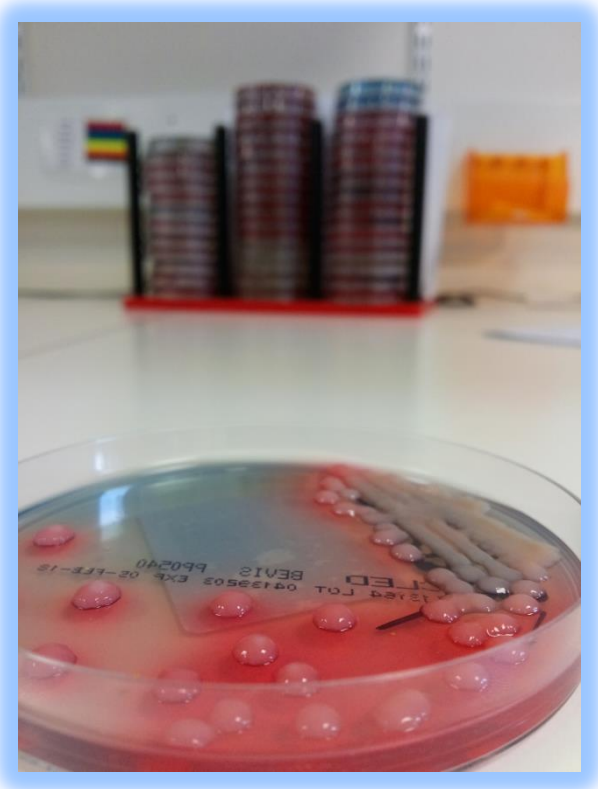
South Tees Hospitals NHS
NHS Foundation Trust

Microbiology Department

Based at: James Cook University Hospital



User Manual

2020/2021

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1. Introduction

The pathology department at South Tees Hospitals NHS Foundation Trust provides laboratory services at the James Cook site for a wide variety of service users, including local and regional hospitals, local GP surgeries and local employers. The laboratory is inspected and accredited by UKAS and Clinical Pathology Accreditation to demonstrate that the specific activities performed by the laboratory meet the criteria set out in the standard.

At James Cook, the Microbiology department is split into Bacteriology and Virology. Bacteriology is responsible for the culturing, isolation and identification of micro-organisms obtained from various parts of the body using several specimen types including swabs, faeces, urine samples, respiratory specimens, blood and cerebrospinal fluid.

Virology is responsible for the detection of viral infections, immunity investigations, toxin detection and outbreak monitoring. In Virology, different techniques are used to perform investigations and confirmation testing on a range of clinical samples. For virology advice see the virology user guide.

This manual provides information about how users can access our service, who to contact for advice, which tests are performed, sample requirements and turnaround times. This information is accurate at the time of issue and is reviewed and updated regularly to incorporate new developments.

If you find any errors within this document or would like to make any comments or suggestions for improvement, please contact elaine.watson4@nhs.net

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If patients are using this guide, please note that any information provided should not be used for self-diagnosis and if you have any concerns about your health, please contact your GP.

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2. Location

Microbiology Department is situated at the James Cook University Hospital, Marton Rd.

Microbiology Department

James Cook University Hospital
Marton Road
Middlesbrough
TS4 3BW

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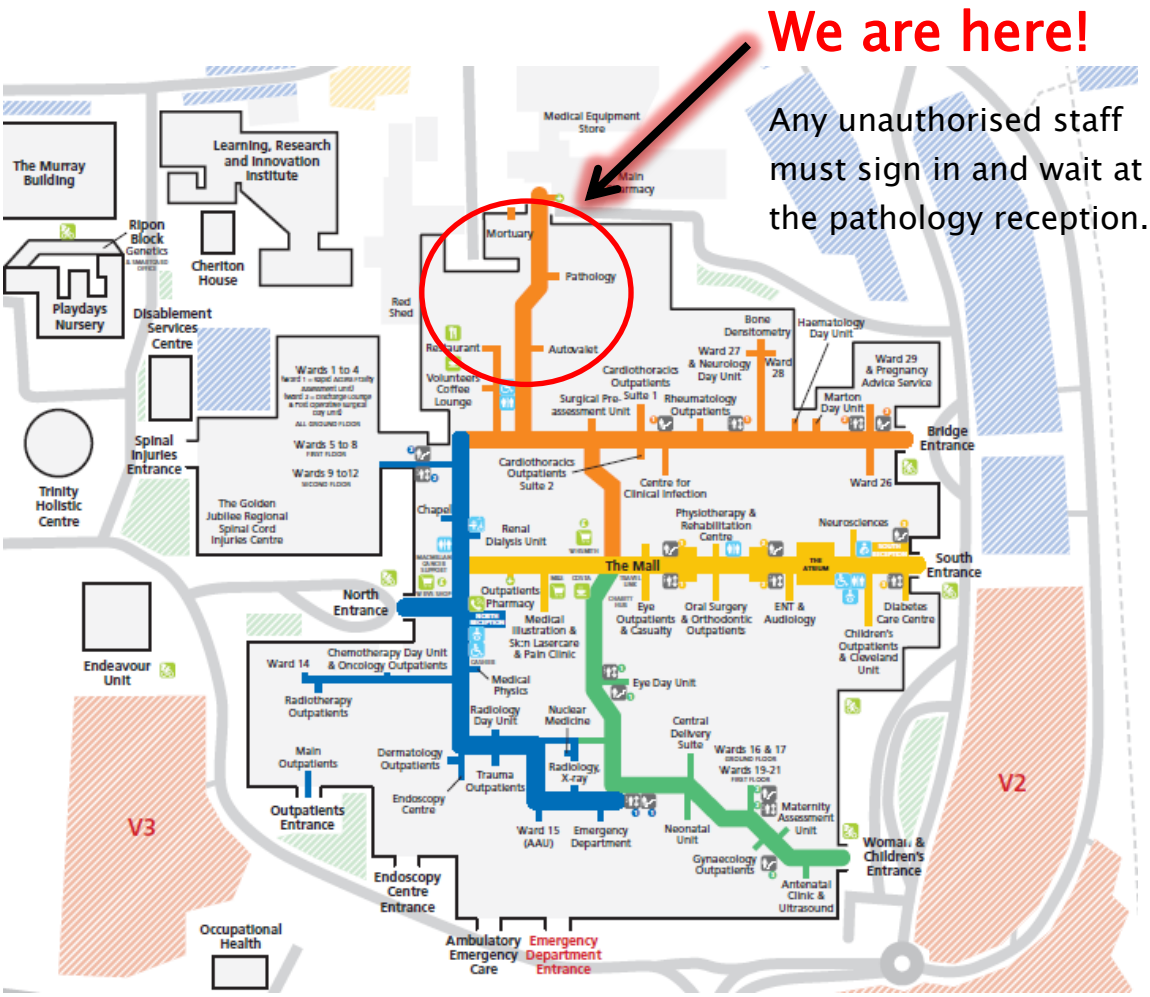
https://www.google.co.uk/maps/search/james+cook+hospital/@54.5519697,-1.2108586,15.5z

https://www.southtees.nhs.uk/content/uploads/James-Cook-campus-map-landscape.pdf



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3. Useful Contacts

Name	Job Title	Contact Details
Elaine Watson	Bacteriology Lead Biomedical Scientist	Ext 55947 elaine.watson4@nhs.net
Sandra Gittins	Virology Lead Biomedical Scientist	Ext 55932 Sandra.Gittins1@nhs.net
Ann Wallis	Pathology Quality Manager	Ext 55239 Ann.Wallis1@nhs.net
	Microbiology secretaries	01642 282604 Ext 52604
Virology Laboratory		Ext 54289
Bacteriology Laboratory		01642 835990 Ext 52606
Pathology Main Reception		Ext 54385

4. Opening Hours

The Bacteriology Laboratory operates a **24 hour shift system 7 days a week**. Out of core hours staff can be contacted by phoning the laboratory on:

01642 850850 Ext **52606**

The Virology department operates **Monday to Friday 9-5 and Saturday and Sunday 9-12**. They can be reached on:

01642 854289

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5. Specimen Requests

All requests for Microbiology investigations must be made by, or on behalf of, a registered medical practitioner, a recognised nurse practitioner or similar, to whom the results will be sent (see below for patient detail requirements). Requests signed by an authorised person (e.g. practice nurse) on behalf of a practitioner are acceptable as long as the origin is stated clearly and the request is fully completed (including relevant clinical details).

If possible requests should be made using the electronic WebICE system, which limits errors in patient identification and speeds up workflow in the laboratory. When making a request please ensure that all the relevant patient identification, clinical details and locations are provided, including the name of the requesting physician. Contact information must be supplied when an urgent request is made.

A request form must accompany all specimens sent to the laboratory.

All Request forms must clearly state the following information:

- Patient Name and Address
- Date of Birth or Age
- NHS number / hospital number
- Patient Gender
- Relevant clinical details including recent foreign travel
- GP practice code – which will be where the result is returned
- Type of specimen
- Date and time of taking the specimen, include who took it
- Risk Status
- Any relevant epidemiological information

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- Any systemic antibiotics that the patient is taking
- Any foreign travel, which is prompted via WEBICE requesting.

It is the responsibility of the requesting person to ensure that request forms are filled out with the adequate information. Failure to do so may result in rejection of the sample.

The specimen container must also be clearly labelled with a self-adhesive sticker and contain the patients full name, date of birth, and / or hospital number. The information on the specimen must directly link with the information on the request form.

Please ensure that all specimen containers are filled with the correct/adequate volume of sample.

For most routine laboratory procedures, consent can be inferred when the patient presents himself/herself at a laboratory, or other suitable primary or secondary care setting, with a request form and willingly submits to the usual collecting procedure.

PLEASE NOTE: If any additional requests are required after the specimen has been sent, please call the laboratory within **2 days and send a request form for the additional tests requested**. The additional laboratory request form must be received in the laboratory prior to the test being carried out.

5.1 High Risk Specimens

Any specimens that are known, or suspected, to be high risk must be clearly labelled with a danger of infection sticker.

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Medical officers responsible for the care of patients have a duty of care towards other members of staff, therefore all specimens from patients who are known to have or strongly suspected of having the conditions listed below must be identified by adding a Danger of Infection label to the specimen container and the laboratory request form:

- HIV
- Hepatitis B & C
- Viral haemorrhagic fever (VHF) of any type
- Microorganisms, (biological agents) in Hazard Group 3 or 4 e.g. TB, *Brucella*, *Salmonella typhi/paratyphi*, Transmissible Spongiform Encephalopathy (TSE)
- Pyrexia of unknown origin (PUO) recently returned from Africa.

Medical officers should also ensure that appropriate information including relevant travel history is provided in order to alert laboratory staff to potential dangers. Clinical details supplied on specimen request forms must contain clear information regarding the nature of the test being requested and sufficient detail to inform laboratory staff upon the safety precautions they need to take in order to process the specimen without risk of infection.

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If, during patient intervention, further information becomes available that has implications for the safety of laboratory staff this must be communicated immediately to the laboratory so that appropriate steps regarding containment can be taken.

6. Collection and Transport of Bacteriology Samples

It is the responsibility of the person taking the sample to ensure that the container used is the appropriate one for the purpose and sterile, is properly closed, and is not externally contaminated by the contents. Specimens in non-approved containers will not be accepted.

Specimens must be placed in the transparent plastic transport bags as soon as they have been labelled, with the attached request form. There must only be one specimen per bag. The transport bag must be sealed using the integral sealing strip and must not be sealed with pins, staples, metal clips etc.

Within the hospital, specimens must be transported in either deep-sided boxes of a smooth impervious material which must not be over-filled, or in specialised carrier pods for the pneumatic tube system. Any specimen transport box or pod must not be used for any purpose other than carrying specimens. The boxes must be cleaned and disinfected each week and whenever contaminated. For urgent samples, ward staff are required to arrange delivery to the laboratory. Specimens must first be placed in the plastic specimen bags together with the completed request form.

The transport of samples from GP surgeries or other primary care locations is carried out by staff that will collect all samples from dedicated collection points.

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Transport for infectious substances by Royal Mail is subject to carriage of dangerous goods regulations 2004 and requires transport and packaging conforming to P650 Packaging.

Patients are able to carry their own specimens via their own or public transport, to the laboratory.

When specimens are transported to the laboratory, efforts should be made to avoid delays. This reduces the risk of samples being lost and of their contents becoming degraded. In addition, storing of specimens on wards after they have been taken should be avoided. This reduces the risk that the specimen may become damaged and its contents released, which could be a potential infectious hazard.

See individual specimen sections for the testing that is carried out on each sample type.

6.1 Urine Samples

Information

Contaminating bacteria from the external genitalia may give rise to misleading results and therefore, we can only accept the following specimens for routine culture:

- Catheter or cystoscopy specimens (please note that catheter tips will not be processed as they do not provide helpful microbiological information)
- Mid-stream urine specimens
- Supra-pubic aspirates

Mycobacterium tuberculosis testing can only be performed if there is a high white cell count in the urine. If so, 3 x 20ml white-topped sterile containers should be used to collect early morning urine on three consecutive days to be tested for TB.

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For **Schistosomiasis** and other parasite testing, collect a urine sample between 10am – 2pm, which is when the concentration of eggs is at its highest. Light exercise should be done before the urine sample is taken e.g. running up a flight of stairs and the volume of urine collected should always be recorded so that an accurate concentration of eggs can be calculated.

Collection

If the patient is able to collect urine without assistance from the nursing staff, they should be instructed as follows:

Females

1. Separate the labia with cotton wool or sponge moistured with water (disinfectant MUST not be used)
2. Wipe the vulva from front to back
3. With the labia still separated, allow some urine to pass into the toilet
4. Without stopping, allow urine to pass into a sterile red-topped borate container (as seen in container section) and fill to the line
5. Pass remaining urine into the toilet

Males

1. Clean the penis with soap and water
2. Commence urination and allow a few millilitres to pass into the toilet
3. Without stopping, allow urine to pass into sterile red-topped borate container (as seen in container section) and fill to line
4. Pass remaining urine into toilet

In elderly or very ill patients, nursing assistance may be required. Ensure that there is minimal chance for contamination of the sample. Specimens showing signs of contamination e.g. with faecal matter, are of no value and will not be cultured.

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Catheter Urines

1. Disinfect the catheter specimen port using an alcohol wipe
2. Clamp tubing below the sampling cuff
3. Clean the sampling cuff with alcohol wipe
4. Aspirate urine using a syringe and transfer to a sterile boric acid container
5. Unclamp the tubing

Container

Use red-topped universal containers that contain boric acid for bacterial culture and microscopy. Paediatric/small volume urines (less than 10ml) should be sent in 7ml red-topped sterile universals with boric acid. If unable to obtain 5ml or more of urine, the accuracy results may be affected due to insufficient dilution of the borate crystals - collect the urine in a white-topped universal container N.B. this type of sample needs to be at the laboratory within 2 hours of collection.

For *Chlamydia* testing and **MRSA** testing, use a white-topped sterile universal bottle (no boric acid) and fill to the line indicated on the label, not to the brim of the container.

Transport

Specimens should reach the laboratory without delay, with a maximum time between sample collection and arrival in the laboratory of 48 hours, to ensure

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accurate results. If there is a delay in the specimen reaching the laboratory, ensure specimens are refrigerated.

6.2 Vaginal and Penile Swabs

Information

- Be sure to obtain a representative sample.
- Label the specimen carefully.
- On the request form, please give all relevant history, especially of current or recent antimicrobial treatment. Please specify:
  - Post-operative
  - Age
  - Post-natal <6wks
  - Miscarriage
  - Pregnancy
  - Toxic shock syndrome
  - Abscess
  - PID
  - Fever

High vaginal swabs are unsuitable for the diagnosis of gonorrhoea or pelvic inflammatory disease.

Collection

1. Cervical swab:
   a. Collect endocervical sample by speculum examination

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b. Remove any mucus or vaginal material from the cervical outer surface
c. Insert the swab into the cervix and leave it inside the cervix for a few seconds
d. Remove swab from cervix and avoid contact with vaginal mucosa when withdrawing the swab

2. Male urethral swabs:
    a. Express exudate from the urethra and collect it on a swab
    b. If no exudate is available insert a swab into the male urethra, rotate and remove it.
    c. Place swab into the orange-capped tube with transport media.
3. Penile swabs
    a. Pre-moisten the swab with transport media
    b. Swab around the penis
    c. Place the swab into the pink-capped tube containing transport medium.

Container


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The pink-capped swab should be used for high vaginal swabs, endocervical swabs and penile swabs, and the orange-capped swab should be used for urethral swabs.

Transport

Please ensure that the specimens reach the laboratory as soon as possible as some bacteria can become unviable when not kept in the correct conditions. This would lead to an unrepresentative result.

Refrigeration if these type of specimens is not advised and if there is a delay should to be stored at room temperature.

6.3 Faeces Samples

Information

- Give all relevant clinical history especially date of onset, food history, foreign travel, abdominal pain, contact with other people and antimicrobial treatment.
- Please consider requesting other investigations such as norovirus or Hepatitis A where seafood has been consumed and GI symptoms and/or clinically relevant features have developed.
- Do not mix urine with stool sample, patients should be encouraged to urinate before giving the faeces sample
- On the request form, it must be stated whether bacterial faecal PCR or Viral PCR is required
- All patients >65 years old and in-patients >2 years old will be tested for *Clostridium difficile* automatically.

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Collection

1. Place a wide mouth container e.g. potty or large empty container in the toilet bowl to prevent specimen falling into the toilet.
2. Cover the potty/large container with clean plastic wrap
3. Pass the stool onto the potty
4. Using a spatula, half-fill the faeces container. Do not fill more than a third full if the specimen is liquid (minimum volume is 2ml).
5. Flush the remainder of the stool sample down the toilet
6. Wash hands thoroughly

Container

Please note: Specimens in toilet paper, nappies, or non-sterile containers are not acceptable and must only be sent in the container as specified above for both bacterial and viral testing.

Transport

Samples need to be transported to the laboratory as soon as possible and should be refrigerated if delay is expected.

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6.4 Sputum Samples/Bronchoalveolar Lavage / cough swabs

Information

- A sputum sample (for non-mycobacterial culture) should be the result of one single expectoration.
- The best specimens are those produced early in the morning from a deep cough, before the patient eats or drinks or clean their teeth.
- If sputum requires testing for ***Mycobacterium tuberculosis***, three separate specimens should be sent on consecutive days- ideally the first specimen of the day. Samples requiring TB testing – will be sent to the reference laboratory for testing and will delay the release of result. See turn-around times for more details.
- For diagnosis of *Pneumocystis carinii* pneumonia (PCP) – induced sputum or bronchoalveolar lavage is more reliable.
- If Legionella or Pneumococcal antigen is to be excluded, please send a urine sample in either a plain universal or boric acid container. See Section 6.1 on how to take a urine sample.
- Cough swabs are only accepted from cystic fibrosis patients.

Collection

1. Approximately 5ml of sputum from the lower respiratory tract needs to be expectorated by deep coughing
2. If cough is dry, physiotherapy, postural drainage or inhalation of aerosol before expectoration might be helpful.
3. The sputum should be collected in the container shown below. No other containers will be accepted.

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Container

Ensure that the container used is sterile; a wide-mouthed container might be easier to use.

Transport

- Sputum samples must be sent in separate bags
- Sputum samples must be sent to the laboratory as soon as possible.
- If delays are expected, specimens should be refrigerated.

6.5 Blood Cultures

Information

- Blood cultures should only be taken when there is a reason to suspect infection. 'Saving Lives' guidance suggests that if bacteraemia is strongly suspected then two sets of blood cultures should be taken at separate times and sites. This is particularly important for patients with septicaemia. Blood cultures should not be taken for routine assessment.
- The blood culture set consists of two bottles, one with an orange label and cap (anaerobic culture), which contains nutrients that will support growth and allow detection of micro-organisms that prefer reduced oxygen environment. The other with a green label and cap (aerobic culture) contains nutrients for micro-organisms that thrive in an oxygen-rich environment.

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These bottles should be sent containing 8- 10ml of the patients' blood. For paediatric samples, use a bottle with a yellow label and cap, which holds a smaller amount (5ml) - if only a small amount of an adults blood can be obtained, use a paediatric bottle.

Two sets are usually collected from different veins, or through existing venous catheters, and sometimes further sets are collected at timed intervals. This is done to detect microorganisms that are present in small numbers or are released into the bloodstream intermittently. It is also done to help ensure that any microorganisms detected are the ones causing the infection and are not present just as contaminants from the skin.

Several samples are also collected from children, but the quantity of each blood sample will be smaller and appropriate for their body size.

If infective endocarditis is considered, please send a total of 3 sets of blood cultures, and discuss with a Consultant Medical Microbiologist. These should be taken via different venepuncture sites.

The Microbiology laboratory at James Cook uses the BacTAlert system, which allows automatic monitoring of the blood cultures. All blood cultures are monitored over a 5 day period (any clinical details of heart problems e.g. valve replacement or endocarditis, get a 10 day incubation period).

Positive results are relayed to the appropriate requester as soon as the Gram stain has been interpreted.

An interim negative report is sent after 48 hours incubation period but all bottles will be monitored until the 5/10 days have passed.

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For patients on antibiotics, a smaller volume of 5ml should be inoculated into each bottle rather than 10ml so that the effect of dilution can be used to neutralise the antibiotics.

Collection

Before the bottles are inoculated, ensure that the sensor at the bottom of each bottle is intact; do not use if it is yellow. The broth in the bottles should be clear; if cloudy do not use that bottle.

1. Kit Preparation:
    a. Label bottles with surname, forename, date of birth and hospital number in the spaces provided. If using printed labels, ensure they do not cover barcodes and do not tear off barcode labels.
    b. Remove caps from bottles and clean with an alcohol wipe.
2. Skin preparation:
    a. Wash your hands with soap and water and then dry
    b. Clean any visibly soiled skin on the patient with soap and water and then dry
    c. Apply a tourniquet and palpate to identify vein
    d. Clean the skin with an alcohol wipe and allow to dry. If a culture is being collected from a central venous catheter, disinfect the access port with an alcohol swab and allow to dry.
3. Sample collection – Needle and syringe method
    a. Wash and dry your hands again or use alcohol hand rub and apply clean examination gloves (sterile gloves not necessary)
    b. Insert needle – do not palpate again after cleaning
    c. Collect the sample and release the tourniquet.
    d. Cover the puncture site with an appropriate dressing.

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e. If blood is being collected for other tests always inoculate the blood culture bottles first.
f. Inoculate the blood into the culture bottles. Do not change the needle between sample collection and inoculation. Inoculate the orange blood culture bottle first and then the green one. Ensure that at least 5-10ml is inoculated into each bottle.
g. Discard the needle and syringe in a sharps container at the point of use.
h. Remove gloves and wash hands with soap and water.
i. Record the procedure in the patient's medical notes including indication, date, time, site of venepuncture and any complications.

4. Sample collection – Winged blood collection method (preferred method)
    a. Wash and dry your hands again (or use alcohol hand rub) and don clean gloves.
    b. Attach the winged blood collection set to the blood collection adapter cap.
    c. Insert the needle. Do not palpate the vein again after cleaning the skin.
    d. Place the adapter cap over the blood culture bottle and pierce the rubber bung.
    e. Hold the bottle upright and use the bottle graduation lines to gauge the sample volume being collected.
    f. If blood is being collected for other tests always inoculate the blood culture bottles first. Follow the instructions for which culture bottle to inoculate first.
    g. Collect the sample and release the tourniquet.
    h. Discard the winged collection system in a sharps container at the point of use.

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i. Cover the puncture site with an appropriate dressing.
j. Remove gloves and wash hands with soap and water.
k. Record the procedure in the patient's medical notes including indication, date, time, site of venepuncture and any complications.

Container

Transport

The blood culture bottles should reach the laboratory as soon as possible, and if delays occur, the specimen should be stored at room temperature – never refrigerate inoculated blood culture bottles.

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6.6 ENT Swabs

Information

Ensure the form includes relevant clinical details, as this will allow the laboratory to culture for specific organisms e.g. *Neisseria meningitidis* or *Bordetella pertussis*.

Collection and Container

PLEASE NOTE: Before taking the sample, please ensure that the swab has been moistened in the transport medium.

Collection of Nasal swabs:

1. Pre-moisten the swab in the transport medium
2. Insert the swab into the nostril and guided it gently and horizontally to the back of the nose.
3. If an obstruction is encountered, withdraw the swab and reinsert it through the other nostril.
4. As soon as the resistance to the posterior wall is felt, withdraw the swab and insert it in the orange-capped container.

The blue-capped pernasal swab should only be used for the culture of *Bordetella pertussis* – using this swab has been proven to increase the yield of any pertussis present due to the specific shape of the swab.

Bacteriology eSwab User Guide

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Selection of eSwabs for Bacterial Investigation

PINK

- Wounds, skin, ulcers and burns.
- High vaginal swab
- Endocervical
- Oral
- MRSA screening

ORANGE

- Ear
- Eye
- Nasal
- Throat
- Urethral

BLUE

- Nasopharyngeal for *Bordetella pertussis* investigation

Step 1

Complete ICE request form
Collect equipment:
- Select appropriate eSwab
- Examination gloves
- Apron

Use sterile examination gloves to avoid contamination with your own skin flora.

Check all items to be used are within expiry date.

Step 2

Please select appropriate eSwab for investigation required, as detailed above.

Take swab supplied with specimen tube and take sample as required for investigation. Please take separate samples for each request / additional sites.

Step 3

Place sampled swab into specimen tube containing liquid transport medium. Break off the swab at the red mark indicated on the swab shaft.

Step 4

Following sampling replace the cap on the specimen tube and screw on firmly to avoid leakage of material.

Step 5

Ensure patient details and swab site are clearly labelled on the specimen tube and match the request form. (It is acceptable to use a small patient ID/ICE label).

Unlabelled specimens will not be processed.

Once complete please send to Microbiology for laboratory investigation.

For further information contact the Microbiology Department on 01642 282606 or extension 52606.

Version 1. Issue Date January 2015. Review Date January 2017.

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Transport

Swabs should be transported in the transport media contained within the swab tube- the samples should reach the laboratory as soon as possible and should be stored in the fridge if there are going to be delays.

6.7 Corneal Scrape

Information

In addition to the routine eye swabs, a swab of the corneal ulcer can be sent but the best sample is where the needle/scalpel is used to inoculate direct into the media plates which are collected from microbiology.

DO NOT send the scalpel or needle used to take the scrape, as this is a sharps risk to laboratory staff.

If *Acanthamoeba* is suspected, then please send the contact lens fluid with or without the contact lens or the needle used flushed with a small amount of sterile saline.

Collection

Can be via a swab, or by using a syringe/scalpel to directly inoculate culture plates.

Container

The scalpel is used to directly inoculate the media plate, the following plates can be ordered from the laboratory by ringing 52606 and asking for a corneal scrape pack. The plates needed are

- Fastidious Anaerobic Agar (FAA) plate,
- blood plate, chocolate plate
- Sabaroud (SAB) plate;

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- a slide is also required for a Gram stain which will come in the plate pack, please label with the patients name on the side of the slide inoculated.

Transport

The slide should come straight to the laboratory so a Gram stain can be carried out to indicate if any micro-organisms are present, along with the direct culture plates, once inoculated, so that they can correctly incubated.

Swabs should be transported in the transport media contained within the swab tube- the samples should reach the laboratory as soon as possible and should be stored in the fridge if there is going to be delays.

6.8 Wound and Pus swabs

Information


The site and origin of the material must be stated on the request form and sample


Anaerobes and fastidious organisms die if subjected to delay or dehydration


Transport medium must always be used for swabs


Pus is preferable to a wound swab


Pus is essential if testing for *M. tuberculosis*

Collection

1. If any volume of pus is present it should be collected using a sterile syringe into a sterile universal container rather than a pus swab
2. Pre-moisten the swab provided with transport medium before swabbing the wound.
3. Replace the swab into the pink-capped tube containing transport medium.

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Container

Transport

Wound swabs should be transported to the microbiology reception as soon as possible. If there is a suspected delay in delivery then the swabs should be refrigerated.

6.9 Skin Scrapings

Information

The edge of a skin lesion is more likely to contain viable fungus

Collection

1. Using a curved scalpel blade, scrape across the inflamed margin of the lesion into the apparently healthy tissue.

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Container

Use a Dermapak to collect skin scrapings.

Transport

Samples should be transported to the microbiology reception via courier and should be kept at room temperature.

6.10 Hair Samples

Information

Cut hairs are unsatisfactory due to the fact that infection usually occurring below the surface near to the scalp

Collection

1. Scalp scrapings should include hair stubs and can be taken using a sterile scalpel
2. Hairs can be plucked from the scalp using sterile epilating forceps

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Container

Transport

Samples should be transported to the microbiology reception via courier and should be kept at room temperature.

6.11 Nail Scrapings

Information

- Clippings should be taken from the discoloured or brittle part of the nail
- The nail should be cut back as far as possible from the free edge as some fungi are restricted to lower parts
- Scrapings can also be taken to supplement the clippings
- Nails should **NOT** be sent to the laboratory in a universal container, due to the nails not being able to dry out, which could result in an over growth of unwanted bacteria. The Dermapak aids the drying out process which preserves the fungus if present in the sample.

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Collection

1. 1. Discoloured , dystrophic or brittle areas should be sampled using pincer style nail clippers
2. If the distal edge is not involved, scrape the affected area using a scalpel blade

Container

Use the Dermapak for nail clippings and scrapings.

Transport

Samples should be transported to the microbiology reception via courier and should be kept at room temperature.

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6.12 Cerebrospinal Fluid (CSF)

Information

- All specimens must be labelled appropriately
- A request form with appropriate clinical history must accompany the specimen
- The laboratory must be notified when a CSF arrives to ensure no delay in the processing of the sample
- Biochemistry will require separate specimens and forms for xanthachromia, protein and glucose. Please contact Biochemistry for special requirements.
- If the specimen is clotted, then a cell count cannot be performed.
- Microbiology require the first and last sample of CSF taken.

Collection

Collection is by lumbar puncture.

Put 1-2ml into a sterile labelled universal container. In cases of suspected subarachnoid haemorrhage collection of CSF should be placed in three containers to compare the degree of bloodstaining. Only one specimen is required if the cell count is the only test required (e.g. neurology patients).

Container

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Please ensure that the universal container is sterile to reduce risk of contamination.

Transport

These samples should reach the laboratory without delay in order to process them and culture any possible bacteria.

6.13 Tissue Samples

Information

- PLEASE NOTE: Specimens in formalin are not suitable for Microbiology testing and will not be processed.
- Ensure that all relevant clinical history is given.
- If more than one tissue or pus sample is being sent, please label them a, b, c etc. and send a separate request form for each specimen.

Collection

1. Place the specimen into a sterile universal container.
2. If there are multiple specimens of tissue, place them into separate containers with separate request forms

Container

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Transport

These samples should reach the laboratory without delay in order to process them and culture any possible bacteria.

6.14 Ascitic Fluid

Information

- Samples should be sent to the laboratory in a set of blood culture bottles
- A separate sterile 20ml universal should contain some of the sample for a Gram stain.
- A separate EDTA sample must be sent to haematology for a white blood cell count

Collection

1. A sterile needle is inserted into the abdomen and a syringe Is used to drain some of the ascetic fluid
2. Place between 5ml and 10ml of the sample into a pair of blood culture bottles and some into a sterile 20ml universal for Gram stain and culture.

Container

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The green bottle is for aerobic culture, orange bottle is for anaerobic culture and the sterile universal.

Transport

These samples should reach the laboratory without delay in order to process them and culture any possible bacteria.

6.15 Pleural Fluid

Information

Cell counts are not performed on pleural fluids, if testing for *Mycobacterium tuberculosis* is also required please send down a separate request form for this investigation with the one sample.

Collection

1. Collect the fluid into a syringe
2. Place up to 20ml into a sterile universal container

Container

Transport

These samples should reach the laboratory without delay in order to process them and culture any possible bacteria.

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6.16 Synovial Fluid

Information

Biochemistry, cytology and TB culture (if requested) will each require separate specimens and request forms

Please give all relevant clinical details on the form, especially of any current or recent antimicrobial treatment.

Collection

1. Collect the fluid into a syringe
2. Place up to 20ml into a sterile universal container

Container

Transport

These samples should reach the laboratory without delay in order to process them and culture any possible bacteria.

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6.17 MRSA Swabs

Information

- MRSA swabs from in-patients are usually inoculated on the ward
- Inoculation is into pink-capped tubes using the provided cotton tipped swabs.
- Do not put two swabs into one container for the nose/groin screen. Follow the guide below on how to take the screening swabs. Two swabs received in one container will result in the specimen being rejected.
- A screen comprises of swabs from: nose/groin, any wounds/IV sites, any skin lesions/eczema etc. or catheter urine if appropriate.
- For emergency cases on specified wards MRSA PCR is performed if requested. This test is carried out using a pink nasal/groin swab only, because the test is only validated for use for nose/groin swabs.

Collection

For correct collection of swabs see appropriate section.

Collection of groin swabs:

1. Take the cotton tipped swab from the package.
2. Gently dip the cotton tip into the culture media to moisten it.
3. Rotate the moistened swab gently but firmly over the area on each side of the groin. Only one swab is necessary
4. Place the cotton tipped swab into the plastic tube with the gel at the bottom.

For Collection of Urine to be screened for MRSA please see 6.1.

MRSA Screening Swabs

18 step guide for taking MRSA screening swabs using the Pink eSwab 490CE.A

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1

Only to be used by staff trained to take samples.

Collect equipment:

- Pink eSwab 490CE.A,
- Non sterile examination gloves and apron.

2

Check all items to be used are within expiry date. NB. Expiry date also present on packaging.

3

Explain the procedure to the patient and prepare them for sampling.

4

Prior to beginning the procedure wash hands with soap and water or disinfect hands using alcohol hand gel.

5

Perform a risk assessment to decide most appropriate PPE use. Put on non sterile examination gloves to avoid contamination of the swabs with your own skin flora.

6

Ensure swab specimen tube is at the top of the pouch before peeling open packaging. If not push tube to the top of the pouch.

7

Peel or rip open packaging level with the specimen tube as indicated in the picture.

8

Either holding the opened pack between thumb and fingers as indicated in the picture step 7, or remove the tube and place it onto a clean tray / stable surface.

9

Open a separate individually wrapped FLOQSwabs Copan flocked swab.

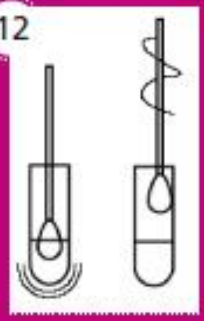
10

Remove the cap from the specimen tube and place inverted on a clean stable surface/tray. Use the separate single wrapped swab to sample both nares first.

11

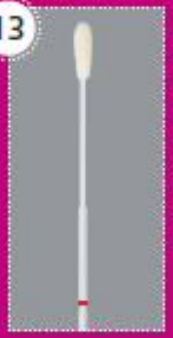
Gently insert tip of the single swab into the anterior nares of nose. Rotate ten (10) times against the mucosal surface. Repeat using the same swab for the other nostril.

12



Swirl the swab in the transport liquid (in specimen tube) for 10 seconds and then press against inside of the container. Discard this (nares) swab into clinical waste bin.

13



Take the swab supplied in the Pink eSwab 490CE.A packaging. Stroke the swab in the groin area and rotate ten (10) times.

14

Place this swab in specimen tube and break off the swab into the tube at the red mark on the swab shaft. Only one swab should now be inside the sample tube.

15

Replace the cap on the tube and screw on firmly to avoid leakage of transport liquid inside tube. Ensure only one swab remains inside tube. Separate swab collection kits must be used for additional sites. Wound, catheter site e.t.c.

16

Ensure patient details and swab site are clearly labelled on the specimen tube and match the request form. (It is acceptable to use a small patient ID/ICE label). Unlabelled specimens will not be processed.

17

Select investigation required on ICE requesting for culture or MRSA PCR. NB. MRSA PCR for emergency screening wards only.

18

Once complete send to mircobiology.

Thank the patient, remove PPE and wash hands.

Ensure the MRSA screening is documented in the patient's medical record/notes.

MCB3819

For further information contact the Microbiology Department on 01642 282606 or extension 52606

Version 3, Issue Date: November 2014, Revision Date: November 2016

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Container

Transport

MRSA swabs should be transported to the Microbiology reception as soon as possible. If there is a suspected delay in delivery then the swabs should be refrigerated.

6.18 Line Tips

Information

- Line tips can occasionally become colonised with bacteria and can go on to cause a blood stream infection which requires clinical intervention.
- This may require the line tip to be removed for successful treatment
- Line tips should only be sent if there is a suspected infection, indications of this include: the site being inflamed, or unexplained fever with a line tip in situ
- The site and duration of line tip should be indicated on request form.

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If the line entry site is inflamed then send a swab of the area also, follow the wound and skin swab section.

Collection

1. Clean the skin in the region of the intravascular catheter
2. Withdraw the catheter with sterile forceps
3. Cut the terminal 5cm of the catheter tip off with sterile scissors
4. Place in a dry, sterile, labelled container to transport to the laboratory
5. If the line tip has been used in total parental nutrition please state on form

Container

Transport

Line tips should be delivered to the Microbiology reception via courier/porter as soon as possible. If immediate transport is not possible then the specimen should be refrigerated.

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7. Acceptance of Specimens

Appropriate, well taken specimens in correct containers, which are promptly delivered to the laboratory, are the key to obtaining the best results. In order to prevent delays relevant clinical detail must be provided on the request form.

In order for specimens to be accepted;

- Specimens must be in appropriate sterile containers
- Antimicrobial agents should not have been administered before the specimen was obtained
- To be able to complete examination an adequate quantity of material should be sent
- The specimen taken should be representative of the disease process
- Sterile equipment and aseptic technique must be used when collecting specimens. Care must be taken to avoid contamination from natural micro-organisms found on the skin or mucous membranes
- Specimens other than blood cultures should be stored between 2-8ºc if immediate transportation to the laboratory is not available. This prevents the over growth of less fastidious organisms.
- A specimen is likely to be rejected if it is so inadequately labelled that the patients identification is in doubt, or if the container has leaked or is at risk of contamination. In these circumstances every effort will be made to inform the requesting doctor.

For samples that are not easily repeated (such as CSF or paediatric samples) the problem will first be discussed with a Biomedical Scientist from the relevant section who will make a decision on whether testing may be allowed to proceed (usually after discussion with the clinician concerned). If the specimen is tested

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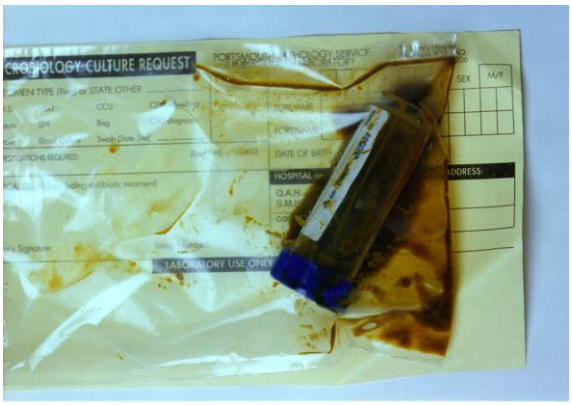
the report will clearly state the nature of the problem as a comment. Alternatively, the requesting clinician will be asked to send a repeat sample.

PLEASE NOTE: Samples of which pose a danger of infection should **NOT** be transported using the hospital pneumatic/ air tube system. These samples will still be issued with a specimen number; however reception staff will not open the sample. Instead it will be handled in the Category 3 laboratory.

Example of leaking specimen:



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8. Testing of Bacteriology Samples

8.1 Urine

8.1.1 Urine Culture

Background of test	Urinary tract infection (UTI) results from the presence and multiplication of bacteria in one or more structures of the urinary tract with associated tissue invasion. This can give rise to a wide variety of clinical syndromes. Infection may spread to surrounding tissues (e.g. perinephric abscess) or the bloodstream (bacteraemia, septicaemia). In contrast the presence of certain types of micro-organisms in urines other than catheter urines (e.g. S aureus including MRSA, Candida) may be a sign of bacteraemia or candidaemia rather than UTI. An important part of the investigation of UTI is bacteriological culture. Microscopy of the specimen is undertaken to quantify the number of white blood cells, red blood cells and also to detect whether there are bacteria or yeast present. Culture and antibiotic sensitivity testing is also carried out, to determine what, if any, bacteria are present and the antibiotic sensitivity pattern. Clinical details provided tailor the tests carried out on the sample.
Associated Diseases	 Acute and chronic pyelonephritis  Cystitis  Urethritis

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	 Epididymitis  Prostatitis  Bacteraemia  Septicaemia  Candidaemia
Turnaround Time	Up to 5 days
Referred Test	No
Referral Laboratory	N/A

8.1.2 Urine Parasites

Background	Microscopic detection of parasitic ova in the urine. This test is carried out when patient has travelled to counties within Africa and Asia or places with poor sanitation.
Associated Diseases	Schistomiasis
Turnaround Time	5 days
Referred Test	No
Referral Laboratory	N/A

8.1.3 Urine Mycobacteria

Background	Genitourinary tuberculosis – As the infection progresses, kidney lesions may caseate, discharging viable AAFB into the renal pelvis and ureter and infections may thus further spread to the bladder. Urinalysis will often show proteinuria, haematuria and sterile pyuria. The urine sample will be sent to the reference laboratory which will perform PCR testing and culture.
Associated Diseases	 Tuberculosis

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	 Glomerulonephritis Renal Failure
Turnaround Time	Negative sample: 6-8 weeks Positive sample: up to 13 weeks after confirmation and sensitivity testing
Referred Test	Yes
Referral Laboratory	TB Reference Laboratory – Freeman Hospital Newcastle

8.2 Vaginal and Penile Swabs

Background	A range of sexually transmissible organisms cause infections responsible for a large number of clinical syndromes. When a specific STI is diagnosed, it is recommended to screen for other infections. Genital testing can also be performed to detect *Trichomonas* infection, yeast infections or any other abnormal bacterial growth. From the swab, the specimen is cultured to attempt to grow any bacteria that are present in the sample. Clinical details provided tailor the tests carried out on the sample.
Associated Diseases	• Gonorrhoea • Trichomoniasis • Vaginal candidosis • Bacterial vaginosis (BV) • Toxic shock syndrome • Lancefield group B streptococcus • Listeria monocytogenes

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	• Septic abortion • Bartholinitis • Mucopurulent cervicitis • Postpartum endometritis • Salpingitis • Pelvic inflammatory disease (PID) • Prostatitis • Epididymitis • Orchitis • Balanitis
Turnaround Time	Up to 5 working days
Referred Test	
Referral Laboratory	

8.3 Faeces

8.3.1 Faeces PCR

Background	Faecal PCR is a test that detects and identifies bacteria that cause infections of the lower digestive tract. The test distinguishes between the types of bacteria that cause disease (pathogenic) and the types that are normally found in the digestive tract (normal flora). The test helps to determine if pathogenic bacteria are the cause of a person's gastrointestinal symptoms (gastroenteritis). The organisms routinely tested for include: *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *E.coli* O157, Shiga toxin, *Entameoba*, *Giardia* and *Cryptosporidium*. If there is an appropriate history of foreign travel, culture for

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	Vibro spp. will be performed, along with *Yersinia* when the correct clinical history is provided.
Associated Diseases	Gastroenteritis
Turnaround Time	5 days
Referred Test	No
Referral Laboratory	N/A

8.3.2 Faeces – *Clostridium difficile*

Background	*Clostridium difficile*, also known as C. diff, is a bacterium that can infect the bowel and cause diarrhoea. The infection most commonly affects people who have recently been treated with antibiotics, but can spread easily to others. *C. difficile* infections are unpleasant and can sometimes cause serious bowel problems, but they can usually be treated with another course of antibiotics. The *C.difficile* testing is done by a line assay which detects whether the bacteria and toxin are present within the sample. *Clostridium difficile* testing will be done on fluid/mucoid/blood stained stools from all inpatients > 2 years old and community patients >65 years of age. Testing for *C.difficile* will not be carried out if:- • The patient is under 2 years old. • two samples have been sent in the last 10 days • A patient has tested positive for *C.difficile* in the last 28 days. Samples tested early in a *C.difficile* infection may test as toxin negative, but all toxin negative samples are tested by PCR to see if the bacteria can potentially produce the toxin.

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	If symptoms continue, and *C.difficile* remains a clinical possibility, please repeat after 48 hours
Associated Diseases	Toxin mega colon
Turnaround Time	4 Hours
Referred Test	No
Referral Laboratory	N/A

8.3.3 Faeces Parasites

Background	Intestinal parasites are parasites that can infect the gastro-intestinal tract of humans. They can live throughout the body, but most prefer the intestinal wall. Means of exposure include ingestion of undercooked meat, drinking infected water, and skin absorption. Parasite investigation will also be carried out where there is a history of foreign travel to Central or South America, Africa or Asia. These investigations will also be carried out in patients with persistent GI symptoms for over 2 weeks, patients with eosinophilia, or if there are query worms in the sample. The laboratory performs this test via microscopy of the faecal sample to detect parasitic cyst, ova or worms.
Associated Diseases	
Turnaround Time	5 days
Referred Test	No
Referral Laboratory	N/A

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8.4 Sputum Samples/Bronchoalveolar Lavage

8.4.1 Sputum Culture

Background	A sputum culture is requested to detect and diagnose bacterial infections such as bacterial pneumonia. A bacterial infection can reach the lungs in several ways. Bacteria may spread from the mouth and throat to upper respiratory tract, bacteria in oral or gastric secretions may be breathed into the lungs as droplets in the air. These droplets are produced when a person sneezes or coughs and can pass into the lungs. Bacteria can also spread to the blood (septicaemia) from a local infection and then be carried to the lungs. Bacterial pneumonia may be a person's main infection, or it may develop after a viral infection such as influenza, a cold or viral pneumonia. The sputum is cultured to identify and bacteria, yeast or fungus present and has antimicrobial sensitivities performed so that options for treatment can be given. Clinical details provided tailor the tests carried out on the sample.
Associated Diseases	 Cystic Fibrosis  Pneumonia  Tuberculosis  Legionnaires disease
Turnaround Time	5 days for routine culture, 8 days if from a patient with cystic fibrosis. Three weeks when fungal culture is requested.
Referred Test	Legionella culture / testing is referred to Colindale reference laboratory in London, if a patient test positive in a legionella urine antigen test.

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Referral Laboratory	Colindale PHE, London.

8.4.2 Mycobacteria

Background	Mycobacterial microscopy and culture is very important in the diagnosis of *Mycobacterium* infection. Correct diagnosis and treatment are essential in the management of the patient; therefore specimens are referred to a regional Mycobacterium Reference Laboratory for microscopy, culture and antimicrobial susceptibility testing by special techniques. Emergency stains can be performed on suspected TB samples; however the sample will still have to be sent to the reference laboratory for confirmation. Testing can be done on many sample types from tissue, fluid samples, Blood, sputum, urine, for advice please contact the laboratory. When blood is being tested please send 2 X 3ml blood samples in lithium heparin tubes (green topped tubes).
Associated Diseases	Tuberculosis
Turnaround Time	3 months
Referred Test	Yes
Referral Laboratory	TB Reference Lab – Freeman Hospital Newcastle & PHE TB reference laboratory in Birmingham when positive at the Freeman.

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8.5 Blood Cultures

Bacterial Culture

Background of the Test	Blood cultures are collected to detect and identify bacteria and yeasts in the blood. Some bacteria prefer oxygen (aerobes), while others thrive in a reduced oxygen environment (anaerobes). Blood cultures are usually collected into two types of media to detect both types of bacteria. If your blood culture is positive, the specific bacteria causing the infection will be identified and antibiotic susceptibility testing will be done to tell your doctor which antibiotics will be effective for treatment. If yeasts are causing the infection, treatment will be given that is appropriate for fungal infections. Infections of the bloodstream are caused most commonly by bacteria (bacteraemia), but can also be caused by a fungus (fungaemia) or a virus (viraemia). The source of the infection is typically a specific site within the body. If the immune defences and white blood cells cannot keep the infection localised at its source it may spread to the bloodstream. When the body shows an early response to this, such as a high or low body temperature, high heart or breathing rates, and a high or low white blood cell count, it results in a condition known as sepsis. The blood cultures are loaded onto a piece of equipment called the BacTAlert, which incubates the blood cultures and detects any bacteria present. Any positive cultures have a Gram stain performed, and are cultured onto plates for bacterial identification and antibiotic sensitivities. At this point the laboratory will phone the positive result to the requesting source.

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Associated Diseases	 Sepsis  Endocarditis
Turnaround Time	Up to 11 days
Referred Test	No
Referral Laboratory	N/A

8.6 Swab Testing

Background of the test	Clinical details provided on the request form and the site of the sample taken tailors the tests carried out on the sample.
Associated Diseases	
Turnaround Time	Up to 5 working days, PVL testing can be up to 2 weeks.
Referred Test	Not for routine culture, only referred when DNA testing is carried out for things like PVL testing
Referral Laboratory	AMRL Colindale, London

8.7 Corneal Scrapes

Background of test	Once the plates are received in the laboratory they are incubated in the correct atmosphere for up to 48 hours, the sabaroud plate is incubated for a further three weeks to detect any fungal growth. Clinical details provided on the request form and the site of the sample taken are used to tailor the tests carried out on the sample.
Associated Diseases	
Turnaround Time	Up to 4 weeks, as all samples are cultured for both bacteria and fungi.

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Referred Test	If acanthomeba testing is required on contact lens fluids etc, this goes to a reference laboratory.
Referral Laboratory	Micropathology, Warwick university

8.8 Skin, hair and nail Scrapings for fungal culture

Background of test	All of these types of specimens received in the laboratory for testing are cultured onto special media to enable fungal growth. When fungal growth is present a microscopy of the growth is performed and then the growth is identified by is microscopic and macroscopic characteristics.
Associated Diseases	
Turnaround Time	Up to 4 weeks.
Referred Test	
Referral Laboratory	

8.9 Cerebrospinal Fluid Samples (CSF)

Background of test	CSF must be sent to the laboratory urgently so that a cell count may be performed to detect the presence of white and red blood cells and a Gram stain performed to detect any micro-organisms. These results are telephoned to the source if an urgent call has been received in the laboratory and placed on the LIMS system and an interim report issued on ICE. Culture is then performed for any bacterial growth and results updated in LIMS and ICE.
Associated Diseases	• Meningitis • Encephalitis

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Turnaround Time	Up to 5 working days for bacterial growth
Referred Test	
Referral Laboratory	

8.10 Tissue, ascitic, pleural, synovial or any fluids Samples

Background of test	All samples have a Gram stain performed for presence of bacteria, and if a suitable sample is received, a cell count is performed. All samples are cultured direct and enriched. Clinical details provided on the request form and the site of the sample taken is used to tailor the tests carried out on the sample.
Associated Diseases	
Turnaround Time	Up to 10 working days for bacterial growth
Referred Test	
Referral Laboratory	

8.11 MRSA Testing Samples

Background	Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of hospital-acquired infections, causing high morbidity and mortality in the UK and throughout the world. The specimen is cultured onto MRSA selective media to detect the presence of this organism.

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	Rapid PCR is performed using the specimen on GeneXpert to detect small quantities of MRSA DNA.
Associated Disease	Methicillin-resistant Staphylococcus aureus (MRSA)
Turnaround Time	5 days or 2 hours for MRSA PCR
Additional Information	
Referred Test	No
Referral Laboratory	N/A

8.12 Line Tips

Background	In order to determine which line tips are likely to be the cause of infection in the blood stream a semi-quantitative technique is used. A segment of the line tip which was terminal to the patient is rolled across a blood agar plate and after this the colonies, if present are counted. Any plates with more than 15 colonies of any organism are commonly accepted in the prediction of line tip sepsis.
Associated Diseases	Sepsis
Turnaround Time	3 days
Referred Test	No
Referral Laboratory	N/A

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9. Reporting of Results

All results are reported via the Pathology LIMS system which feeds into the WebICE system.