscopy result ("fungal elements seen") is an indication for treatment but does not guarantee that infecting fungus will grow on laboratory culture.
2. If positive microscopy result reported but culture proves negative, this may be due to sampling tissue where fungus is no longer viable or patient has already received antifungal treatment. Up to 35% of dermatophyte infected nails fail to grow on culture; therefore microscopy is of paramount importance in making the diagnosis.
3. A negative microscopy followed by a positive dermatophyte culture result is most commonly due to sampling of healthy tissue along with an infected area and treatment is still indicated.

xiv) Gentamicin, Vancomycin and Teicoplanin assays

Assays for monitoring these antibiotics are performed in the Biochemistry Department. Clotted blood (yellow topped tube) should be sent with an accompanying Biochemistry form giving full details of the antibiotic regimen, when the last dose of antibiotic was given and when the blood sample was taken. Results are immediately entered on the "ICE" computer system but any advice on the use of these antibiotics must be obtained from the Microbiology medical staff. See Rx Guidelines app or the Trust Antimicrobial Website for further details on gentamicin, teicoplanin and vancomycin monitoring.

Occasionally patients may be on other antibiotics such as tobramycin amikacin or Daptomycin and antifungal agents such as voriconazole that require monitoring. These tests are sent away to the Antimicrobial Reference Unit in Bristol and advice on when and how to monitor may be obtained from the microbiologists or see relevant guidelines on Trust Antimicrobial Website.

xv) Semen analysis

a) For infertility investigations

An appointment booking system operates. The patient's GP or hospital consultant must refer the patient by completing a hard copy of a Microbiology request form or **PRINTING OFF** a hard copy of the ICE request form **(i.e. please do not refer solely via the ICE system as requests for semen analysis made electronically cannot be accessed by the Microbiology Department).** Please include the individual's full address and ask for a semen analysis test for infertility to be arranged. The form must be sent to the Microbiology Secretaries at Stoke Mandeville Hospital either via post or email (buc-tr.micro@nhs.net) and at the same time the patient should be given a semen analysis collection kit. On receipt of referral the secretaries will place the patient on a waiting list and a letter will be sent out when appointments becomes available requesting the patient telephone in to make an appointment on a date they prefer. The sample should

then be brought to either the Microbiology Department at Stoke Mandeville Hospital or the Cellular Pathology Department at Wycombe Hospital. Clear instructions are given in the collection kits for appropriate production of the sample and its delivery to the laboratory for examination.

b) Post vasectomy checks

Post vasectomy specimens DO NOT require an appointment. However, there is a specific collection kit for post-vasectomy samples which should be issued. Patients can deliver these specimens to Pathology reception at either hospital Monday to Friday before midday – please ensure the accompanying request form clearly states that it is a "post vasectomy" check.

GUIDELINE FOR TESTING OF URINE

Background information:

- Consider whether urine culture is needed (see following tables). Do not send urines in asymptomatic patients unless antenatal.
- In elderly, bacteriuria is common and not related to increased morbidity or mortality. Only send sample if at least 2 of the following symptoms are present – dysuria, pyrexia >38^{o}C, new incontinence, increased confusion.
- 15% adult women get UTI each year but 50% of women with UTI symptoms have negative culture. Symptoms may be due to inflammation of the urethra – "urethral syndrome" – which does not respond to antimicrobials.
- Do not send routine catheter urine samples, as bacteriuria is usual. Only send for culture if features of systemic infection or new acute haematuria are present.
- In sexually active young men and women, remember that chlamydia infection may produce UTI symptoms.

Urine microscopy/dipstick testing:

Microscopy is no longer routinely performed on all urine samples. Studies have shown that near patient dipstick testing, with the detection of leukocyte esterase (LE) and/or nitrite (N) has a sensitivity and specificity equivalent to microscopy for predicting positive urine culture.

Urine microscopy will be performed on all paediatric samples <3 years age as per NICE guidance. In infants > 3 years, dipstick testing for leukocyte esterase and nitrite is diagnostically as useful as microscopy and culture, and can safely be used. However, microscopy may be performed in >3 year infants if they have at least 1 of the following risk factors:

- Infants and children who are suspected to have acute pyelonephritis/upper urinary tract infection
- Infants and children with a high to intermediate risk of serious illness
- Infants and children with recurrent UTI
- When clinical symptoms and dipstick tests do not correlate.

Microscopy will also be performed on request for suspected glomerular/tubular renal disease, localisation of bleeding and possible schistosomal infection.

Samples to be collected - in adults an MSU, CSU or clean catch urine sample should be obtained. In infants and young children, a clean catch is best but a urine collection pad sample, a catheter specimen or a suprapubic bladder aspirate may also be used. Bag urine specimens are the least favoured due to high contamination risk. If there is likely to be a delay in culture (>4 hours), the sample must be refrigerated.

Assessment of patient:

Each patient should be clinically assessed as "high" or "low" likelihood of having a urinary infection. The appropriate type of urine sample is then collected and a dipstick test is performed by the clinician. According to the results of the clinical assessment and LE/N dipstick tests, the patient and their urine sample should be managed as detailed in the following tables.

NB C.S.U. samples will always give a positive dipstick test – treatment should only be considered when patient is systemically unwell or has new acute urinary symptoms e.g. haematuria.

Urine culture and empirical treatment:

1. Adults (>16yr)

High likelihood of UTI – dysuria, frequency, loin pain, haematuria
Low likelihood of UTI – vague lower abdominal pain

Dip stick test result	High likelihood of UTI	Low likelihood of UTI
LE positive N positive	Regard patient as having UTI. **Start empirical antibiotics** e.g. trimethoprim/nitrofurantoin. **Do not send sample** to the laboratory for culture and sensitivity UNLESS recurrent/non responsive infection or male or pregnant.	
LE negative N positive or LE positive N negative	**Start empirical antibiotics** e.g. trimethoprim/nitrofurantoin. **Do not send sample** to the laboratory for culture and sensitivity UNLESS recurrent/non responsive infection or male or pregnant.	Send sample to the laboratory for culture and sensitivity. Subsequent management will depend on results of urine culture and clinical review.
LE negative N negative	Send sample to the laboratory for culture and sensitivity. Subsequent management will depend on result of urine culture and clinical review.	Send sample to the laboratory for culture and sensitivity **ONLY IF** pregnant or male otherwise UTI excluded discard urine

specimen.

2. Children: A <3yrs, B 3yr -16yr (See Guideline 380)

Children <3mths: With clinically suspected UTI should be referred urgently to secondary care.

Children 3mths - 3 years: With clinically suspected UTI, use urine dipstick testing (see NICE Guideline 54 2017). If child systemically unwell refer to secondary care for treatment pending culture results.

High likelihood of UTI –

Infants <3 months of age: Fever, vomiting, lethargy, irritability, poor feeding, weight loss/poor weight gain. Less commonly jaundice, haematuria, offensive urine, abdominal pain.

Infants 3 months and older, preverbal children: Fever, abdominal pain, loin tenderness, vomiting. Less commonly lethargy, irritability haematuria, offensive and cloudy urine.

Verbal children: Urinary frequency and dysuria. Less commonly dysfunctional voiding, retention / incontinence, loin tenderness, abdominal pain. Less likely still, fever, malaise, vomiting, haematuria, offensive/cloudy urine.

Low likelihood of UTI – other source of symptoms identified.

A. Children <3years (See Guideline 380)

Dip stick test result	High likelihood of UTI	Low likelihood of UTI
LE positive N positive	Regard child as having UTI. Send definitive sample to the laboratory for culture and sensitivity. **Start antibiotics**.	
LE negative N positive or LE positive N negative	Send definitive sample to the laboratory for culture and sensitivity. **Start antibiotics** (if dipstick test done on a fresh sample of urine). Subsequent management will depend on result of urine culture.	Send sample to the laboratory for microscopy, culture and sensitivity. Subsequent management will depend on results of urine culture and clinical review. If likelihood of UTI increases send definitive sample and **start antibiotics.**
LE negative N negative	Send definitive sample to the laboratory for microscopy, culture and sensitivity. Subsequent management will depend on result of urine culture and clinical review. If likelihood of UTI increases send definitive sample and **start antibiotics.**	

B. Children 3years – 16years (See Guideline 380)

Dip stick test result	High likelihood of UTI	Low likelihood of UTI
LE positive N positive	Regard child as having UTI. Send sample to the laboratory for culture and sensitivity. **Start antibiotics**.	Send sample to the laboratory for culture and sensitivity only. Subsequent management will depend on results of urine culture and clinical review. **Start antibiotics** if likelihood of UTI increases.
LE negative N positive or LE positive N negative		Send sample to the laboratory for microscopy, culture and sensitivity. Subsequent management will depend on result of urine culture and clinical review. **Start antibiotics** if likelihood of UTI increases.
LE negative N negative	Send sample to the laboratory for culture and sensitivity. Subsequent management will depend on result of urine culture and clinical review.	UTI excluded discard urine specimen.

GUIDELINE FOR ENTERIC PCR

The laboratory no longer routinely cultures stools for common enteric pathogens but uses a molecular automated platform for the detection of enteric pathogens directly from stool samples. The test targets *Salmonella* sp, *Shigella* sp, *Campylobacter* sp, Verotoxin-producing *E.coli* (VTEC), *Cryptosporidium* and *Giardia* in a PCR assay.

The negative samples will be reported as:

Giardia species DNA	Not Detected
Cryptosporidium species DNA	Not Detected
Salmonella species DNA	Not Detected
Shigella species / EIEC DNA	Not Detected
Campylobacter species DNA	Not Detected
VTEC (Verotoxic E.coli) DNA	Not Detected

Where a target is detected for *Salmonella* sp, *Shigella* sp and *Campylobacter* sp, culture will be performed and if the organism is viable, full identification and sensitivity tests will be carried out. *Salmonella* sp and *Shigella* sp will be sent to the Gastrointestinal Bacteria Reference Unit (GBRU) for confirmation and typing. If VTECs are detected the stool sample will be sent directly to GBRU for full investigation. The laboratory will culture for *E. coli* O157 but verotoxin producing strains other than *E.coli* O157 can also cause diarrhoea.

The *ipaH* plasmid target for *Shigella* may also be present in Enteroinvasive *E. coli* (EIEC) so the assay would not be able to differentiate between the two organisms and on culture Shigella may not be isolated. EIEC is a recognised cause of travellers' diarrhoea, similar to that caused by *Shigella,* and is usually self-limiting.

Occasionally the organism will not grow on culture as it may not be viable but the system will still detect small amounts of DNA. In these cases we will issue the report as NOT ISOLATED.

The system is not designed to detect asymptomatic carriage of Salmonella sp. If this test is required please indicate on the request form.

We aim to be able to provide a same day service for results on samples that we receive before 2:30pm Monday – Friday and before 10:30am at the weekends and bank holidays.

PATIENTS WITH NOTIFIABLE INFECTIOUS DISEASES

There is a legal requirement that patient's seen in hospital or the community with a confirmed (or suspected – see below) notifiable infectious diseases are reported to the Proper Officer of the Local Authority. The Proper Officer is either the Consultant in Communicable Disease Control (CCDC) or Consultant in Health Protection (CHP) who are responsible for the appropriate action being taken. The CCDC/CHP is based at Thames Valley HPT (South East), Public Health England, Chilton, Didcot, Oxon. OX11 0RQ and can be contacted either directly during normal working hours (Tel No. 0344 225 3861) or via the Wycombe/Stoke Mandeville Hospital switchboards out of hours. If the case is in hospital, the Trust's Infection Prevention & Control Team should also be informed. The responsibility for notification lies with the Clinician (Registered Medical Practitioner – RMP) in charge of the patient. This may either be a Hospital Consultant or General Practitioner.

List of Notifiable Infections. The list of notifiable diseases with further clinical details and guidance on degree of urgency for notification is given below.

For all these infections, a notification form must be completed and sent to the Proper Officer at the address given on the form. In the hospitals the form is available on the Trust's Intranet (mailto:http://swanlive/sites/default/files/guideline_242.pdf) All notifications are medically confidential.

Health Protection (Notification) Regulations 2010

The new Regulations also require RMPs to notify the CCDC/CHP if there are "reasonable grounds for suspecting" that the patient:

- Has an infection which in the view of the RMP presents or could present significant harm to human health (e.g. emerging or new infections): or
- Is contaminated (such as with chemicals or radiation) in a manner which, in the view of the RMP could present significant harm to human health: or
- Has died with, but not necessarily because of a notifiable disease, or other infectious disease or contamination that presents or could have presented significant harm to human health.

The RMP should not wait for laboratory confirmation or results from other investigations in order to notify a case of suspected infection or contamination. There are clear time limits for notification by the RMP, including provision for urgent reporting:

- Urgent – as soon as possible after clinical suspicion or diagnosis, and always **within 24 hours**. Verbal notification needs to be followed by written notification **within 3 days** using the form (via fax or email).
- Non urgent – the RMP notifies the Proper Officer in writing **within 3 days** using the form (via fax or email).

NB A contact number of the notifying RMP should be provided at the time of written notification (preferably a mobile number). Email notifications must be password protected with the password being sent in a second email. They should only be used for non-urgent notifications.

Additional Reporting to Infection Prevention & Control Team

In addition to the list of notifiable infections, the Trust's Infection Prevention & Control Team should be informed if any of the following proven or suspected infectious disease cases occur in the hospital setting:

- More than two patients on a ward with unexplained diarrhoea with/without vomiting.
- MRSA or other multi-resistant organism (e.g. ESBL producing coliform, CPE or VRE) causing infection or colonisation.
- Candida auris (recent admission discharge from Royal Brompton Hospital or Neuro ITU at JR, Oxford)
- Any suspected influenza.
- Unusual disease associated with possible deliberate release of pathogen/toxin.

Notifiable diseases, with explanatory notes and guidance on the need for urgent notification

Notifiable disease	Definition / comment	Urgent?
Acute encephalitis		No
Acute meningitis	Viral and bacterial	Yes, if suspected bacterial infection
Acute poliomyelitis		Yes
Acute infectious hepatitis	Close contacts of acute hepatitis A and hepatitis B cases need rapid prophylaxis. Urgent notification will facilitate prompt laboratory testing. Hepatitis C cases known to be acute need to be followed up rapidly as this may signify recent transmission from a source that could be controlled.	Yes
Anthrax		Yes
Botulism		Yes
Brucellosis		No, unless thought to be UK-acquired
Cholera		Yes
Diphtheria		Yes
Enteric fever (typhoid or paratyphoid fever)	Clinical diagnosis of a case before microbiological confirmation (e.g. case with fever, constipation, rose spots and travel history) would be an appropriate trigger for initial public health measures, such as exclusion of cases and contacts in high risk groups (e.g. food handlers).	Yes

Notifiable disease	Definition / comment	Urgent?
Food poisoning	Any disease of infectious or toxic nature caused by, or thought to be caused by consumption of food or water (definition of the Advisory Committee on the Microbiological Safety of Food)	Yes, if clusters or outbreaks
Haemolytic uraemic syndrome (HUS)	E. coli 0157 would be the most common associated pathogen	Yes
Infectious bloody diarrhoea		Yes
Invasive group A streptococcal disease (IGAS) and scarlet fever		Yes if IGAS No if scarlet fever
Legionnaires' Disease		Yes
Leprosy		No
Malaria		No, unless thought to be UK-acquired
Measles	Post-exposure immunisation (MMR or HNIG) does not provide total protection for contacts. Infection may still be acquired but illness is reduced	Yes
Meningococcal septicaemia		Yes
Mumps	Post-exposure immunisation (MMR or HNIG) does not provide total protection for contacts. Infection may still be acquired but illness is reduced	No
Plague		Yes
Rabies		Yes
Rubella	Post-exposure immunisation (MMR or HNIG) does not provide total protection for contacts. Infection may still be acquired but illness is reduced	No
Severe Acute Respiratory Syndrome (SARS)		Yes
Smallpox		Yes
Tetanus		No, unless associated with injecting drug use
Tuberculosis		No, unless healthcare worker or suspected cluster or multi drug resistance.

Notifiable disease	Definition / comment	Urgent?
Typhus		No
Viral haemorrhagic fever (VHF)	e.g. Ebola, Congo-Crimean, Marburg and Lassa viruses	Yes
Whooping cough		Yes, if diagnosed during acute phase
Yellow fever		No, unless thought to be UK-acquired

MICROBIOLOGY TESTS PERFORMED, SPECIMENS REQUIRED AND AVERAGE TURN-AROUND TIMES

PLEASE NOTE:

- All average turn-around times given represent normal laboratory working days.
- Turn-around times may be extended for all bacteriology samples when potentially significant pathogens isolated require further identification and sensitivity testing.
- For all tests marked *, more urgent examination may be performed following consultation with microbiologist e.g. Hepatitis B test following needlestick injury.
- For all specimens sent to the microbiology departments, only use laboratory approved containers, swabs, bottles etc.
- Significant positive results including those from preliminary investigations on in-patients that may change management are phoned to clinical teams by the consultant microbiologist on blood cultures, sterile fluids including CSF samples and faecal specimens.
- In line with good antimicrobial stewardship, the sensitivities reported to clinicians/GPs will reflect the narrowest spectrum of agents appropriate for treatment and will take into account possible penicillin allergy.

A) BACTERIOLOGY – MOST COMMON SPECIMENS

Blood cultures

Specimen required:	Pair of culture bottles. Single paediatric bottle for neonates/infants.
Average turn-around time:	Incubated for 5 days but all positives reported directly to clinician at time of detection. Prolonged incubation of 14 days is performed in specific clinical circumstances e.g. investigation of endocarditis.

Bronchial washings / tracheal aspirates

Specimen required:	Sample in sterile container.
Average turn-around time:	2 days.

Cerebral Spinal Fluid (CSF)

Specimen required:	Fluid in sterile conical bottom containers (usually samples 1 and 3 if three aliquots collected at lumbar puncture with sample 2 to biochemistry for protein/glucose/other measurements). Minimum 1ml CSF required, ideally 2-3ml in each bottle.
Average turn-around time:	Microscopy (and PCR if appropriate) performed immediately. Microscopy results will be phoned as soon as possible, the PCR takes a minimum of 1hr and again will be phoned. Culture – minimum 2 days. All microscopy results and positive cultures reported directly to clinician.

Chlamydia PCR

Specimen required: Endocervical/urethral/vaginal/conjunctival/rectal thin swab in chlamydia/viral transport medium.
"First pass" urine (10-15 mls) in sterile conical bottom container (mainly for male patients)

Average turn-around time: Negatives – 2 days. Positives – 3 days. Samples batched but run daily. If rectal swab positive, sample sent for lymphogranuloma venereum strains of Chlamydia. Results take 7-10 days.

Ear/Nose/Throat swab

Specimen required: Swab in charcoal transport medium.

Average turn-around time: 2 days - nose and throat swabs. 3 days – ear swabs.

Eye swab

Specimen required: Swab in charcoal transport medium.

Average turn-around time: 2 days.

Faeces

Specimen required: Loose (Type 5-7) faeces in **blue sterile container with spoon** or standard sterile container. Fill container one third full.

Average turn-around time: Cl. difficile toxin/GDH detection – 1 day. Enteric PCR – 1 day for initial result, further culture may be required. Culture longer for few specific pathogens e.g. Vibrio cholera
*Parasites – up to 3 days. Samples batched. OCP concentration procedure only undertaken if history of foreign travel to high risk areas or other parasite specific clinical information is supplied.
All significant isolates reported directly to clinician if in-patient.

Fluids/Aspirates/Pus

Specimen required: Sample in sterile conical bottom containers.

Average turn-around time: Preliminary report – 3 days. Extended culture - 5 days but further report will only be issued if significant growth found.

Fungi

Specimen required: Skin scrapings/nail clippings/hairs with roots in 'Dermopak' envelope collection kit.

Average turn-around time: Microscopy – up to 7 days. Samples batched. Culture – up to 3 weeks.

Genital tract swab (cervix, urethra, vagina excluding chlamydia test)

Specimen required: Swab in charcoal transport medium.

Average turn-around time: 2-3 days – dependent on clinical details given.

Intravascular line tips

Specimen required:	Distal 2-3cm of line tip cut aseptically at time of line removal and put in sterile container. Only send tips if clinical suspicion of line-related sepsis, together with relevant blood cultures. Give good clinical details on accompanying form. Tips will be processed if positive blood culture detected 7 days before to 7 days after line removal, or if there is other good clinical evidence of line sepsis.
Average turn-around time:	If processed, 3 days.

IUCD

Specimen required:	Coil in sterile container.
Average turn-around time:	Microscopy – 2 days. Culture – up to 2 weeks for Actinomyces isolation.

Legionella antigen

Specimen required:	Urine in sterile container. Always give CURB-65 score on request form and state that there is X-ray confirmation of pneumonia.
Average turn-around time:	1 day.

MRSA Screen

Specimen required:	Set of screening swabs from various sites in specific “eswab” transport medium or individual swabs in charcoal transport medium. Urine in sterile container if catheterised.
Average turn-around time:	Negative – 1 day. Positive – 2 days.

Neisseria gonorrhoeae PCR (GUM patients only)

Specimen required:	Endocervical/urethral/vaginal/throat/rectal thin swab in chlamydia/viral transport medium. “First pass” urine (10-15mls) in sterile conical bottom container (mainly for male patients).
Average turn-around time:	Negatives – 2 days. Positives (need confirmatory test) – 4-5 days. Samples batched but run daily.

Ophthalmic samples

Specimen required:	Contact lens/fluid, corneal biopsy/tissue.
Average turn-around time:	Preliminary report – 3 days – Acanthamoeba PCR – sent to reference laboratory turnaround up to 4 working days.

Pernasal swab

Specimen required:	Small swab on flexible wire. Difficult collection procedure probably performed best by paediatric specialist.
Average turn-around time:	Up to 7 days due to prolonged incubation. Positive result reported directly to clinician.

Pneumococcal antigen

Specimen required:	Urine in sterile container. Always give CURB-65 score on request form and state that there is X-ray confirmation of pneumonia.
Average turn-around time:	1 day.

Screens (e.g. for VRE, ESBL, CPE, MRAB but excluding MRSA)

Specimen required:	Swabs from various sites in charcoal transport medium. Other relevant specimens in sterile containers e.g. sputum, urine, faeces.
Average turn-around time:	Negatives – 2 days. Positives – 3 days.

Semen analysis

Specimen required:	Semen in sterile container. Test performed for infertility reasons by appointment only.
Average turn-around time:	Sperm count and motility assessment – same day (should be examined within 1 hour of production). Morphology for normal forms – 1 day.

Sputum/respiratory aspirates

Specimen required:	Sample in sterile container. Only purulent samples <24hr old will be processed.
Average turn-around time:	2 days. In cystic fibrosis patients – up to 5 days due to prolonged incubation for specific pathogens e.g. Burkholderia spp. If Legionella culture performed, up to 7 days due to prolonged incubation.

Tuberculosis

Specimen required:	Sterile container (type depending on specimen). For possible urinary tuberculosis, send whole of 3 consecutive early morning urine collections in sterile 250 ml containers available from laboratory. For pulmonary tuberculosis, send three consecutive early morning sputum samples. Label all specimens and forms as 'High Risk'. For suspected TB meningitis, minimum 10ml CSF ideally required. May be sent for additional PCR testing. Samples of blood or bone marrow need to be collected into a lithium-heparin vacutainer which is then set to the Reference Laboratory for investigation. If blood cultures are required for the investigation of endocarditis then please discuss with a Microbiology Consultant.
Average turn-around time:	*Microscopy – 2 days. Samples batched. Culture – up to 8 weeks. Positive microscopy and culture results reported directly to clinician. If sent for PCR test – 1-2 days.

Tissue/Biopsy

Specimen required:	Sterile container, occasionally with added sterile saline if small sample to keep moist environment.
Average turn-around time:	Preliminary report – 3 days. Full report – 7 days.

Urine

Specimen required:	Sterile container (MSU, CSU, CCU, SPU).
Average turn-around time:	Negative cultures – 1 day. Positive cultures – 2 days.

Wound swab

Specimen required:	Swab in charcoal transport medium. Routine ulcer/sore swabs not cultured unless evidence of surrounding erythema or cellulitis given on form.
Average turn-around time:	3 days. For some clinical circumstances – up to 5 days.

For any other bacteriological (or parasite/fungal) investigation, contact laboratory for advice on collection of specimens.

B) VIROLOGY/SEROLOGY PERFORMED IN-HOUSE

PLEASE NOTE:

- New positive blood borne virus/acute hepatitis and any other significant positive serology/PCR results that may affect immediate management are phoned to clinicians/GP by the consultant microbiologist.

Antibiotic assay (Gentamicin, Vancomycin and Teicoplanin)

Specimen required:	5ml clotted blood. Collect from separate peripheral vein, not through line by which antibiotic given. (Assays performed in Biochemistry Department).
Average turn-around time:	Same day. Results reported immediately onto computer. See Trust Antimicrobial Website for further details on antibiotic monitoring.

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Specimen required:	5 ml clotted blood.
Average turn-around time:	4-5 days. Samples batched

Cytomegalovirus antibody (IgM+IgG)

Specimen required:	5ml clotted blood.
Average turn-around time:	*2-3 days

Epstein-Barr virus nuclear antigen (EBNA) IgG antibody

Specimen required:	5ml clotted blood.
Average turn-around time:	*2-3 days for positive EBNA IgG. Viral capsid antigen (VCA) IGM will be added if EBNA IgG is negative (4-5 working days)

Hepatitis A IgM antibody

Specimen required:	5ml clotted blood.

Average turn-around time: *3 days.

Hepatitis A IgG antibody
Specimen required: 5ml clotted blood.
Average turn-around time: 3 days.

Hepatitis B surface antigen
Specimen required: 5ml clotted blood.
Average turn-around time: *3 days. First time positive results require confirmation with second sample.

Hepatitis B Surface antibody/Core antibody
Specimen required: 5ml clotted blood.
Average turn-around time: 3 days.

Hepatitis B "e" antigen/antibody
Specimen required: 5ml clotted blood.
Average turn-around time: 5 days.

Hepatitis C antibody
Specimen required: 5ml clotted blood.
Average turn-around time: 3 days. Two separate assays available in-house, a second sample will be required to confirm first positive result.

Hepatitis E antibody
Specimen required: 5ml clotted blood.
Average turn-around time: 7 days.

Herpes simplex PCR test
Specimen required: Swab in C/VTM. CSF in sterile container.
Average turn-around time: 5 days. Herpes simplex PCR test on CSF done same day if appropriate.

HIV antibody
Specimen required: 5ml clotted blood.
Average turn-around time: *3 days for negatives. Three assays available in-house. Second sample will always be required to confirm first positive result.

Influenza A & B PCR

Specimen required:	Respiratory sample (NPA, BAL, nose/throat swab) NB if an extended viral screen is required then this needs to be sent to the Reference Laboratory
Average turn-around time:	24 hrs

Measles/Mumps IgG antibody

Specimen required:	5ml clotted blood.
Average turn-around time:	3 days. This test confined to occupational health checks. Contact microbiologist before sending any samples.

Mycoplasma IgM & IgG antibody

Specimen required:	5ml clotted blood.
Average turn-around time:	3 days. Paired sera sometimes required.

Norovirus PCR

Specimen required:	Faeces in blue sterile container with spoon or standard sterile container.
Average turnaround time:	1 day. This investigation is only performed when suspected outbreak needs confirmation and requires authorisation by the Infection Prevention and Control Team or the Microbiologist.

Parvovirus IgM and IgG antibody

Specimen required:	5ml clotted blood.
Average turn-around time:	1 week. Paired sera sometimes required. Saved antenatal serum can be used for parvovirus investigations in pregnancy.

Respiratory syncytial virus

Specimen required:	Nasopharyngeal aspirate. RSV season usually from October to March.
Average turn-around time:	*1 day. Results reported directly to ward. Near patient EIA membrane test used by paediatrics in their department. Training support provided by serology lab staff and paediatrics take part in NEQAS quality assurance programme.

Rotavirus antigen

Specimen required:	Faeces in blue sterile container with spoon or standard sterile container.
Average turn-around time:	1 day. Positive results reported directly to clinician or ward.

Rubella IgM and IgG antibody
Specimen required: 5ml clotted blood.
Average turn-around time: *5 days. Paired sera sometimes required.

Syphilis antibody (Confirmed at PHE Colindale)
Specimen required: 5ml clotted blood.
Average turn-around time: 3 days. Positive samples require confirmation and more detailed testing by reference laboratory - 1-2 weeks.

Toxoplasma IgM and IgG antibody (Confirmed atPOR)
Specimen required: 5ml clotted blood.
Average turn-around time: 3 days. Positive samples require confirmation by reference laboratory – 1-2 weeks.

Varicella zoster virus IgG antibody
Specimen required: 5ml clotted blood. Saved antenatal serum can be used for chickenpox investigations in pregnancy.
Average turn-around time: *1 day.

Varicella zoster virus PCR test
Specimen required: Vesicle swab in C/VTM.
Average turn-around time: *3days. Currently only validated for use on vesicle swabs.
CSF samples processed same day if clinically appropriate

C) VIROLOGY/SEROLOGY SENT TO REFERENCE LABORATORIES

PLEASE NOTE:
- Any significant positive serology/PCR results that may affect immediate management are phoned to clinicians/GPs by the consultant microbiologist.

Adenovirus PCR test (FIN)
Specimen required: Swab in C/VTM, EDTA blood, respiratory samples
Average turn-around time: 3-4 days.

Antibiotic assay (Amikacin, Tobramycin, Daptomycin, Voriconazoleetc.) (SME)
Specimen required: 5ml clotted blood. Collect from separate peripheral vein, not through line by which antibiotic given. Assays sent to Antimicrobial Reference Laboratory, Bristol.
Average turn-around time: 2 days. Results usually phoned by Reference Laboratory then entered onto computer with clinical interpretation.

"Atypical pneumonia" antibody screen (e.g. (BRI)

Chlamydia, Coxiella)
Specimen required: 5ml clotted blood.
Average turn-around time: 1–2 weeks. Paired sera usually required.
Bartonella antibody No longer available

Bordetella antibody (RSI)
Specimen required: 5ml clotted blood.
Average turn-around time: 1-2 weeks. For late diagnosis of whooping cough. Discuss with local Public Health England and microbiologists before sending samples.

Brucella antibody (BRU)
Specimen required: 5ml clotted blood.
Average turn-around time: 1–2 weeks. Paired sera occasionally required.

Cytomegalovirus PCR test (FIN)
Specimen required: 3ml EDTA blood. Fresh urine in sterile container. Respiratory tract secretions/washings in sterile container.
Average turn-around time: 3 days. Discuss with microbiologist before sending samples.

Dengue antibody (POR)
Specimen required: 5ml clotted blood.
Average turn-around time: 1-2 weeks. Paired sera sometimes required.

Enterovirus PCR test (FIN)
Specimen required: Throat swab in C/VTM. CSF in sterile container, stool, EDTA blood.
Average turn-around time: 3 days.

Epstein-Barr PCR / viral load test (FIN)
Specimen required: 3ml EDTA blood.
Average turn-around time: 3 days.

Hepatitis B PCR / viral load test (FIN)
Specimen required: 3ml EDTA blood.
Average turn-around time: 3days.

Hepatitis C PCR / viral load / Genotype test (FIN)
Specimen required: 3ml EDTA blood.
Average turn-around time: PCR/viral load – 3 days. Genotype test – 1 week.

Hepatitis E IgM and IgG antibody/PCRtest (BBV)
Specimen required: 5ml clotted blood – antibody test. 3ml EDTA blood – PCR test.
Average turn-around time: 1-2 weeks.

HIV PCR / viral load test (FIN)
Specimen required: 6ml EDTA blood. Requests for HIV viral load should only be sent via Dept of G U Medicine.

Average turn-around time:	3 days.
HIV pro-viral DNA PCR test for maternal transmission	(BBV)
Specimen required:	1-3ml EDTA neonatal cord blood plus 3ml maternal EDTA blood.
Average turn-around time:	1-2 weeks.
Leptospira antibody	(POR)
Specimen required:	5ml clotted blood.
Average turn-around time:	1-2 weeks. Paired sera sometimes required.
Measles/Mumps IgM/IgA antibody	(BBV)
Specimen required:	Saliva in specific collection kit.
Average turn-around time:	1-2 weeks. If either acute measles or mumps case suspected, contact local Public Health England to obtain saliva collection kit.
Meningococcus/ Pneumococcus PCR test	(FIN)
Specimen required:	3ml EDTA blood. CSF in sterile container.
Average turn-around time:	3 days.
Mycobacterium tuberculosis lymphocyte activation test	(CHU)
Specimen required:	Quantiferon – set of blood samples in specific kit supplied by laboratory. Elispot – 5ml heparin blood. By arrangement with
Average turn-around time:	laboratory only1-2 weeks
	.
Parasitic antibodies (helminths, amoeba etc.)	(HTD)
Specimen required:	5ml clotted blood.
Average turn-around time:	1-2 weeks.
Respiratory viral PCR screen (e.g. influenza / parainfluenza / Adenovirus / RSV / metapneumovirus)	(FIN)
Specimen required:	Nose/throat swab in VTM. Respiratory tract secretions/washings in sterile container.
Average turn-around time:	3 days.
Rickettsial antibody (typhus)	(POR)
Specimen required:	5ml clotted blood.
Average turn-around time:	1-2 weeks. Paired sera usually required.

Viral haemorrhagic fever (VHF) PCR/antibody (Ebola/Lassa etc)	(POR)
Specimen required:	5ml clotted blood and 3ml EDTA blood.
Average turn-around time:	1 day. **Always** contact microbiologist before sending any samples.
Yersinia antibody	(LEP)
Specimen required:	No longer available.

For any other virology/serology investigation, contact laboratory for advice on collection of specimens.

Volumes of EDTA blood for neonatal/paediatric cases can be reduced by using the specially designated paediatric bottles. For clotted blood, the adult yellow-topped gel-containing tubes are preferred and can be used with a reduced volume of blood. If in any doubt, discuss sample requirements with the laboratory before taking.

Details of Reference Laboratories used for specialist testing

If a referral laboratory is used to undertake specialist testing then an ID code has been provided adjacent to the specific test concerned. The details of the relevant laboratories are given below.

Code	Laboratory Name and Details
BBV	Public Health England Colindale 61 Colindale Avenue Colindale London. NW9 5HT
LEP	Enteric Pathogens Laboratory, Colindale
RSI	Respiratory & Systemic Infections Lab, Colindale
LHCAI	Healthcare Associated Infections and Antibiotic Resistance Testing, Colindale
STBL	Sexually Transmitted Bacteria Laboratory, Colindale
BRI	PHE Laboratory, Southmead Hospital, Bristol
SME	Antibiotic Testing Reference Laboratory, Southmead Hospital, Bristol BS10 5NB
SOU	Southampton HPA, Tremona Road, Southampton SO16 6YD
BRU	Special Diagnostic Unit, Royal Liverpool and Broadgreen Hospital, Liverpool L7 8XP
HTD	Department of Clinical Parasitology, Hospital for Tropical Diseases, Mortimer Market, London WC1E 6JA
FIN	Micropathology UK Limited, Sir William Lyons Road, Coventry, CV4 7EZ
POR	Porton Down, Rare and Imported Pathogens Lab, Salisbury SP4 0JG
CHU	Dept. of Immunology, Churchill Hospital, Oxford University Hospitals, Old Road, Headington, Oxford, OX7 7LJ

Infections, Appropriate Specimens and Tests Available

Acanthamoeba	Rare but potentially sight-threatening cause of ocular keratitis. Usually associated with contaminated soft lenses. (Not performed in-house, sent to reference laboratory,)
Actinomycosis	Actual pus always preferable to swabs. Look for 'sulphur granules' within lesion/pus. Pelvic infection associated with use of IUCD's.
Adenovirus	Causes conjunctivitis, acute respiratory disease and infantile gastroenteritis. For diagnosis, send eye or throat swab in viral transport medium (VTM).
Amoebae	Causes amoebic dysentery, amoebic abscess (usually hepatic), amoeboma of gut. Travel history important. Examination of 'hot stool' necessary if amoebic dysentery considered. Send clotted blood for amoebic antibodies if invasive disease suspected. See **Faeces, Ova / Cysts / Parasites**.
Anaerobic vaginosis	Associated with Gardnerella vaginalis/anaerobes. Offensive vaginal discharge showing characteristic 'clue cells' on microscopy. Secretions collected on vaginal speculum have pH≥5. See **Vaginal swab**.
Antibiotic assays	Vancomycin, teicoplanin and gentamicin are the main antibiotics which must be monitored. Follow advice on Trust Antimicrobial Website for both the administration and monitoring of these antibiotics. Clotted blood sample required for antibiotic assays. Do not take sample for assay from same line through which antibiotic has been given. Assays performed in Biochemistry Dept.
Antistreptolysin O (we are potentially dropping this, WH to check with ref lab)	Measures antibodies to 'O' haemolysin produced by Strep. pyogenes. Helpful in diagnosis of rheumatic fever, post-streptococcal glomerulonephritis and severe cellulitis.
Arthritis	Send joint effusion in sterile container for microscopy and direct culture. Joint fluid may also be injected into blood culture bottles for enrichment. Send serum for antibody levels if viral or reactive arthritis suspected.
Ascites	If bacterial peritonitis suspected, aspirate 40mls ascitic fluid aseptically and send 20mls neat in sterile container for cell count, Gram stain and culture. Divide other 20mls between pair of blood culture bottles (10mls each bottle) to act as enrichment.

Aspergillus Fungal mould which can cause ear, respiratory, and occasionally systemic infections in immunocompromised patients. Send relevant specimen for culture. Serological tests for Aspergillus, including galactomannan often useful for investigation of respiratory or systemic disease but discuss with microbiologists first.

Bilharzia See **Schistosomiasis**.

Borrelia burgdorferi Cause of Lyme disease. Send clotted blood 6-8 weeks after tick bite for antibody tests. If case suspected, discuss first with microbiologists.

Bronchiolitis See **RSV**.

Brucellosis Indigenous cases in UK extremely rare. Must have history of contact with cattle or consumption of unpasteurised dairy produce. Cause of PUO in patient with relevant travel or exposure history. In acute infection, may grow from blood cultures. Send paired sera for antibody tests. Consult microbiologists, if case suspected.

Campylobacter Common cause of gastroenteritis. Send faeces for culture. Most infections mild, but severe infections may cause acute abdominal pain and pyrexia with bloody diarrhoea. Post infectious reactive arthritis and Guillain-Barré syndrome can occasionally follow – serology test available for investigation. See **Diarrhoea, Faeces**.

Candida Common cause of itchy vaginal discharge, nappy rash in infants. Send vaginal or skin swabs for microscopy and culture. May cause more serious invasive disease in immunosuppressed patients, or following major abdominal surgery. Serology may be helpful in invasive infection (beta-D-glucan). Also see **Vaginal swab.**

Cellulitis Spreading erythema often accompanied by pain and swelling of subcutaneous tissues. Most commonly due to haemolytic streptococci and Staph. aureus. Send skin swabs if there is any broken skin area and clotted blood for ASO titres in severe cases. If affecting legs, check between toes for evidence of primary fungal infection allowing portal of entry for cellulitis pathogen.

Cervical swab Essential for investigation of pelvic inflammatory disease. Two swabs should be taken from endocervical canal. The first put in routine charcoal transport medium, for gonococcal culture in particular; the second, placed in chlamydia/viral transport media (C/VTM), for chlamydia PCR test. Take cervical swabs under direct vision, using a speculum, and in good light. See **Chlamydia, Gonococcal infection, Vaginal swab**.

Chickenpox See **Varicella-zoster virus**.

Chlamydia *C. pneumoniae* and *C. psittaci* cause respiratory tract infections. Send paired sera for antibody titres. C. trachomatis causes urogenital tract infections, pelvic inflammatory disease and conjunctivitis in both adults and babies. Send thin steel swab in C/VTM or "first pass" urine for PCR test. Antibody testing not helpful in diagnosis of acute C. trachomatis infection. When both chlamydia and routine bacteriological genital swabs taken, complete separate microbiology forms for each specimen. See **Eye swab**.

Clostridium difficile Normal flora in neonates but cause of antibiotic associated diarrhoea/pseudomembranous colitis in adults. Send faeces for toxin/GDH detection. See **Diarrhoea, Faeces**.

Conjunctivitis Consider bacteria, chlamydia and viruses (especially Herpes simplex, adenovirus). See **Eye swab**.

Coxiella burnetii See Q fever.

Coxsackie Cause aseptic meningitis, Bornholm disease, myocarditis, pericarditis, skin rashes including hand, foot and mouth disease. Viral RNA detection via PCR test from throat or vesicle swab (in C/VTM), faeces and CSF. Send paired sera (clotted blood) for antibody titres no longer available.

Cryptococcus Rare fungal infection. Cryptococcal meningitis seen mainly in AIDS and other immunosuppressed patients. Send serum for Cryptococcal antigen and CSF for culture and antigen testing. Consult microbiologists if case suspected.

Cryptosporidium Protozoal parasite causing gastroenteritis especially in children. Send faeces. Diagnosis made by microscopy. See **Diarrhoea, Faeces**.

Cyclospora *Cyclospora cayetanensis* is a foodborne and waterborne parasitic cause of diarrheal illness in children and adults. Diagnosis is established by the detection of oocysts via stool microscopy (performed in-house).

Cytomegalovirus Can cause congenital infection, glandular fever type illness but most infections are asymptomatic. Can also cause acute hepatitis and pneumonia in severely immunocompromised. Send EDTA blood, fresh urine, respiratory secretions (in sterile container) for CMV PCR test. Send clotted blood for antibody titres.

Dengue Relatively common cause of PUO in returning traveller. Headache and thrombocytopenia are major features, but may also cause more serious haemorrhagic fever. Travel history vital - mainly SE Asia/Pacific/India/parts of Caribbean. Send clotted blood (paired sera) for antibody and consult microbiologists if case suspected.

Dermatophytes Most common fungal pathogen affecting skin, nail and hair. Several species including Trichophyton sp., Microsporum sp. and Epidermophyton sp. common in UK. Send scrapings of skin from edge of lesion, hairs including roots and nail clippings as far proximally from affected nail as possible, in specific envelope kits for microscopy (Dermapak) and culture.

Diarrhoea Send faeces sample (Type 5-7 sample). In community acquired cases, potential pathogens routinely looked for include Salmonella, Shigella, Campylobacter, E.coli O157, Giardia and Cryptosporidium. Clostridium difficile toxin/GDH test performed on all suspected hospital associated infections and some community based faecal samples. In children, rotavirus looked for. Other organisms such as Yersinia, Vibrio, Parasites examined for if relevant clinical/travel history. See **Faeces**.

Diphtheria Consult microbiologists immediately if case is suspected clinically. Send throat swab in routine charcoal medium. Immunisation and any relevant travel or exposure history is important to obtain. See **Throat swab**.

Ear swab Place patient in sitting position and introduce swab gradually. Deep ear swabbing should be carried out by medical staff using a speculum. Record any antimicrobial treatment used in the ear on request form.

Enteric fever Typhoid (S. typhi) and paratyphoid (S. paratyphi A,B,C) fevers. In acute stages, blood cultures are best method of diagnosis. Faeces and urine may become culture positive later in the disease. The Widal serological test is no longer performed due to its poor sensitivity and specificity.

Epstein-Barr Cause of infectious mononucleosis (glandular fever) and occasionally hepatitis. Send clotted blood for specific antibody tests. Paul-Bunnell test, for heterophile antibody present in serum of many patients with EBV infection, performed in Haematology Dept. is no longer available

Eye swab Send to laboratory as soon as possible. For bacterial culture, send routine swab of pus collecting at inner canthus of eye. For viral investigation send thin steel swab in C/VTM. Conjunctival scrapings often superior especially for chlamydia investigation. If ophthalmia neonatorum suspected contact ophthalmologists and/or paediatricians.

Faeces

Use either sterile blue container with 'spoon' attached in lid or standard 60 ml sterile container to collect specimen (Type 5-7). Fill either one third full with sample. If stools are liquid, 10 mls is adequate. A rectal swab is not an appropriate substitute for faecal examination. With all specimens of faeces, relevant travel history and any antibiotic treatment must be recorded on request forms. See **Diarrhoea**.

Giardia

Gduodenalis (G. lamblia and *G. intestinalis)* may cause acute gastroenteritis-type symptoms or be associated with chronic steatorrhoeic diarrhoea and failure to thrive symptoms in children. It is essential to provide this clinical information on the form.
See **Diarrhoea, Faeces, Ova/Cysts/Parasites**.

Gonorrhoea infection

In suspected female genital infection, endocervical swabs are superior to vaginal swabs for detection of N. gonorrhoeae. Best results obtained with direct plating and PCR testing as performed in the GU Medicine department. Send endocervical/urethral/rectal/throat swabs as soon as possible to laboratory. For male infection, send urethral/rectal/throat swabs where clinically appropriate. A urine sample may be submitted from a Sexual Health clinic patient. If ophthalmia neonatorum suspected, contact ophthalmologists and/or paediatricians.

Helicobacter pylori

Organism associated with peptic ulcer disease, gastric MALT lymphoma and gastric cancer. Non-invasive test used in both hospital and CCG setting is urea breath test. Antibody tests neither sensitive nor specific enough for diagnosis, but a stool sample may be submitted for an antigen test. Culture and sensitivity nolonger performed in-house

Hepatitis

Send clotted blood for all initial investigations of suspected hepatitis. The following viral causes may be relevant depending on clinical background: Hepatitis A, B, C, E, CMV, EBV. Hepatitis C is most commonly associated with IV drug use or past blood transfusion. Give all relevant information on accompanying request form.

Herpes simplex

Cause of cold sores, whitlows, kerato-conjunctivitis, encephalitis, genital tract infections. Diagnosed by PCR test. Send swab of lesion in C/VTM or neat CSF. HSV antibody tests not generally helpful in diagnosis of acute infection.

Human Immunodeficiency virus (HIV)

Send clotted blood for antibody test. All samples from HIV suspected or known positive patients should be marked as 'high risk'. Positive result always requires confirmation with second sample, which can be accompanied by two EDTA bloods for HIV viral load and CD4 lymphocyte count.

Hydatid disease	Caused by Echinococcus granulosus. Hydatid cysts can develop in liver, lungs, brain etc. Send clotted blood for antibody test if suspicious cysts seen radiologically and patient has relevant travel, occupational and/or exposure history.
Infectious mononucleosis	See **EBV, CMV, Toxoplasma**.
Influenza	Send a nasopharyngeal swab in VTM for PCR tests. In the hospital, respiratory aspirates in sterile containers can be sent if influenza lower respiratory tract infection suspected. If an influenza epidemic occurs, diagnosis becomes based on clinical findings.
Legionella	Cause of 'atypical' pneumonia, especially in smokers, immunocompromised patients and recent travellers abroad. Send urine for Legionella antigen test. In highly suspected case, send sputum for Legionella culture.
Leptospirosis	Influenza-like illness with or without meningitis or haemorrhagic jaundice caused by Leptospira spp. Need relevant exposure history to animals/water etc. Usually require paired sera for antibody titre, although IgM tests are available. If case suspected, contact microbiologists.
Listeria	Causes bacteraemia / meningitis especially in immunocompromised patients. Also important pathogen during pregnancy and neonatal period. Diagnosis made via blood cultures, CSF and placental tissue examination where appropriate. Serology not helpful in suspected acute infection.
Lyme disease	See ***Borrelia burgdorferi***.
Malaria	Initial investigations, diagnosis performed in-house via Haematology Dept. Clinical advice from the ID department at the JR
Measles	Notifiable disease. Contact local Public Health England if suspected acute case seen. They will supply special saliva collection kit for measles or mumps IgM/IgA antibody diagnostic test. The diagnosis may also be made by nose and throat swabs sent in VTM. Routine measles and mumps IgG antibody testing in blood only indicated for few specific circumstances e.g. health care worker at high risk of exposure to measles with no vaccine history. In-house antibody test available but always contact microbiologists before sending request.

Meningitis

If case of meningitis suspected by GP in community, stat dose of parenteral penicillin or cefotaxime? usually advisable as soon as possible. Helpful to take throat swab before antibiotic is given, as subsequent blood and CSF cultures in hospital may be negative following antibiotic. Swabs can be sent in with the patient. In the hospital setting, if no antibiotic has yet been given IV dexamethasone should be given before first dose of antibiotic. Send blood cultures, CSF, throat swab, EDTA blood (for bacterial PCR tests) and clotted blood (saved for possible antibody tests) in suspected meningitis case.

MRSA

Screening programme carried out in hospitals for methicillin resistant *Staph. aureus*. Specific infection control precautions including suppression therapy are taken if any patient known to harbour MRSA is admitted to hospital. MRSA screen consists of swabs from nose, throat, perineum, any other broken skin area and urine if catheterised.

Mumps virus

See **Measles**.

Mycobacteria

Send sputa on 3 consecutive days for pulmonary TB investigations. Collections of whole early morning urines on 3 consecutive days are necessary for investigation of renal tract infection. Specific sterile 250ml containers are available from the laboratory for these specimens. If 'atypical' mycobacterial infection is suspected, e.g. fish tank granuloma, contact microbiologists.

For blood and bone marrow samples please send specimens in a lithium-heparin tube. Do NOT use any tube containing EDTA.

If immunological blood tests for latent *M.tuberculosis* infection are considered, contact the serology laboratory for further advice.

Mycoplasma

Common cause of 'atypical' pneumonia, but can occasionally cause otitis media, skin rashes (especially erythema multiforme), arthritis, myocarditis, pericarditis. Send clotted blood for antibody test.

Nasopharyngeal secretions

Aspirate from nasopharynx collected in sterile container mainly for investigation of suspected respiratory syncytial virus infection in infants. Can also occasionally be used for Bordetella pertussis culture if pernasal swab not possible. See **RSV**, **Pernasal swab.**

Nose swab

Sit patient facing strong light source and tilt head back. Insert swab into both nostrils and sample with gentle rotating movement. Care should always be taken in threading swabs into nose especially in babies.

Ophthalmia neonatorum

Notifiable disease. Neonatal eye infection caused by Neisseria gonorrhoeae or chlamydia and acquired at birth from an infected maternal genital tract. Consult ophthalmologists and/or paediatricians if case suspected. See **Chlamydia, Eye swab, Gonorrhoea infection**.

Ova/Cysts/Parasites

For gastrointestinal parasite infections (e.g. Ascaris, Enterobius, Trichuris, hookworms, tapeworms, Giardia, Entamoeba) send faecal specimen. Send terminal urine sample for Schistosoma ova. In suspected invasive parasitic infections e.g. Amoeba, Strongyloides, Hydatid, Schistosoma, antibody tests available - send clotted blood sample and consult microbiologists. It is essential to provide relevant travel history. See **Faeces**.

Parvovirus

Causes fifth disease in children (slapped cheek syndrome). In adults, especially females, infection can cause arthritis with or without rash. Can also cause foetal mortality/morbidity if mother infected during early pregnancy. If history of exposure to parvovirus in pregnancy, early antibody test essential. Send clotted blood for parvoviral IgM/IgG tests, or contact laboratory if routine antenatal “booking” serum has already been taken as this sample can also be tested.

Pernasal swab

For diagnosis of whooping cough (*Bordetella pertussis*), special thin wire swab with charcoal transport medium is available from laboratory. Pass swab on flexible wire through nostril along floor of nasal cavity to posterior nasal space. Swab rotated gently, withdrawn and placed in transport medium. Send to laboratory immediately and inform microbiologists.

Pinworm (threadworm) infection

Due to Enterobius vermicularis. Worm sometimes seen in faeces by patient. To look for ova deposited around perianal area wipe moistened swab around anus and break off into small volume of sterile saline in universal container. Procedure should be performed first thing in the morning. Microscopy of spun deposit will show characteristic eggs of pinworm if present. Previous method using “sellotape slide” should no longer be used.

Pneumocystis jirovecii

Cause of pneumonia in HIV and other severely immunosuppressed cases. Diagnosis confirmed by Pneumocystis PCR test. Contact Microbiologists before sending broncho-alveolar lavage or induced sputum for pneumocystis investigation. Testing of serum for beta-D-glucan can be useful.

Pneumonia	Send only good quality purulent sputum (i.e. not just saliva) in sterile containers. Urine antigen test available for pneumococcal and legionella infection but must be evidence of pneumonia on CXR. Always give CURB-65 score on request form. Consider possibility of TB, Pneumocystis or fungal infection in compromised host. See **Chlamydia**, **Legionella**, **Mycoplasma, Q fever, Sputum**.
Pseudomembranous colitis	See ***Cl. difficile***.
Psittacosis	*C. psittaci* infection via inhalation of infected aerosols from birds. Causes influenza-like illness or 'atypical' pneumonia. Send paired sera for antibody titres. See **Chlamydia**.
Pus	Wherever possible collect pus with sterile syringe and send to laboratory in universal container. Only send pus swab if no actual fluid can be obtained.
Q. fever	Infection due to *Coxiella burnetii*. Acquired following exposure to infected animals/animal products or consuming unpasteurised dairy products. Causes influenza-like symptoms followed by 'atypical' pneumonia and in some cases can progress to endocarditis. Send paired sera for antibody titres.
Rabies	Contact microbiologists immediately if history of animal bite abroad.
Rotavirus	Causes gastroenteritis mainly in children. Send faeces for rotavirus antigen test. See **Diarrhoea**, **Faeces**.
RSV	Respiratory syncytial virus most common cause of bronchiolitis in infants. (RSV season most commonly late October to March). Nasopharyngeal aspirate is specimen required for virus detection. See **Nasopharyngeal secretions**.
Rubella	Routine rubella antibody test in pregnancy - send clotted blood sample. For possible rubella contact in pregnancy - send paired sera for antibody titres. Timing of samples will depend on date of contact - ring laboratory for further advice.
Salmonella	Group of bacteria responsible for gastroenteritis and more systemic illness. See **Diarrhoea**, **Enteric fever**, **Faeces**.
Scabies	Caused by mite, *Sarcoptes scabiei*. Diagnosed clinically and by extracting mite from characteristic burrow in skin of hands, wrists, perineal or genital areas. In hospital consult dermatologist and control of infection nurse if case suspected. Affected patient must be isolated and contacts may also require treatment.

Schistosomiasis Invasive tropical infection due to Schistosoma spp. Relevant travel history required. Diagnosed 1) by sending terminal urine samples for ova detection via microscopy, 2) histological examination of rectal biopsy specimens, 3) serology - send clotted blood for antibody test. Contact microbiologists if case suspected.

Semen Analysis Semen analysis performed both for infertility and post-vasectomy checks. For infertility investigations, very important for patient to have booked time to deliver sample to laboratory as microscopy should be performed within 1 hour of production. For details on how appointment booking system operates, see **Section xv in Guidelines for Specimen Collection** in this Microbiology Handbook.

Shigella Cause of bacillary dysentery, gastroenteritis. In UK, *S. sonnei* most common species producing mild illness. *S. flexneri* associated with outbreaks especially in institutions. Other shigellae usually imported and may cause severe infection. See **Diarrhoea**, **Faeces**.

Shingles See **Varicella-zoster**.

Sputum Best obtained first thing in morning. Physiotherapy helpful to obtain good quality specimen. Unless obviously purulent, sputum examination has limited value in COPD patients. Mucoid or salivary specimens and samples >24hr old will not be examined. Collect in sterile container. For tuberculosis investigations send three early morning samples. See **Mycobacteria**, **Pneumonia**.

Syphilis Send clotted blood sample for serological tests. *Treponema pallidum* EIA antibody test performed as routine screen and if positive testing is performed in-house with RPR and TPPA. .If test(s) positive, serum sent to Treponemal Reference Laboratory for more detailed investigations.

Tetanus *C. tetani* causative organism of this notifiable disease. Send tissue/pus in sterile container and clotted blood sample for toxin detection. Contact microbiologists immediately if condition clinically suspected.

Threadworm See **Pinworm**.

Throat swab Have good light source and use tongue depressor if available. Rub swab quickly but gently over pharyngeal wall and/or tonsillar fossa. For bacteriology, place swab in charcoal transport medium; for virology, place swab in VTM.

Toxocara Consider this worm infection especially in child with unexplained eosinophilia and significant contact with dogs or cats. Send clotted blood for antibody test.

Toxoplasma Natural parasite of cats. Humans are intermediate hosts. Infection most commonly asymptomatic but can cause glandular fever-like syndrome and congenital infection if acquired in pregnancy. Send clotted blood for antibody test.

Trichomonas Protozoal parasite responsible for vaginal discharge. Must be considered sexually transmitted disease and can cause urethral discharge in male contact. See **Urethral swab, Vaginal swab.**

Tuberculosis See **Mycobacteria**.

Typhoid See **Enteric fever**.

Urethral swab For investigation of urethritis. Send swab in transport medium appropriate to investigation required. See **Chlamydia**, **Gonorrhoea infection**, **Herpes simplex**, **Trichomonas.**

Urine Specimens should be examined as soon as possible after collection, to avoid bacterial overgrowth. If delay likely, specimen must be refrigerated. Routine urine microscopy no longer performed. New patient dipstick testing together with clinical assessment must be used to manage patient and decide on whether urine culture is appropriate. See Guideline for Testing of Urine in earlier section of Microbiology handbook.
MSU - collect specimens in sterile containers half full.
CSU - collect by sterile needle aspiration from sampling port of tubing. Do not collect from bag or end of catheter.
For infants and children a clean catch during stream is usually the most suitable method.
For chlamydia PCR test, collect 10-15 mls of "first-pass" urine. Initial morning sample is optimal choice but urine must have been held in bladder for minimum of 3 hours.
For TB investigations, send 3 consecutive early morning urines (whole sample) in specific sterile containers available from laboratory.

Vaginal swab Take swab from upper vagina, using speculum, for investigation of vaginal discharge. Differential diagnosis will include Candida, Trichomonas, anaerobic vaginosis. In children, vulval or low vaginal swab may detect other pathogens e.g. anaerobes, haemolytic streptococci, Haemophilus sp. Vaginal swab can be used for chlamydia PCR test but optimal specimen is endocervical swab. See **Anaerobic vaginosis**, **Cervical swab**, **Chlamydia, Gonorrhoea infection, Trichomonas.**

Varicella-zoster

Causes Chickenpox and shingles. Clinical diagnosis usually obvious but if confirmation required send swab from base of vesicle in VTM for PCR test. Very important if chickenpox in mother around time of labour - life threatening neonatal infection may follow if baby not given hyper immune immunoglobulin. For advice on chickenpox in pregnancy contact microbiologists. Chickenpox antibody tests if required in pregnancy can be performed on stored antenatal booking blood.

Whooping cough

Caused by *Bordetella pertussis* and is a notifiable disease. Optimal method of diagnosis is culture from nasopharynx early in illness. If suspected diagnosis delayed, *B. pertussis* antibodies may be helpful but should be discussed with Public Health England before sending blood sample. See **Pernasal swab**.

Worms

If patient claims to have passed actual worm, send it to laboratory in sterile container if still available. Arrange for examination of faeces for ova etc. See **Faeces**, **Ova/Cysts/Parasites**.

Wound swab

Obtain at beginning of dressing procedure, after old dressing removed and prior to any antiseptic cleansing of wound. Apply swab to infected site avoiding surrounding skin or mucous membranes; place in charcoal transport medium. If significant pus present, aspirate with sterile syringe and send in sterile container.

Do not send routine swabs from wounds or ulcers, only if there is surrounding erythema, cellulitis or the patient is systemically septic.

Yersinia

Y. enterocolitica and *Y. pseudotuberculosis* can cause diarrhoea, abdominal pain, mesenteric lymphadenitis, leading to reactive arthritis in some cases. In acute stage, blood cultures and faeces are required for bacterial isolation but clinical details given must be consistent with possible Yersinia infection. Yersinia antibody testing for the investigation of suspected reactive arthritis is no longer available. See **Faeces.**