Torbay and South Devon NHS
NHS Foundation Trust

MICROBIOLOGY DEPARTMENT USER MANUAL

Contents

1.0 Aim of User Manual

The Microbiology Department is committed to providing a high quality, safe, efficient and cost-effective service. It is aware of and considers the needs and requirements of its service users. These include patients and staff of Torbay and South Devon NHS Foundation Trust, Primary Care Trusts, and other agencies within the district.

This guide is intended to enable all users to make best use of the various services provided, ensuring an accessible and efficient service.

This is a controlled document. The most up to date version will only be available from the Department.

2.0 General Information

The Microbiology Department is located within Torbay Hospital.

The scope of the service offered by Microbiology includes a full analytical and advisory service appropriate for a District General Hospital. More complex or rare investigations will be referred to other appropriate laboratories. A scope of the services offered is also available in the Laboratory Medicine's quality manual; a copy can be provided on request.

3.0 Quality Assurance

UKAS MEDICAL
8916
Accredited to ISO 15189:2022

3.1 Accreditation

The Microbiology department is a UKAS accredited medical laboratory No. 8916. Accreditation provides assurance that the laboratory is working to international standards and providing results that are of the highest possible quality.
The full scope of the tests that are accredited can be viewed on the UKAS website. The department strives to continuously update and for this reason some tests may not be currently included on the scope of accreditation. Tests are validated or verified before bringing into use and wherever possible a suitable Quality Assurance scheme is in place. Current tests offered that are outside scope are noted in Appendix A, and will also be noted on the patient test report.

The department has an extensive Quality Management system and Governance procedures in place to control all the processes within the laboratory. We participate in a number of internal and external quality assurance schemes to ensure the quality of our results. Where differences are identified, the impact of those differences on biological reference intervals and clinical decision limits shall be evaluated and acted upon, with users informed if any

clinically significant differences are identified. If you require any further information, please contact the Laboratory Manager.

3.2 Providing Feedback, Compliments, and Complaints

The department is committed to continually improving the service it provides to patients and users. The department has a quality policy which all members of staff are aware of and strive to follow. This combined with a detailed quality improvement policy and complaint management process ensures the department is continually focused on the level of service it provides.

If you wish to provide feedback/compliments/complaints regarding the service provided by Microbiology please use the Patient Advice and Liaison Service (PALS) available on the trust's website.

3.3 Protection of Personal Information

We support the Torbay and South Devon NHS Foundation Trust in continuous compliance with legislation of data processing and handling of sensitive information.

Our vision is to create an environment of confidentiality, integrity, and quality of information with the organisation - we achieve this by providing a high-quality service within a framework of tools that enable new ways of working and the provision of high quality care.

We work to ensure the right information is available to the right people when and where it is required.

4.0 Requesting Tests

4.1 Request Forms and Specimen Labelling

Request forms and samples must be clearly labelled with patient details (minimum name and date of birth) and the date and time the sample is taken.

4.2 Rejection Criteria

Certain samples will be rejected.
The following will be rejected:

- Unlabelled samples (except unrepeatable samples)
- No unique patient identifiers
- Leaking and broken specimens
- Empty or incorrect specimen pots
- Specimens in expired specimen containers
- Mis-matched samples and forms (except unrepeatable samples)
- Incomplete request forms including no tests requested
- Forms with no sample(s)
- Specimens with no forms
    - Unrepeatable samples - replacement form is written making it clear that the details were obtained from the specimen
    - Repeatable samples - specimen discarded
- Specimens received outside of specified time frame (e.g. TB TSpot), microbial requests over 72 hours old.
- Samples that have not been collected on ICE

Unacceptable Specimens and Requests

- Urinary catheters
- Whole samples such as toes or large lumps of bone
- Swabs for TB – (are discussed with consultant before rejecting)
- Small amounts of urine for TB investigations
- Vomit

Rejected Requests:

- Norovirus request when outbreak already identified
- Norovirus request when 6 specimens already sent from that area
- Norovirus request when not notified of any outbreak
- Parasite requests when no details of foreign travel
- Multiple specimens of the same type received on the same day
- Repeat serological tests within inappropriate timescale (dependent on the test requested)

4.3 Specimen Containers & Sample Collection

Ensure that the correct specimen container is used for the sample type. A guide to using the correct specimen containers is available throughout the Specimen Guidance. An "at a glance" section of what to use and what not to use for Microbiology tests is included in Appendix A.

4.4 Requesting Additional Tests

Specimens are retained for the following time periods after processing, additional examinations may be requested at any time during that period.

Please note:

Microbiology specimens will often deteriorate with time, so if further examinations are required then it is better to submit a fresh specimen, if possible.

How long samples are stored for:

- Swabs - 7 days
- CSF - current month + 1 month
- Fluids - current week + 1 week
- Faeces - 7 days
- Norovirus - up to sample being 2 days old.
- Urines - 3 days
- Chlamydia - 1 month
- Respiratory - 5 days
- AFB samples - 8 weeks
- Needle stick serum - 2 years (minimum)
- Serology (original blood tube) - 4 weeks*
- Serology (non blood) - 2 days
- Antenatal serum - 3 years

*Please note: Serology tests can only be added to bloods already tested by the serology laboratory, if tests are required to be added to samples from other pathology disciplines, a fresh sample will be requested.

4.5 Patient Consent

It is the responsibility of the person taking the sample/requesting to gain patient consent.
If obtaining consent is not possible in emergency situations, the lab may carry out necessary procedures, provided they are in the patient's best interest.

5.0 Results

Some final reports can take between 2 to 10 days depending on culture for unusual/atypical organisms. Preliminary reports are sent out at 2 days. See Appendix A (Quick Guide) for the turnaround times of individual tests.

6.0 Investigations, Turnaround Times & Sample Requirements

6.1 Turnaround Times

Some final reports can take between 2 to 10 days depending on culture for unusual/atypical organisms. Preliminary reports are sent out at 2 days.
For standard culture and microscopy - 48 hours for the majority of samples. Further work on positive samples may take another 24 hours.
Further reports may be issued at 5, 7, and 10 days if further investigations are required.
Some samples are referred to reference laboraties – the turnaround times for these tests varies.

6.2 Laboratory Portfolio

The laboratory provides a number of tests performed in-house:

Bacteriology tests
Bacteriology tests to provide microscopy, culture and antibiotic susceptibility testing for specimens from/of:

- Gastrointestinal tract
- Upper respiratory tract
- Ear, eye, nose, skin
- Blood
- CSF
- Wound
- Tissue, pus or fluid
- Genital tract
- Urine
- Sputum
- Organism screening eg MRSA screening
- Mycobacteria culture
- Any other body site or specimen for culture

Mycology tests
Mycology tests to provide microscopy, culture testing for fungi and yeasts and some anti-fungal susceptibility testing for yeasts.

Parasitology tests
Parasitology tests to provide microscopy and identification of medically important ova, cysts and parasites.

Serological tests

Tests performed on site include:

- HBsAg screen and confirmation
- anti-HBc total
- anti-HBs (HBsAb)
- HCV antibody screen and confirmation
- HIV 1&2 antibody screen
- Syphilis antibody screen
- HAV IgM
- Rubella virus IgG
- Measles virus IgG
- Mumps virus IgG
- CMV IgM and IgG
- EBV - EBNA, VCA IgG, VCA IgM
- Borrelia burgdorferi (Lymes disease) IgM and IgG
- Toxoplasma gondii total IgG/M
- VZV IgG
- Treponema pallidum RPR
- H.pylori antigen (faeces)
- Legionella antigen (urine)
- Streptococcus pneumoniae antigen (urine)
- Clostridium difficile toxin detection (faeces)

Molecular detection tests

Tests performed on site include:

- SARS CoV2
- Influenza virus A & B and RSV
- Extended Respiratory virus/bacterial PCR profile performed on the Biofire (https://www.biofiredx.com/products/the-filmarray-panels/filmarrayrp/)
- Extended CSF panel performed on the Biofire (BioFire FilmArray Meningitis/Encephalitis Panel (ME) | BioFire Diagnostics (biofiredx.com)
- Enteric PCR (Salmonella, Shigella, VTEC/O157, Campylobacter, Giardia, Cryptosporidium
- Norovirus 1&2
- Chlamydia trachomatis and Neisseria gonorrhoeae
- Toxigenic Clostridium difficile
- HIV Viral Load
- Hepatitis C Viral Load
- VRE Screening
- Carbapenemase Screening
- Mycobacterium TB complex PCR
- MRSA PCR

7.0 Specimen Guides

7.1 Blood Cultures

WHAT DO I NEED?

Storage before use: At room temperature out of direct sunlight

- Adult: Aerobic (Blue top) and Anaerobic (Purple top)
- Maximum fill 10ml, 5-10ml is best
- Children: Paediatric bottles (Pink topped)
- Maximum fill of 4ml, with no minimum fill

Please do not obscure the bottle's barcodes with patient labels

HOW DO I TAKE THE SPECIMEN?

Check discolouration, leakage, bulging or depressed septa, and the expiry date.
Drawing of blood:
To take blood cultures always make a fresh stab. Do not use existing peripheral lines or sites immediately above peripheral lines. Collect blood cultures BEFORE other blood tests.

1. Clean tops of culture bottles with 70% alcohol impregnated swab, allow to dry
2. Apply tourniquet and palpate to identify vein
3. Clean skin with 70% alcohol impregnated swab and allow to dry
4. Prior to venepuncture screw the BLUE adapter cap provided to the grey end of the collection tube attached to the Butterfly needle
5. Apply clean gloves
6. Do not palpate vein again AFTER cleaning
7. Insert Butterfly needle into vein site
8. Start with the aerobic bottle, place BLUE adapter cap onto upright bottle to pierce the septum. The bottle must be below the patient's arm to prevent flowback.
9. Maintain the luer connection into the bottle by securing it between thumb and forefinger.
10. Continue to hold bottle upright and use graduation lines marked on bottle to accurately gauge sample volume and collect sample.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

- Transport to the lab as soon as possible after being taken.
- Working hours - Send via the air tube (Station 2) or left at specimen reception
- Outside working hours – leave in the out of hours box, do not refrigerate.
- PLEASE DO NOT OBSCURE THE BOTTLE'S BARCODES WITH PATIENT LABELS

ANYTHING ELSE?

- Taken when bacteraemia is suspected.
- Minimum of 2 sets taken, separated according to clinical urgency of starting treatment, e.g. 10 minutes apart or hours apart.
- ? Endocarditis – minimum of 3 sets taken, at intervals of at least an hour. State the time each set was taken on the request form.
- If fungal or unusual infections, such as *Brucella*, are suspected please inform the laboratory as prolonged incubation and/or special techniques/precautions may be necessary.
- Please state any recent foreign travel, as there may be pathogens such as *Salmonella typhi*, which pose a risk to laboratory staff.

WHAT WILL AFFECT THE RESULTS?

- All specimens should be taken before antimicrobial treatment is started as this may give a false negative result.
- If too little blood is added to the vial it may give a false negative result.
- If too much blood is added to the vial these can be flagged as false positives.
- Contamination by skin flora is common resulting from incorrect inoculation of the vial.
- Delay in loading bottle onto machine can lead to non-viability of some organisms, in particular *Streptococcus pneumoniae*.
- Organisms are often few and/or may appear intermittently in the blood stream; therefore, several consecutive blood samples should be collected from each patient.
- If possible, particularly when endocarditis is suspected, the blood cultures should be taken when the patient is spiking a temperature.
- Infrequently false positives may occur due to a very high number of white blood cells being present in the blood sample.

7.2 Chlamydia and (N. gonorrhoea)

WHAT DO I NEED?

- Female Swabs (vaginal, cervical, and vaginal self-take)
- Eye swabs
- Throat, rectal, and male urethral swabs
- Urine - 15-20mls in a white or yellow capped universal.
- Dry Copan Self take vulvo-vaginal swabs (white tops) – these are acceptable but have a reduced sensitivity.

HOW DO I TAKE THE SPECIMEN?

Yellow topped swabs: After the swab has been taken, insert the swab into the tube and snap the shaft of the swab at the scored point. Please only send one swab to the laboratory - the other is intended only to clean the mucus off the cervix and should be discarded.

Instruction Guides (how to take samples):

How to take a self-take vaginal swab – Appendix D
How to take a endocervical swab – Appendix E
How to take a urine – Appendix F

Note: for eye swabs, if fluorescein eye drops are used, wash eye thoroughly prior to sample collection.

Urines: The patient should not have passed urine for at least an hour prior to collection. It is important to take first voided urine, NOT midstream.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

- Swabs: Send as soon as reasonably practicable.
- Urines and dry swabs: Must be sent to the lab within 24 hours and must be refrigerated during this time.

ANYTHING ELSE?

- Eye swabs will be processed however the test has not been validated for this sample type.
- Rectal swabs will be processed however the test has not been validated for this sample type.
- This is detected by PCR using automatic molecular technology. This test cannot be used to assess the therapeutic success or failure following antibiotic therapy, since DNA may persist for up to 6 weeks. Submitting a repeat sample to test if the patient is cured must be done 6 weeks after treatment has commenced.

WHAT WILL AFFECT THE RESULTS?

- If fluorescein eye drops are used, the eye must be thoroughly washed out prior to collection of the sample, or the test may be inhibited.
- False negatives - Bloodstained or very dark samples may give false negative results, these samples may be rejected.
- Inhibitory - Certain substances inhibit amplification and/or detection. Common inhibitory substances are: urinary protein, perfumed substances, fluorescein, excess amounts of blood, white cells and cervical mucus which has not been removed. This occurs in about 0.2% of samples and the sample needs to be retaken. However, it may be worthwhile attempting a different type of sample: if a cervical swab yielded an inhibitory result, submit a urine sample next time, and vice versa.
- Clots in the sample - the analyser will reject samples with clots and these will be repeated once by the laboratory.
- Urines: should be first void, not in boric acid, taken 1 hour after last passing urine, and placed in preservative within 24 hours.
- Female swabs: the best sample is a properly taken endo-cervical swab. Self-take vulvo- vaginal dry Copan swabs are acceptable but probably have a reduced sensitivity. No validation has been done on these samples.
- Non-genital sites: although this laboratory processes eye swabs in this way, the method is not validated for these sample types. A disclaimer stating this will be automatically added to the report.
- Every PCR method/specimen type has a limit of detection. Please contact the laboratory if this information is required.

7.3 CSF (Cerebrospinal Fluid)

WHAT DO I NEED?

- Sterile plastic universals – white topped (yellow topped if white not available)
- 3 (or 4 for SAH) consecutively taken samples NUMBERED 1, 2, and 3 (and 4)
- Samples 1 and 3 - send to microbiology for cell count and culture.
- Sample 2 - to Biochemistry for protein.
- Glucose - send sample 2 in a fluoride tube (grey-topped).
- If subarachnoid haemorrhage (SAH) is suspected, send sample 4 to Biochemistry and inform them. This must be protected from light (see Policy below). Samples 1 and 3 must still be sent to microbiology for a cell count.
- Cytospin (oncology patients) – sent to Microbiology for cell count, sample is then sent to Haematology Laboratory for cytospin if >5 white blood cells.
- If bacterial meningitis is suspected - take blood cultures as well
- If meningococcal disease suspected – take an EDTA blood sample (purple top) as well
- If Mycobacterium Tuberculosis (TB) or CJD is suspected discuss with Consultant Microbiologist

HOW DO I TAKE THE SPECIMEN?

See local policies

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

- The sample should be sent to the laboratory as soon as possible after being taken.
- Always inform the laboratory when a CSF sample has been taken and is on its way.
- Send the sample with a porter. Do NOT use the pneumatic air tube for CSFs.
- Out of Hours: The microbiology biomedical scientist must always be contacted through switchboard.

ANYTHING ELSE?

- Results: In all cases, significant results, cell counts, and Gram stains will be phoned as soon as they are available. This includes Referral Laboratory results; these can take up to a week.
- Cell count: Performed on all but clotted & PM samples. Differential count will be performed on raised WCC.
- Normal values: These values represent the upper and lower limits of normality. Bacterial or viral infection may still need to be considered where leucocyte counts are near the upper normal limits in neonates and young children.

Abnormalities associated with bacterial meningitis

- Reduced glucose concentration: <60% blood glucose (CSF: serum ratio <0.6)
- Elevated protein concentration
- Raised white blood cell (WBC) count: 10^1 - 10^4 predominantly polymorphs
- Elevated intracranial pressure

Culture: All samples – culture and Gram stain (except cytospin samples)

Meningitis/Encephalitis PCR Panel:
Irrespective of WBC count all infants up to 1 year have a PCR test
Bacterial and Viral PCR & WCC <5 - Tested after discussion with the microbiology consultant.
CSF Viral PCR requested and WCC >5 – Microbiology consultant to lead decision

CSF PCR targets: E.coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae, CMV, Enterovirus, HSV 1&2, HHV-6, Human parechovirus, VZV, Cryptococcus neoformans/gattii.

TB: Requires at least 2-3mls of CSF to optimise the chance of isolation or DNA detection.

CJD: If suspected discuss with the microbiology consultant prior to the collection of the specimen. If necessary, the laboratory will store the sample at -80°C until the Referral Centre in Edinburgh has been contacted.
Amoebae and Cryptococcus – Please discuss with Consultant Microbiologist.

WHAT WILL AFFECT THE RESULTS?

- Optimal time for specimen collection is prior to antimicrobial therapy.
- Patient is likely to have received β-Lactam antibiotics (in cases of suspected meningococcal meningitis/septicaemia) prior to the specimen being taken.
- Specimens should be transported and processed as soon as possible to minimise clot formation.
- CSF samples must NOT be sent via the Air-Tube System.
- Samples for xanthochromia must be protected from light.
- Time delay >2 hours from taking sample to processing can result in significant deterioration of cells. This can make it difficult or impossible to perform a differential white count. A time delay of >30 minutes can prejudice a xanthochromia result.
- Refrigeration prior to specimen processing is desirable.
- CSF may be received in inadequate volumes which may impede the recovery of organisms.
- CSF samples may be received unnumbered or incorrectly numbered.
- Evaluation and significance of findings assumes that adequate care and aseptic technique is taken during specimen collection, transport and microbiological processing so that detected organisms are from the source of infection and not contaminants.

7.4 Ear Swabs

WHAT DO I NEED?

- Special flexible thin wire swabs (orange topped).
- Charcoal swabs (black topped) are also acceptable but orange topped swabs are preferred.
- Sterile universals (or sterile yellow topped containers) can be used to transport fluid samples
- For investigation of fungal infection, scrapings of material from the ear canal are preferred (see skin, hair, and nail guide) although swabs can also be used.

HOW DO I TAKE THE SPECIMEN?

Swabs - Place into in the ear canal and rotate gently. Place the swab in the charcoal transport medium.
Fluids/skin scrapings – Aseptically transfer fluids to a sterile container or skin scrapings to a dermapack/sterile yellow topped pot as appropriate.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Store in the refrigerator until transported to the laboratory as soon as reasonably practical.

ANYTHING ELSE?

Please also see the ENT department page for further information

WHAT WILL AFFECT THE RESULTS?

- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted.
- An external ear swab is not useful in the investigation of otitis media unless there is perforation of the eardrum
- All specimens should be fresh and taken before antimicrobial treatment is started.
- It is preferable for swabs to be transported in Amies transport medium with charcoal.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed (beyond overnight).

- Specimens may be cultured up to 48 hours after collection; delays of over 48 hours are undesirable. But if not processed on the same day, care should be taken with the interpretation of results.
- Skin flora contamination of ear swabs specimens is common.

7.5 Eye Related samples, Swabs and Corneal Scrapes

WHAT DO I NEED?

Eye Swab
For bacteria – black charcoal swab
For viruses – green topped viral swab
Corneal Scrape Kit
Kits are available in the fridge in the pathology specimen reception area. They can be collected at any time when required.
Each kit contains: 4 culture plates, 1 enrichment broth, 1 glass slide, 1 viral swab.
Aqueous and Vitreous Humour - These specimens are usually received in the syringe.
Contact Lens - Send in its case (or sterile container) with the cleaning fluid (or sterile saline).

HOW DO I TAKE THE SPECIMEN?

Eye Swab: Gently open the lower eyelid to expose the conjunctival membrane. Rub the swab gently over the membrane, avoiding the cornea. Place the swab in the transport medium.

Corneal Scrape:

1. Inoculate the enrichment broth first.
    a. Use aseptic technique, remove the bottle cap, tilt the bottle until the liquid is just below the opening.
    b. Introduce the needle tip / scalpel blade into the edge of the fluid and agitate gently to remove the sample material into the fluid. Do not drop whole needle into the broth.
2. Inoculate plates:
    a. Open each plate only when ready to inoculate and immediately replace lid.
    b. Stroke the flat side of the needle / blade gently across the surface of each plate.
3. Finally, inoculate glass slide, pressing flat side to the glass.
    a. Air dry and label with patient's name on the same side as the smear.
4. If viruses are suspected use the green topped swab, using the technique described for 'Eye Swab'.
5. Label all plates and broth with patient's name.
6. Return all items to the box.
7. Complete a request form and place this inside the plastic bag and then into the box as well.
8. Contact the laboratory (or on call scientist via switchboard if out of hours) once the sample has been taken and is on its way.

Contact Lens: Send in its case (or sterile container) with the cleaning fluid (or sterile saline). Please explain to the patient that their lenses and case cannot be returned to them.

Acanthamoeba:
From a corneal scrape: Put the scalpel blade/needle into a plain universal containing sterile saline.
From a contact lens: send the lens in cleaning fluid.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Sent to Laboratory as soon as reasonably possible, refrigerate overnight if necessary.
For corneal scrape kits contact the laboratory (or on call scientist via switchboard if out of hours) once the sample has been taken and is on its way.

ANYTHING ELSE?

Contamination of eye swabs with normal skin flora is common and these organisms will only be followed up if clinically indicated e.g. post-surgery, foreign body.

WHAT WILL AFFECT THE RESULTS?

- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted.
- All specimens should be fresh and taken before antimicrobial treatment is started.
- It is preferable for swabs to be transported in Amies transport medium with charcoal.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed beyond overnight.
- Specimens may be cultured up to 48 hours after collection; delays of over 48 hours are undesirable. But if not processed on the same day, care should be taken with the interpretation of results.
- Skin flora contamination of the eye and associated specimens is common.

7.6 Faeces

WHAT DO I NEED?

Stool - Use sterile faeces pots (white, brown or blue)
Threadworm – Perianal dry swab in sterile universal
Whole/Part worm – Sterile Universal
Antibiotic Resistance Screening (VRE and CPE) -
Rectal Swab using special red topped dual swabs available from Microbiology department.

HOW DO I TAKE THE SPECIMEN?

Stool: submit a 5g portion of faeces (about 2-3 teaspoonful's).
See Patient guide, Appendix C

Amoebae: Where a patient has visited undeveloped countries and amoeba are suspected a 'hot' stool is required. Stool must reach laboratory within 30 minutes of being passed. Notify the laboratory that such a sample is on its way.

Enterobius vermicularis (threadworm/pinworm): Perianal specimens are best obtained in the morning before bathing or defecation. Three specimens should be taken on consecutive days before pinworm infection is ruled out.
Cotton-wool swab in white topped sterile universal container should be used for collection. Spread buttocks apart, and rub the moistened cotton-wool swab over the area around the anus, but do not insert into the anus. Place swab into a white-topped sterile universal container, trimming the length of the swab if necessary. Occasionally, an adult worm may be collected from a patient and sent in saline or water for identification.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Send to laboratory as soon as reasonably possible. Refrigerate until transportation.
Hot stools for amoeba must be received within 30 minutes of being passed. Notify the laboratory that such a sample is on its way.

ANYTHING ELSE?

Examinations are performed based on clinical information and patient demographics. Please provide as many relevant clinical details as possible.

Examinations

- Immunocompetent hospitalised patients >3 days only have C. difficile testing on loose or liquid stools.
- All other patients are tested for Salmonella, Shigella (ipaH gene) and verotoxin (E.coli 0157), Campylobacter, Giardia and Cryptosporidium by PCR (see below). N.B. Verotoxin detection on bloody samples are sent to the reference laboratory for investigations.
- In addition to the above, other examinations can be performed based on clinical details including - parasitology, Vibrio spp (including Aeromonas and Plesiomonas spp) culture and Yersinia.

Key clinical details

- Travel outside Europe or N. America (specify which countries visited)
- Worms seen in stool
- Eosinophilia
- Previous or suspected parasites
- Rice water stool
- Contact with non-tap water
- Mesenteric adenitis, terminal ileitis, pseudo-appendicitis, reactive arthritis
- Shellfish consumption

Toxigenic Clostridium difficile testing

Ideal samples are those that are taken and tested within 24 hours.

Additionally to the above, stools of Bristol Stool Chart 5 to 7 will be tested for the presence of toxigenic C.difficile by PCR from:

- Hospitalised patients >2 years
- Any patient >65 years
- Patients on antibiotics
- Immunocompromised patients (e.g. from Turner ward, RGDC, on chemotherapy, post-transplant, monoclonal antibody therapy, HIV).

We do not test specimens that:

- Have been tested in the previous 7 days (unless the patient deteriorates and/or C. difficile associated diarrhoea is strongly suspected – please discuss with microbiologist).
- Have tested toxin positive in the last 28 days (unless the patient has clinically relapsed). The test can remain positive, even in asymptomatic patients, making interpretation difficult.
- Are formed, unless discussed with laboratory or ?parasites.

Viruses

- Norovirus testing will only be carried out during an outbreak, under guidance from the Infection Control department, or if norovirus is suspected on admission.
- Helicobacter pylori antigens can be detected in faeces samples. Please read the limitations.

WHAT WILL AFFECT THE RESULTS?

Molecular testing

- The detection of a virus is dependent on specimen collection, handling, transportation, storage and preparation. Failure to follow procedures may lead to incorrect results.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of C.difficile tcdB gene variants, resulting in false negative results.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown norovirus variants resulting in a false negative result.
- With norovirus assay, the presence of Benzalkonium (≥ 0.2% w/v) and Bismuth (5% w/v) may affect results.
- Bloody samples for verotoxin may be sent to the reference laboratory for investigations.

Time between sample collection and processing

- Specimens of faeces should be transported to the laboratory and processed as soon as possible - Shigella species may not survive and C.difficile toxin may deteriorate, even on refrigeration.

Multiple samples

- Faecal pathogens and parasites are shed sporadically thus the chance of isolating a pathogen is greatly increased if more than one sample is sent. Generally, three samples are recommended (taken over 3 different days) for each bout of diarrhoea.
- In the case of a food handler further stool samples may be sent once symptoms subside to check for pathogen clearance – usually require 3 x negative samples. N.B. it may take up to 6 months for clearance.

Negative results

- Because bacteria and parasite are shed sporadically, a negative culture or parasite result does not mean that infection is not present.

Parasites

- Liquid samples do not concentrate well, small parasites and cysts do not concentrate well.
- Parasites are shed sporadically, and for this reason patients are often advised to submit three separate stools, taken on different days.
- Amoeba are sensitive to temperature, therefore must reach lab within 30 minutes.

H.pylori antigen

- False negative results may be obtained if the patient has been taking antibiotics, proton pump inhibitors (PPI's) or bismuth preparations. Stool samples should be sent 4 weeks after cessation of antibiotics, and 2 weeks after termination of ingestion of PPI's or bismuth.
- Epidemiological studies have shown that infection with H.pylori is prevalent throughout the world, but prevalence rates vary with age and ethnic background. e.g. in the USA, the prevalence increases with age at approx 1% per year. In Europe and USA, 25-50% of the population carries H.pylori, but prevalence rates for Asia, Africa and South America have been reported as 70-90%.

Quick Reference Guide to PCR Results

Salmonella detected: The target gene detected is specific for Salmonella enterica spp, positive PCR indicates the presence of salmonella species. Chronic carriage of very low numbers of organism can occur so PCR may remain positive in this setting. Culture will be attempted on all PCR positive samples; occasionally, due to low bacterial numbers, the organism may not grow and therefore we will not be able to confirm or speciate the organism.

Shigella (ipaH gene) detected: The gene target is known as ipaH. This is present on Shigella species as well as certain Enteroinvasive E.coli species (EIEC), which causes similar symptoms as Shigellosis. Chronic carriage does not usually occur; therefore a positive PCR is highly suggestive of Shigella enteritis or EIEC. All positive samples will be cultured for Shigella. We are unable to identify EIEC's by culture.

Campylobacter detected: The target gene is present in Campylobacter jejuni, coli and lari species. Chronic carriage does not usually occur. Positive samples will not be routinely cultured.

Verotoxin (incl. E.coli O157) detected: A positive result indicates the presence of the stx1 and/or stx2 gene target. These genes are responsible for causing haemolytic-uraemic syndrome (HUS) and are found in E.coli species such as O157. The same genes are also present in Shigella dysenteriae and other non- O157 E.coli. A positive test indicates the presence of a gene capable of causing HUS but is not diagnostic of the syndrome itself. Positive samples will be cultured for O157.

Cryptosporidium detected: A positive result indicates the presence of the target gene specific for C. parvum and/or hominis in the sample.

Giardia lamblia detected: A positive result for Giardia indicates the presence of the target gene specific for G. lamblia in the sample.

7.7 Genito-Urinary Specimens

Genito-urinary samples include:
High vaginal swab (HVS), low vaginal swab (LVS), vulval swab, labial swab, cervical swab, endocervical swab, penile swab, urethral swab, screening swabs for N. gonorrhoeae, aspirates from Bartholin's gland, fallopian tube, tubo-ovarian abscess, pouch of Douglas fluid, intra-uterine contraceptive device (IUCD), and products of conception.
Ano-vaginal swabs for GBS screening.

WHAT DO I NEED?

- Female swabs - standard charcoal swabs.
- Urethral swabs - thin orange topped swabs.
- IUCD (Intrauterine contraceptive device) - use plain sterile universal containers (white or yellow topped).
- For Chlamydia – See Chlamydia Guide.
- For Viruses – green topped viral swab.

HOW DO I TAKE THE SPECIMEN?

Collect specimens before antimicrobial therapy where possible.
Always provide relevant clinical details, as these will influence the processing of the sample.

Neisseria gonorrhoeae (females) and pelvic infection: Take a cervical swab. HVS is suboptimal for recovery of this organism.
For Trichomonas, the posterior fornix, including any obvious candidal plaques should be swabbed.
High vaginal swabs - After the introduction of the speculum, the swab should be rolled firmly over the surface of the vaginal vault. Avoid vulval contamination.
Cervical swabs - After introduction of the speculum to the vagina, the swab should be rotated inside the endocervix. Avoid vulval contamination.
Urethral swabs – Avoid contamination from the vulva or the foreskin. The patient should not have passed urine for at least one hour. For males, if a discharge is not apparent, attempts should be made to "milk" exudate from the penis. The swab is gently passed through the urethral meatus and rotated.
Intrauterine contraceptive devices (IUCDs) - The entire device should be sent.
Fluids and pus - These are taken from the fallopian tubes, tubo-ovarian, and Bartholin's abscesses etc. during surgery.
Group B Streptococcus screening – performed on vagino-rectal swabs at 35-37 weeks in patients with previous GBS in pregnancy. EDD or gestation dates MUST be included in the

clinical details. Sampling involves swabbing the distal vagina (vaginal introitus), followed by the rectum. A single swab for both sites of collection is rational, but two different swabs can be used. Because lower vaginal as opposed to cervical cultures are recommended, cultures should not be collected by speculum examination.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Place all swabs into transport medium and send samples straight to laboratory. Any delay, store the sample in the fridge.

ANYTHING ELSE?

Standard screen includes (age and gender appropriate):

- Culture for yeasts, Staphylococcus aureus, and significant Beta haemolytic streptococci
- Cervical and Urethral will additionally have culture for *Neisseria gonorrhoeae*.

Other samples based on clinical details could have:

- Full culture including long term anaerobes
- Culture for *Neisseria gonorrhoeae*
- Extended incubation for Actinomyces spp.
- Culture for Listeria spp.
- Microscopy for Bacterial vaginosis and *Trichomonas vaginalis* will only be performed on specific request (please include relevant clinical details)

WHAT WILL AFFECT THE RESULTS?

- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted (e.g. only an HVS for ?STIs, when a cervical swab should be submitted for *N.gonorrhoeae* investigation).
- All specimens should be fresh and taken before antimicrobial treatment is started.
- It is preferable for swabs to be transported in Amies transport medium with charcoal.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed, specimens may be cultured up to 72 hours after collection, delays of over 72 hours are undesirable.
- Faecal contamination is common, especially in children or post-menopausal women
- S.aureus is part of normal perineal flora, and the significance of growth can be difficult to interpret in vulval swabs
- Vaginal swabs are unlikely to yield the same microbes as those on the IUCD.
- Vaginal contamination of the IUCD is common.

7.8 Screening – MRSA / MSSA

WHAT DO I NEED?

For an MRSA screen, send the following samples using charcoal transport swabs:

- Nose (one swab for both nostrils)
- If MSSA screen required – take separate Nose swab
- Throat (not neonates)
- Groin
- Lesions or sites of abnormal skin including ulceration, eczema, pressure areas (even if the skin is intact), umbilicus (in neonates), site of indwelling vascular cannula, suprapubic catheter, tracheotomy, PEG sites, and other manipulated sites.
- Sputum if a productive cough, urine if catheterised, vaginal discharge if present.

For URGENT MRSA swabs (Pre-assessment implant surgery, urgent admissions to Trauma & Orthopaedics, or specific request by Microbiology Consultants) use the red-topped COPAN Dual Rayon Swab. These can be obtained on request from the Microbiology Department x254990

HOW DO I TAKE THE SPECIMEN?

Swabs used to sample skin sites should first be moistened with sterile saline and then rubbed over the sample site (NOT necessary for nose and throat). Place all swabs in transport medium and send to the microbiology laboratory.

If the patient shows signs of infection, relevant samples should be submitted for routine examination, referring to the possibility of MRSA infection on the request form.

MSSA/SAS (Methicillin Sensitive Staph Aureus/Staph Aureus Screen) - All patients with implant surgery have an MRSA screen as above. If an MSSA screen is also required a separate nose swab should be taken.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

If there is a delay in getting samples to the laboratory, store the samples in the refrigerator.

ANYTHING ELSE?

WHAT WILL AFFECT THE RESULTS?

- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted.

- All specimens should be fresh and taken before antimicrobial treatment is started.
- It is preferable for swabs to be transported in Amies transport medium with charcoal.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed.
- Inoculation of MRSA agar before SAS agar can result in MSSA growth being inhibited – risk of this happening is increased when both MRSA and SAS are requested but no separate nose swab is received.

7.9 Mycobacterium

For Sputum, Bronchial washings, lavage and pleural fluids follow Sputum Guide.

WHAT DO I NEED?

- **Fluids, pus, tissues, biopsies, CSF** - Use sterile yellow topped pots or white topped universal containers as deemed appropriate to the size or volume of specimen. N.B. Pleural fluid and Pericardial fluid are sub-optimal and may not rule out infection – Concurrent pleural or pericardial biopsy taken with the fluid is more useful.

- **Sputum and bronchial washings** – For mycobacterial investigations on sputum three early morning samples should be taken on three consecutive days. Sputum and respiratory washing samples should be submitted in sterile universal containers (white or yellow topped). Saliva or nasal discharge samples are not suitable for TB investigation and will be rejected. Small sputum samples <5ml are sub-optimal and may not rule out TB infection.

- **Bone marrow and Blood** – 2x citrated blood tubes (blue topped)

- **Urine** - 3 large volume containers using the 'TB Urine Pack' - available from Microbiology department on specific request. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative. **NB Boric acid is toxic to mycobacteria so red topped boric acid universal containers should not be used for this investigation.**

- **TSpot** – 2 x Lithium heparin tubes (green topped) (must be received in the Microbiology lab before 2pm Monday to Friday. Specimens for T-spot should not be submitted over the weekend).

HOW DO I TAKE THE SPECIMEN?

NB: For all suspected TB samples, do NOT send these in the air tube. Please mark clearly on the request form that they are High Risk samples.

For Sputum, Bronchial washings, lavage and pleural fluids follow Sputum Guide.

- **Bone marrow & Blood:** Collect these samples according to local protocols.
- **Tissue and biopsies:** Collect these according to local protocols. If the sample is very small, keep it moist by adding a small amount (1ml) of sterile saline to prevent drying out. Never add formalin to these samples.
- **Pus:** Aspirate pus or fluid with a sterile syringe.
-

- **Swabs:** Swabs are not suitable for TB culture, due to the paucity of bacteria available. If there is a special reason why a swab should be cultured for TB, please call the consultant microbiologist to discuss.
- **CSF:** Please consult the CSF guide.
- **Tuberculosis urine:** Using the TB urine pack (3 large universals) take 3 consecutive early morning urine samples. As boric acid is toxic to mycobacteria any urine samples received in red topped boric acid universal containers requesting mycobacterial investigations will be rejected.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

If there is a delay in all but blood/bone marrow samples to the laboratory, store them in the refrigerator.

T-spots must be received in the laboratory by 2pm Monday to Friday.

ANYTHING ELSE?

Microscopy: Performed on all samples (except urines) using a fluorescent stain for acid and alcohol fast bacilli (AAFB).
Culture: Performed on all samples, incubated at 37ºC (for mesophilic *Mycobacterium* spp incl *M.tuberculosis*. If cutaneous Mycobacteria (e.g. *M.marinum*) are suspected, culture at 30ºC will also be performed. This additional culture will be performed on all skin biopsies.
MTB PCR: A consultant led molecular test is available when a result is needed urgently. MTB PCR can be used to detect M. tuberculosis complex DNA, and to detect Rifampicin resistance.
TSpot: Blood test for difficult to diagnose TB infections, such as latent disease. The T-SPOT TB test is an *in vitro* diagnostic assay that measures T cells primed to *Mycobacterium tuberculosis* (MTB) antigens. The test was developed for diagnosing both latent TB infection and TB disease in humans. Please discuss this test with a consultant microbiologist, or the TB nurse (ext 55117).
Liquid culture: Bone marrow and blood samples are referred for liquid culture.
Urine: Culture of three total early morning urines establishes the diagnosis in 80%-90% of cases. This percentage is drastically reduced when smaller volume samples are submitted.
Mycobacterium chimaera: Infections associated with cardiopulmonary bypass must be discussed with a Microbiology Consultant prior to requesting. Samples require specialist collection equipment and protocols.

WHAT WILL AFFECT THE RESULTS?

- The quality and volume of the sample will affect the isolation rate. Some samples, particularly CSF have a very low load of organisms, and large volume samples are required.
- MTB PCR: The presence of inhibitory substances in body products makes this a less sensitive test than culture.
- MTB PCR: As this test detects DNA, patients on treatment could have a positive result, even though the bacteria are no longer viable.
- Microscopy: This will detect all acid fast organisms present. This does not mean they are always viable, and in cases where patients are undergoing treatment these may be non-viable.
- All specimens should be fresh.
- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted (eg. Inadequate tissue samples or CSF, Sputum <5ml volume.)
- If less than 40 ml of urine is processed it will greatly reduce the recovery rate.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in bacterial overgrowth.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed.
- Samples are decontaminated, in order to eliminate contaminating bacteria, before being cultured for TB.
    - The decontamination step is inevitably somewhat toxic to mycobacteria but processes are in place to minimise loss of mycobacteria while simultaneously maximising the elimination of as many other microorganisms as possible.
    - Occasionally the decontamination process will fail, and the culture will be overgrown with contaminants - in this case you will be asked to repeat the specimen.

7.10 Nose Swabs

Nasal swabs: To investigate carriage of S. aureus and Group A streptococci in patients with repeated skin infections, or boils. NB Simple nose swabs are not suitable specimens for the investigation of acute or chronic and fungal sinusitis - See NICE management guidelines. Laboratory investigations into the aetiology of sinusitis are only instigated under ENT specialist request with antral washout or sinus aspirate being the required specimen types.

For SAS screening (Staph aureus in patient screening/PVL screening) see wounds guide.

Nasopharyngeal / nose and throat swabs: To diagnose whooping cough. Swabs will be investigated for Bordetella pertussis and Bordetella parapertussis.

WHAT DO I NEED?

Nasal swab - Charcoal Trans Swab (black topped)
Nasopharyngeal / nose and throat swabs (for investigation for Bordetella pertussis/parapertussis) – Virocult viral transport medium (green topped VTM)
Take the throat swab first and use the same swab to sample from the nose.

HOW DO I TAKE THE SPECIMEN?

Nasal swabs: Swab the anterior nares by gently rotating the swab over the mucosal surface.
Throat swabs: Quickly but gently rub the swab along the back of the throat, behind the uvula (posterior pharynx) and over the tonsillar areas on both sides of the throat. Remove the swab gently without touching the teeth, gums, or tongue.

Nasopharyngeal swabs: Insert the dry swab through one nostril straight back (NOT upwards), along the floor of the nasal passage until you reach the posterior wall of the nasopharynx. Rotate the swab gently then leave in place a few seconds. Carefully remove the swab without touching the sides of the nostril.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

If there is any delay in transporting the swab to the laboratory, store the swabs in the refrigerator.

ANYTHING ELSE?

Swabs for B.pertussis:

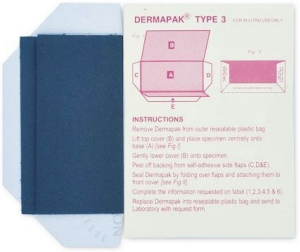
- Include duration of symptoms in clinical details to aid the laboratory to perform appropriate testing.

- Laboratory confirmation of clinically suspected cases at Torbay Hospital can be made by PCR or serological tests (which usually only provide a late or retrospective diagnosis).
- Culture is conventionally performed to confirm infection with B. pertussis and B. parapertussis. The method is highly specific but sensitivity is low 20-40%. Culture is also more likely to be unsuccessful the longer the time since the onset of illness, which is why we have moved to PCR methods for diagnosing infection. PCR is more sensitive than culture as it does not require organisms to be viable. Serology is particularly useful in diagnosing infection in patients who have been coughing for four weeks, when PCR would be anticipated to be unhelpful.

WHAT WILL AFFECT THE RESULTS?

- Nasal carriage of S.aureus is intermittent.
- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted.
- All specimens should be fresh and taken before antimicrobial treatment is started.
- It is preferable for swabs for culture to be transported in Amies transport medium with charcoal.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed.
- Specimens may be cultured up to 48 hours after collection; delays of over 48 hours are undesirable. But if not processed on the same day, care should be taken with the interpretation of results.
- A green-topped VTM swab is the preferred swab type for the investigation of Bordetella spp. Other swab types received requesting Bordetella spp investigation may be rejected by the laboratory.
- PCR is less likely to be positive in patients with symptom duration of more than 4 weeks.
- Serology may be helpful to confirm the diagnosis of whooping cough in patients with cough duration of more than 2 to 3 weeks, when PCR is unlikely to yield positive results.

7.11 Skin, Hair and Nails



WHAT DO I NEED?

For all samples - use special Dermapaks (flat black card packs)
Nails in sterile universal containers (yellow or white topped) will be accepted.

HOW DO I TAKE THE SPECIMEN?

Skin Scrapings: Using a scalpel blade, gently shave off material from the active edges of the lesion.
Nails: Nail clippings should include the full thickness of the nail and should be clipped as far back from the edge of the nail as possible.
Hair: Hairs should be plucked from the affected areas as close to the root as possible. If there are associated scalp lesions, include skin scrapings from these with the sample.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Samples must be kept at room temperature awaiting transport to the laboratory.

ANYTHING ELSE?

Dermatophytes - Infection is cutaneous and generally restricted to the non-living cornified layers in patients who are immunocompetent as they are generally unable to penetrate tissues which are not fully keratinised. Classification is divided into 3 genera: Epidermophyton sp., Microsporum sp., and Trichophyton sp.

Non-dermatophytes - can infect nails damaged by physical trauma, disease, or pre-existing infection with a dermatophyte. These will only be reported in accordance with relevant clinical details.

Yeasts - have been implicated as a significant cause of nail infections.

WHAT WILL AFFECT THE RESULTS?

- If the patient has already started anti-fungal treatment, the fungi may be too damaged to grow on culture media.
- Collection - Specimens should ideally be collected in Dermapaks and of sufficient quantity.
- Transport - Samples should be transported to the laboratory as soon as possible. If there is a delay in transport specimens should be kept dry and at room temperature.
- Test selection - multiple samples – it is important to examine material from each piece received in Dermapak.
- It is important that an adequate portion of sample is selected for microscopy and for culture to ensure optimal results.

- Owing to the random distribution of fungal hyphae throughout the skin in dermatophyte infections a negative microscopy can be associated with a positive culture and vice versa.
- Hair samples taken far away from the scalp are unlikely to yield useful information – the sample should be taken as near to the root as possible.

7.12 Sputum and other Respiratory Samples

Bronchial aspirates, bronchoalveolar lavage, transthoracic aspirate, transtracheal aspirate, bronchial brushings, bronchial washings, endotracheal tube specimen, pleural fluids, sputum expectorated, cough swabs.

WHAT DO I NEED?

Sterile universal pot (white or yellow topped).
Cough swabs – black topped charcoal swab

HOW DO I TAKE THE SPECIMEN?

Record clinical details, in particular: cystic fibrosis, immunocompromised, bronchiectasis, ?atypical pneumonia, aspiration pneumonia.

Take fresh sample, before antimicrobial therapy where possible.

Sputum: Expectorated sputum by coughing, not saliva, is required. If the patient has difficulty producing sputum, ask a physiotherapist to assist in obtaining a sample. Do not collect shortly after eating or drinking.

Bronchial washings, lavage, and **pleural fluids**: Collect these specimens according to local protocols. Submit samples in a sterile universal.

Mycobacterium species - Early-morning, freshly expectorated sputum collected on at least 3 consecutive days. See Mycobacteria Guide for further information.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Transport to the laboratory as soon as possible. If there is an unavoidable delay, it is preferable to refrigerate the sample.

ANYTHING ELSE?

Culture

- **Sputum** – Routine pathogens only. Further investigations are clinical detail lead.
- **Pleural fluids** – Any common pathogen, further investigations are clinical detail lead. Mycobacterial culture must be requested if required.
- **Bronchial washings or lavage** - Cultured for routine pathogens and TB. Further investigations are clinical detail lead.
- **Cough swabs** and **Cystic fibrosis samples** – Cough swabs should be submitted from children with cystic fibrosis and primary ciliary dyskinesia (PCD). Cultured for routine pathogens and non-routine pathogens in line with current UK Cystic Fibrosis Trust

guidelines. In addition, culture for TB and non-tuberculous mycobacteria will be carried out at Annual Review (this needs to be indicated on the request form).

Report of the UK Cystic Fibrosis Trust : Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis (2022) Consensus documents (cysticfibrosis.org.uk)

WHAT WILL AFFECT THE RESULTS?

- Oral contamination of sputum and occasionally of Bronchial samples can lead to overgrowth of pathogens by oral flora.
- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted (eg. salivary or mucosalivary specimens).
- All specimens should be fresh and taken before antimicrobial treatment is started.
- Prior administration of antibiotics can lead to overgrowth of unusual organisms.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Sputum may be refrigerated for up to 2-3 hours without appreciable loss of pathogens. Any further delay may allow overgrowth of Gram negative bacilli and Haemophilus species, and S. pneumoniae may be rendered non-viable.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed.
- Specimens may be cultured up to 48 hours after collection, delays of over 48 hours are undesirable. But if not processed on the same day, care should be taken with the interpretation of results.
- Upper respiratory tract flora contamination of the sputum and associated specimens is common.
- A “no growth” result is inappropriate for routine sputum specimens due to the dilution step in culturing sputum.

7.13 Throat, Mouth and Tongue

WHAT DO I NEED?

All sites use a charcoal trans swab (black topped).
Virology – Green topped viral swab. (HSV or respiratory pathogens only as clinically indicated).

HOW DO I TAKE THE SPECIMEN?

Throat swabs should be taken from the tonsillar area and/or posterior pharynx, and any areas of ulceration, exudation or membrane formation, avoiding the tongue and uvula with the aid of a good light and tongue depressor.
Throat culture should not be taken if the epiglottis is inflamed as sampling may cause serious respiratory obstruction.
Mouth and tongue swabs – Swab area as required.
Virology - Take the swab in the same way as other throat swabs.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

The swab should be stored in the fridge if transport to the laboratory is delayed.

ANYTHING ELSE?

- Throat swabs - screened for significant Beta-haemolytic streptococci. Other organisms will only be investigated if the following clinical details are provided:
-

Clinical Data provided	Target Organism
Immunocompromised, or on antibiotics	Yeasts
Epiglottitis	Haemophilus influenzae type B
Persistent sore throat	*Fusobacterium necrophorum*
Vincent's angina	Vincent's organisms
Membranous pharyngitis, overseas travel in last 10 days	C. diphtheriae
Raw milk consumption, works with farm animals	*C.ulcerans*
Quinsy, or any abscess	*S.aureus*, Anaerobes
Invasive meningococcal disease, non-blanching rash	*N.meningitidis*
If specifically requested, or as part of a GU screen	*N.gonorrhoeae*
Rash, recurrent tonsillitis, treatment failure	*Arcanobacterium haemolyticum*

- Tongue and mouth swab – Screened for yeast organisms. S. aureus and significant Streptococci will be investigated with certain clinical details.

WHAT WILL AFFECT THE RESULTS?

- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted.
- All specimens should be fresh and taken before antimicrobial treatment is started.

- It is preferable for swabs to be transported in Amies transport medium with charcoal.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed.
- Specimens may be cultured up to 48 hours after collection; delays of over 48 hours are undesirable. But if not processed on the same day, care should be taken with the interpretation of results.
- Oral flora contamination is common.
- Yeasts are normal flora in the mouth - a light growth of yeasts can be difficult to interpret.
- *Fusobacterium necrophorum* is a strictly anaerobic organism and as such is very oxygen sensitive. Although the transport medium contained within a transwab tube will maintain viability of anaerobes over short periods any delay in processing will affect their recovery.
- Swabs of dental abscesses may be contaminated with superficial commensal flora and only useful if taken from a disinfected site. Aspiration of dental abscess may be taken to assist in identification of the causative organism(s).

7.14 Urine

For TB – See Mycobacterium Guide
For Chlamydia – See Chlamydia Guide

WHAT DO I NEED?

- **Urine for culture** - Specimen pot containing boric acid crystal (red topped).
- **Very small volume samples (<5ml) for culture** - Universal containers without boric acid (white topped).
- **Legionella and Pneumococcal Antigens** - either container.
- **Schistosomes** - Special "Schistosome Urine Pack" (large silver topped container available from pathology reception).
- **Urine for congenital CMV** - Universal containers without boric acid (white topped). 2 samples within first 3 weeks of life.

HOW DO I TAKE THE SPECIMEN?

Collection of the sample is critical for mid-stream samples. Please instruct patient to clean round the urethra as thoroughly as possible and only submit the mid-stream portion of urine.
Instructions for the patient can be found in the patient user guide, Appendix C.

Boric acid container - Fill to the line indicated on the container label (approx 25 ml of urine), securely fasten the cap, and gently invert the container three times to help the boric acid crystals dissolve.
Non-boric acid containers – fill with no more than 5ml of urine.
Nephrostomy urine samples, Supra Pubic Aspirated urine, Ureteric urine Samples, Cystoscopy urine Samples, Ileal conduit / Urostomy urine samples and Prostatic Massage specimens – follow local protocol.

Specimens showing obvious signs of contamination, e.g. with faecal material, are unsuitable for culture and will not be processed.

Schistosomes
The optimal urine sample for recovery of Schistosoma ova needs to be taken:

- After light exercise (e.g. walking around at a normal pace for 10 minutes)
- Between 10 am and 2 pm
- Only the last part of the urine is required (terminal urine)

Special large volume containers with full instructions for the patient are available from pathology's Central Reception Office on request.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Boric acid pots - Deliver to the laboratory as soon as possible. If there is to be a delay it is not necessary to refrigerate the specimen.
Non–boric acid pots - Deliver to the laboratory as soon as possible, refrigerate if delayed.

Urine for CMV: Must be received in the laboratory before 3pm on the day of taking the sample and not to be taken on a Saturday/Sunday

ANYTHING ELSE?

Clinical Details – Please provide.
Please also state test required, samples may be rejected if it is unclear what the sample is for.

Microscopy
In accordance with the latest guidelines microscopy is deemed no more sensitive than a dipstick test and is only helpful in certain cases. Accordingly, this laboratory performs microscopy on selected patients only i.e. children under 3, diagnosis of pyelonephritis with associated symptoms, specially taken urine or on special request.

Laboratory microscopy for red cells is less accurate than dipstick due to red cell lysis in transport.

Culture and sensitivity
All samples receive a culture.

- 10,000 cfu /mL is considered a significant growth, all growths above this level are reported with a sensitivity.
- In specially taken urines (Prostatic massage, SPA and ureteric urines) any growth is deemed significant.
- Usually, only pure growths are reported.
- Asymptomatic bacteriuria in the elderly is common, and should not be treated, as it does not reduce mortality or prevent symptomatic episodes.

WHAT WILL AFFECT THE RESULTS?

- Contaminating bacteria from the external genitalia may give rise to misleading results.
- Bag urines from infants are frequently contaminated with skin flora or faecal flora.
- If antibiotic therapy has commenced prior to the taking of the specimen, bacteria may fail to grow.
- Boric acid inhibits fastidious organisms.
- Non boric acid urines which have not been stored in the fridge will overgrow, making colony counts unreliable.
- Asymptomatic bacteriuria in the elderly is common, and should not be treated, as it does not reduce mortality or prevent symptomatic episodes.
- Indwelling catheters inevitably result in bacteriuria due to biofilms. Most catheter results will not be released with an antibiotic sensitivity, unless there is an indication in the clinical details of systemic illness (e.g. fevers, rigors).
- Catheter samples should never be sent from urine decanted directly from the bag – urine should be sampled through the port only.

7.15 Serology – Blood Samples

WHAT DO I NEED?

Blood tubes:
Clotted (Gold top): Most samples.
EDTA (Purple or Pink top): HIV Viral Load. CMV, HSV, Adenovirus, HHV8 PCR.
Heparin (Green): TSpots (must arrive in the laboratory by 2pm on the day they are taken)

NB. The same volume of sample per test is required for most paediatric samples. Please send sufficient samples to cover the number of tests requested.

HOW DO I TAKE THE SPECIMEN?

Follow local protocol for taking blood samples.

Please provide on forms - **Full clinical details including onset of illness**

Serology (antibodies and antigens) - usually, 5-10 ml of clotted blood should be sent. If multiple tests are requested, 2 tubes should be sent.

Molecular serology (viral loads and PCR):

Test	Sample	Special conditions
HIV viral load **HIV resistance** **HBV viral load**	EDTA blood	To reach the lab ASAP. Refrigerate if delays are unavoidable.
HCV PCR **HCV Viral Load** **HCV genotype**	Clotted blood (or EDTA).	To reach the lab ASAP. Refrigerate if delays are unavoidable.
CMV, EBV, AdV, HSV PCR EDTA blood	EDTA blood	To reach the lab ASAP. Refrigerate if delays are unavoidable.

T-SPOT – This test is performed by Oxford Diagnostics
Test needs *two* heparin tubes (lithium or sodium heparin – green topped). Can be one tube for children under 2 years. Transport to microbiology laboratory immediately. Samples must arrive in the laboratory by 2pm on the day they are taken and cannot be received on a Saturday or Sunday.
Required samples volumes:

- Adults & children >10yo: 6ml
- Children >2-<10yo: 4ml
- Children <2yo: 2ml

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

See above.

ANYTHING ELSE?

Turnaround times

Results which require immediate action will be conveyed by telephone. Any test which has been arranged as an urgent test with the microbiology consultants will also be prioritised.

Routine tests performed on site will be reported within 72 hours.

Work sent to reference laboratories will be subject to delays. Sometimes these can be as long as 2 weeks, as they often only perform expensive tests in batches. However, in the Reference Laboratory section of this user guide contact details are provided if you wish to contact the Ref Lab directly for a speedy result.

Add-on tests

We are unable to accept requests for add-on tests to blood samples which have been sent to other departments in pathology. There are a few reasons for this.

- Insufficient sample – there may be insufficient sample remaining for the test requested.
- Insufficient sample for follow up – there may be sufficient sample for the test requested, but if this requires further work based on the result of the add-on test, there may then be insufficient sample remaining.
- False positive results – although rare, when testing small samples of saved serum, this can generate false positive results for blood-borne virus assays.

For these reasons, we ask that, where possible, a new sample be sent for any additional tests required. If this is not possible, please discuss with a Consultant Microbiologist.

WHAT WILL AFFECT THE RESULTS?

- As diagnosis often depends on the demonstration of a rise in titre it is best if paired sets are sent, one in acute stages and one approximately ten days later in the convalescent stage.
- Haemolysed and lipaemic blood samples may give inaccurate results.
- Bloods for viral loads should be received in the laboratory as soon as possible.
- False positive IgM results are common with any viral tests.
- IgG antibodies can take several weeks to appear and retesting may be appropriate.
- The detection of a virus is dependent on specimen collection, handling, transportation, storage and preparation. Failure to follow procedures may lead to incorrect results.
- Lack of clinical /relevant details on request forms may mean the correct tests are not performed.
- Illegible writing on forms may mean the correct tests are not performed.
- For specific tests, please telephone the laboratory.

7.16 Virology – Non- Blood Samples

WHAT DO I NEED?

Viral Swab – green or red topped swab **(CHECK EXPIRY DATE)**
Nasopharyngeal aspirate – in a sterile container (white topped).
Urine CMV PCR in neonates - send a freshly taken urine sample in a sterile universal container (white or yellow topped). Two samples are required for neonatal CMV testing.

HOW DO I TAKE THE SPECIMEN?

Eye - **For HSV & VZV & Adenovirus**– See Eye guide
Skin Swab - For HSV & VZV – Select a recently developed vesicle, and using a scalpel or needle, gently remove crust. Using the green topped virology swabs, rub the swab into the vesicle fluid. Place the swab into the tube, snap the swab at the breakpoint, discard the residual shaft) and tighten the cap.
Genital- **For HSV & VZV** – See Genito-urinary Guide

Viral Respiratory swabs – (take both nose and throat as described below)

Nasal swab (collected from the nasal septum, not just the anterior nares)

1. Stand at the side of the patient; ensure the patient's head is resting against the wall, Place your hand on the patient's forehead and the thumb at the tip of the nose.
2. Insert the swab into the closest nostril horizontally, approximately 2–3 cm
3. Place sideways pressure on the swab in order to collect cells from the midline nasal septum
4. Rotate the swab twice (2 × 360° turns) collecting the epithelial cells (not mucous)
5. Place swab into the labelled tube snap the swab at the breakpoint, discard the residual shaft.

Throat swab

1. Stand at the side of the patient, ensure the patient's head is resting against the wall, place your hand on the patient's forehead (non-dominant hand), ask the patient to open their mouth widely and say 'argh'.
2. Insert the swab into mouth avoiding any saliva
3. Place sideways pressure on the swab in order to collect cells from the tonsillar fossa at the side of the pharynx.
4. Rotate the swab twice (2 × 360° turns) collecting the epithelial cells (not mucous)
5. Place swab into the same tube as the nose, snap the swab at the breakpoint, discard the residual shaft) and tighten the cap.

Naso-pharyngeal aspirate - Take following local protocols.

Urine CMV PCR in neonates - send a freshly taken urine sample.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Any delays in transport to the laboratory should be minimised, refrigerate if delays.

NPA – Sent to the laboratory. **DO NOT** use the air system (pod). Discuss with Consultant Microbiologist before sending.

ANYTHING ELSE?

Screen	Target Organisms
SARS-CoV-2 PCR	SARS-CoV-2
Resp Quad Swab (Covid/Flu/RSV)	SARS-CoV-2, Influenza A & B and RSV
Extended respiratory screen for immunocompromised, respiratory consultant led and children with Negative RSV results.	Adenovirus, Coronavirus, Mers-CoV, SARS-CoV-2, Human metapneumovirus, Rhinovirus / Enterovirus, Influenza A, Influenza B, Parainfluenza, RSV, Bordetella pertussis, Bordetella parapertussis, Chlamydophila pneumoniae & Mycoplasma pneumoniae.
Eye swab for viruses	HSV 1 & 2, VZV, Adenovirus
Genital and skin swabs	HSV 1 & 2, VZV

WHAT WILL AFFECT THE RESULTS?

- Detection depends greatly on the specimen; correct pre-examination storage, correct sampling (e.g. ensuring material from vesicles obtained, not just surface sweep), point of illness at which sample is taken and/or low levels of virus shedding. This may result in false negative results.
- Negative results from patients with suspected disease may not preclude actual disease.
- A positive PCR result does not necessarily indicate a viable virus.
- The effect of interfering substances has only been evaluated for those listed within the kit insert, none of which showed interference.
- Cross-reactivity with respiratory tract organisms other than those described here can lead to erroneous results.
- **Recent patient exposure to FluMist or other live attenuated influenza vaccines may cause false positive results.**

7.17 Sterile Fluids Requiring a Cell Count (e.g. Joint Fluids, Ascitic Fluids and CAPD)

For example: Joint Fluids, Ascitic Fluids and CAPD

WHAT DO I NEED?

Sterile universal container (white or yellow topped)
EDTA sample (for Ascitic Fluids - cytospin)

HOW DO I TAKE THE SPECIMEN?

All fluids should be taken aseptically according to local clinical protocols.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Transport immediately to the laboratory.

If an urgent cell count and Gram stain is required, the laboratory must be informed when the sample has been taken and is on its way. If out of hours, the on-call microbiology scientist can be contacted through switchboard.

ANYTHING ELSE?

Joint fluids, ascitic fluids and CAPD fluids will all routinely have a cell count (unless clots present), routine culture and enrichment culture. Differential WBC counts are only performed on request.

Joint Fluids for crystal examination
Synovial fluid is examined under polarising microscopy for Sodium urate and/or calcium pyrophosphate crystals indicating gout or pseudogout.

Ascitic Fluids for Cytospin
Ascitic fluids should be accompanied by an EDTA tube.
If the WBC >250 /mm^3, the sample is passed on to Haematology for a cytospin.
The decision to process samples will be at the discretion of the Haematology Biomedical Scientist depending upon workload – for cytospin results or queries please contact Haematology.

WHAT WILL AFFECT THE RESULTS?

- Joint fluids are often heavily blood stained or clotted - this invalidates the cell count.
- Organisms may be present in very small amounts and so may not be seen on the Gram stain or grow on culture.

- Prior administration of antibiotics may lead to false negative culture results.
- Old samples often display amorphous crystalline debris that preludes identification of urate or pyrophosphate crystals.
- Optimal time for specimen collection is ideally prior to antimicrobial therapy.
- Specimens should be transported and processed as soon as possible to minimise clot
- formation.
- If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48h are undesirable.
- Fluids such as synovial fluids may be received in inadequate volumes which may impede the recovery of organisms.
- Evaluation and significance of findings assumes that adequate care and aseptic technique is taken during specimen collection, transport and microbiological processing so that detected organisms are from the source of infection and not contaminants.
- Ward-based contamination during sampling and inoculation of blood culture bottles with Ascites and CAPD fluids.
- The results of enrichment methods must be interpreted with caution to determine if a significant isolate or a sampling/ laboratory contaminant.

7.18 Wounds, Tissue and Pus

WHAT DO I NEED?

Wound and skin swabs – charcoal transport swab.
Pus, Tissue – Sterile Universal container (white or yellow topped)
Revision tissues/fluids – Sterile universal container and bead broth containers

HOW DO I TAKE THE SPECIMEN?

Pus: Pus is preferable to a wound swab. Aspirate pus or fluid with a sterile syringe and transfer to a sterile universal container.
Swabs: If insufficient pus to aspirate, remove any superficial debris, then rotate a swab firmly on the advancing edge of the lesion. Place the swab in charcoal transport medium.

In the absence of surrounding cellulitis, swabs of ulcers seldom yield useful information, due to the large amount of colonising flora present.

Tissues: Take according to local protocols. Do not send whole appendages e.g. toes.

Revision tissues/fluids: Taken in theatre. A fluid (or first tissue sample taken) should be sent in a white topped sterile container and subsequent tissues should be sent in bead broth containers.

Site: Always state the site and nature of the wound. This gives the laboratory an indication of what normal flora may be present, and when to perform extra investigations.

HOW SHOULD I TRANSPORT SPECIMEN TO LAB?

Pus and Tissue - Transport to laboratory as soon as possible. Refrigerate in case of delays.
Swabs - If there is any delay in getting the swab to the laboratory, store the swab in the fridge.

ANYTHING ELSE?

Toxin testing: *Staphylococcus aureus* with virulence factors such as PVL (Panton-Valentine Leukocidin) or SST (scalded skin toxin) can cause highly contagious infections (e.g. folliculitis) in healthy children and young adults. More serious infections such as necrotising fasciitis and pneumonia are increasingly described. If you suspect your patient may have one of these infections, contact the microbiology consultant and the isolate can be sent for toxin testing.

Clinically important isolates will always be phoned from the laboratory to the requestor, or to the Infection Control Department.

WHAT WILL AFFECT THE RESULTS?

- Insufficient clinical information can lead to the laboratory failing to perform the necessary extra testing.

- The tissue specimen should, ideally, be large enough to carry out all microscopical preparations and cultures required.
- If whole appendages, eg. Toes, are received by the laboratory they will be rejected.
- Tissue specimens received in formalin or formalin pots are not suitable for culture and will not be processed by microbiology.
- Revision tissue specimens received in sterile containers, instead of a bead broth, are then transferred to bead broths by laboratory staff – this can increase the risk of contamination.
- Appropriate specimens are often difficult to obtain, and as a result incorrect or suboptimal specimens are often submitted.
- All specimens should be fresh and taken before antimicrobial treatment is started.
- It is preferable for swabs to be transported in Amies transport medium with charcoal.
- The volume of fluid specimens influences the transport time that is acceptable. Larger volumes of purulent material maintain the viability of anaerobes for longer.
- Delayed transport of specimens, or incorrect storage during transportation, especially over weekends, could result in loss of pathogen viability for fastidious organisms.
- Refrigeration is preferable to storage at ambient temperature when processing may be delayed.
- Specimens may be cultured up to 48 hours after collection; delays of over 48 hours are undesirable. But if not processed on the same day, care should be taken with the interpretation of results.
- Skin flora contamination of the wound and associated specimens is common.
- Swabs are unsuitable for TB culture, and this should only be performed after consultation with consultant, in containment level 3.

7.19 Relevant Clinical Details

Certain clinical details added to a request can aid appropriate sample processing and are required to ensure correct Health and Safety procedures are followed when processing samples.
If the patient has any of the following clinical details, please make sure they are included and stated in the request.

ALWAYS INCLUDE IN CLINICAL DETAILS WHEN:

- Immunocompromised
- Foreign Travel and the countries visited / arrived from
- IVDU

TABLE 1: SAMPLE INFORMATION & ASSOCIATED MICROOGANISMS

Sample Type	Clinical Details	Microorganism considered
Blood Cultures	Undulant fever	*Brucella* species
Blood Cultures	Foreign travel especially if associated with fever	*S. typhi/ paratyphi*
Blood Cultures	Consumption of unpasteurised dairy products	*Listeria monocytogenes*
All samples (especially: psoas pus; lymph nodes; neck lump/ abscess; discitis fluid)	HIV positive; patient from Africa; IV drug user	Risk of TB
CSF	Risk of CJD/Prion & associated symptoms	CJD/Prion
Wound swabs	Animal bites or exposure	*Pasteurella* species *Capnocytophaga species*
Wound swabs	Water associated (swimming injury, etc)	*Vibrio* species *Aeromonas* species
Wound swabs	Chronic non-healing ulcer/lesion & at least 1 of the following: Foreign travel; high-risk contact; clinical micro staff	*Corynebacterium diphtheria*
Throat swabs	Membranous pharyngitis; consumed raw milk; farm worker	*Corynebacterium diphtheria*
CSF & Throat swab	Petechial/ non-blanching rash	*Neisseria meningitidis*
Pernasal swab	Whooping cough	*Bordetella pertussis*

TABLE 2: CLINICAL INFORMATION THAT MAY SUGGEST POTENTIAL INFECTION BY HG3 PATHOGENS

Clinical Details	High Risk Occupations	High Risk Sports /Pastimes
IV drug Abuser	Hospital or Laboratory staff (exposure incident)	Outdoor water sports
Return travel / visitor from abroad where HG3 pathogens are endemic	Veterinary or Animal worker	Caving or pot-holing
Consumption of unpasteurised products (milk/dairy)	Farming or visit to farm	Camping & hunting in endemic area
Psoas abscess / cold abscess	Slaughter house/ abattoir worker	Animal hide drum playing/making
Enteric fever	Horse caretakers	
HUS (haemolytic uremic syndrome)	Equine butchers	
Consumption of raw or undercooked meat products	Industrial processing of wool, hide or hair	
	Meat packing or rendering plant employees	

Other clinical details that may suggest potential CL3 organisms that are not listed in Table 2:

- Ground glass X-ray
- MTB, TB, Mycobacteria, miliary
- Melioidosis
- Has an HPZ number
- Exposure to bird or bat droppings

WHAT WILL AFFECT THE RESULTS?

Not including relevant clinical details as described above

Appendix A: Quick Guide

Any tests marked with * are not accredited by UKAS

Test and Info Guide	Sample Container	Storage / Transport	Turnaround	General Information	Information for Patients
Antibiotic Assays	6ml clotted blood (Gold Top)	Fridge	Vanc, Gent, Tobramycin daily in Biochemistry Lab Others: 2 days (Referred)		
Antibiotic Resistance Screening VRE and CPE Rectal Swab ESBL* and MRAB* - rectal swab MRSA PCR* – throat/groin swab	Special Red topped dual swab (for PCR), one for each test Black topped bacterial swab Red topped dual swab – test performed point of care on the ward	Fridge	Report in 2 days Report in 2 days Report in 2 days	When to test: VRE: Any patient transferred from another Hospital. CPE: Any patient transferred from outside Devon and Cornwall. MRSA: Any patient before urgent orthopaedic surgery. ESBL and MRAB: After discussion with microbiology consultant. VRE: Vancomycin resistant enterococci CPE: Carbapenamase producing enterobacteriacae ESBL: Extended spectrum Beta-lactamase MRAB: multi-resistant *Acinetobacter Baumanii*	

Blood Cultures	8-10ml blood (Anaerobic/Aerobic set) 1-3ml blood (paediatric bottle) Using collection kit.	Take straight to main Pathology reception. Out of hours: Leave in the box on top of the Bactec FX40 mini blood culture incubator, entrance to Pathology. Do not refrigerate	Ward notified as soon as organisms are detected	Direct antimicrobial susceptibility tests are performed on the majority of blood culture isolates. The laboratory follows EUCAST recommendations and accepts responsibility for these results.	
Chlamydia and Gonorrhoea PCR Genito-urinary swabs Oropharyngeal swabs Ano-rectal swabs* Eye swabs* First voided urine	Yellow topped Cobas swabs Plain universal sterile container (not metal topped)	Fridge	3 working days	How to take a endocervical swab How to take a urine	Self-take patient guide
C.S.F. Bacterial culture and microscopy Viral PCR	Plain universals x 3, or plain universals x4 if ?SAH	Inform laboratory specimen has been taken (x55257 or on-call BMS out of hours), Transport immediately to laboratory.	Cell count :1 hour post receipt Culture: Report in 2 days Viral: Report within 1 day of requesting, unless referred for uncommon pathogens.	Never send in air tube. Sample containers must be clearly numbered in the order they were taken.	

Ear	ENT swab (orange topped)	Fridge	Report in 2 days		
Eye Swabs Bacterial culture Corneal Scrape	 Black topped swab Corneal Scrape kit	Fridge	 Report in 2 days Report in 2 days	Corneal Scrape kits -available on top of specimen fridge outside reception. Acanthamoeba (referred): • From a corneal scrape: Put the scalpel blade/needle into a plain universal containing sterile saline. • From a contact lens: send the lens in cleaning fluid.	
Environmental testing*	Air sampling samples for bacterial and fungal colony counts	Sent directly to lab – no storage	1 week		
Faeces Culture Gastro panel PCR *C.difficile* PCR & toxin testing Norovirus Parasites *H.pylori* antigen	 Blue topped, or white topped universal	Fridge	 48 hours 24 hours 18 hours 12 hours 48 hours 48 hours	Tests driven by Clinical Details.	Patient Guide

Genito-Urinary Bacterial culture HVS & cervical Urethral IUCD, Fluid	 Black topped swab Orange topped swab Sterile universal container	Fridge	Report in 2 days	Tests driven by Clinical Details	
MRSA and MSSA screening	Black topped swabs	Fridge	Negative result within 24 hours		
Mycobacterial* culture and microscopy	Dependant on sample type	Fridge	AFB Smear - 1 working day Culture - 9 to 12 weeks		
Nose bacterial culture	Black topped swab	Fridge	Report in 2 days		
Skin, Hair and Nail – Mycology culture	Dermapak, *or* plain universal	Room temp	Microscopy: 1 week Culture: 2-3 weeks		
Sputum and other respiratory Bacterial culture	Plain universal, or wide mouthed container	Fridge	Report in 2 days*	Tests driven by Clinical Details	
Sterile Fluids Culture and microscopy, including cell count Crystal Microscopy* on joint fluids	Plain universal, or wide mouthed container	Fridge	Cell count :1 hour post receipt Report in 2 days	Telephone Laboratory if Urgent.	
Throat, Mouth, Tongue Bacterial culture	Black topped swab	Fridge	Report in 2 days	Tests driven by Clinical Details	

Urines Bacterial culture Legionella and Pneumococcal antigens* Mycobacteria/TB* Schistosoma CMV PCR	Red topped boric acid filled to line *or* plain universal for small volumes. Plain sterile universal TB and Schistosome urine packs available from Microbiology Plain sterile universal	Boric - room temp All others - fridge	Report in 2 days Report in 1 day 9-12 weeks 24 hours 1 week (referred)	Microscopy performed on a limited number of samples dependant on clinical details.	Patient Guide
Wound, Pus and Tissue (Non-Revision, non-sterile) Bacterial culture and microscopy	Black topped wound/skin swab Pus/fluid/tissue in a sterile universal	Fridge	Report in 2 days		
Revision Tissues Bacterial culture and microscopy, including cell count	Joint fluid (or first tissue if a joint fluid is not taken) into a sterile sample pot. Place subsequent tissue samples into a broth with glass beads in the theatre.	Fridge	Cell count :1 hour post receipt Report in 2 days		
Line tips eg CVP or Hickman lines, swabs of cannula insertion sites	Disinfect the skin around the cannula entry site, remove cannula using aseptic technique, and ideally cut off 4cm of the tip into an appropriate CE marked leak proof container using sterile scissors.	Fridge	Report in 2 days	**Note**: Peripheral lines are not suitable specimens for cultures and should not normally be sent to the laboratory for testing.	

Virology Routine serology Immunity screening (Measles, Mumps, Rubella, VZV, Hepatitis B)	Clotted gold topped blood sample	Fridge	All results within 2 working days unless stated otherwise (results may take longer if confirmation is required)		
Antenatal screening (HIV, Syphilis, Hepatitis B)	Clotted gold topped blood sample	Fridge			
Hepatitis screening (Hepatitis B, Hepatitis C, Hepatitis A, Hepatitis E, CMV, EBV)	Clotted gold topped blood sample	Fridge	Hepatitis E: 14 days (referred)	Hepatitis A and E are only tested when certain criteria are met.	
Hepatitis B screening (Surface antibody, surface antigen, core antibody)	Clotted gold topped blood sample	Fridge			
Sexual health screen (HIV, Syphilis)	Clotted gold topped blood sample	Fridge			
Zoonosis (Lyme disease, Toxoplasma)	Clotted gold topped blood sample	Fridge			
T Spot	Green topped heparin blood x2	Room temp	7 days (referred)	Must be received in the lab by 2pm on the day the sample is taken.	

Virology Non-routine serology Various other tests	Various	Fridge	Various	Various non routine tests can be performed at specialist reference laboratories.	
Virology Viral loads HIV Hepatitis C Hepatitis B EBV CMV Enterovirus	Purple topped EDTA blood	Fridge	Report in 1 day Report in 7 days (referred)		
Virology (non-blood) Eye swab – HSV PCR Skin swab – HSV/VZV PCR Respiratory swab – Bordetella PCR Respiratory swab – Flu/Covid/RSV panel Respiratory swab – Extended viral panel	Green topped viral swab	Fridge	1 week (referred) 7 days (referred) 14 days (referred) 2 days (if meets testing criteria) <3 hours (if meets testing Criteria)	Testing criteria is subject to change throughout the year – may require discussion with a microbiology consultant. The Flu/Covid/RSV panel is tested point of care* by emergency department staff in the winter months.	

Appendix B: Urine Collection Guide

Torbay and South Devon NHS
NHS Foundation Trust

How should I collect a urine sample?

You should:

- collect your urine sample in a completely clean (sterile) container
- store it in a fridge in a sealed plastic bag if you can't hand it in straight away

Collecting a urine sample

Your doctor or another healthcare professional should give you a container and explain how you should collect the urine sample.

You can collect a urine sample at any time of day, unless your GP or practice nurse advises you otherwise.

The types of urine sample you might be asked for include a random specimen, first morning specimen or timed collection.

To collect a clean urine sample:

- label the container with your name, date of birth and the datewash your hands
- start to urinate and collect a sample of urine "mid-stream" in a sterile screw-top container (to the fill line, if possible)
- screw the lid of the container shut
- wash your hands thoroughly

Follow any other instructions your doctor gives you.

What's a mid-stream urine sample?

A mid-stream urine sample means you don't collect the first or last part of urine that comes out. This reduces the risk of the sample being contaminated with bacteria from your hands or the skin around the urethra, the tube that carries urine out of the body.

https://www.nhs.uk/common-health-questions/infections/how-should-i-collect-and-store-a-urine-sample/

09/01/2019

Appendix C: Faeces Collection Guide

Torbay and South Devon NHS
NHS Foundation Trust

How should I collect a stool (faeces) sample?

You should:

- collect your stool (faeces) sample in a clean container
- store the container in a fridge in a sealed plastic bag if you can't hand it in straight away

Collecting a stool sample

Your GP or another healthcare professional, such as a nurse, should explain how to collect the stool sample. It should be collected in a clean, dry screw-top container.

Your doctor or a member of staff at the hospital will give you a blue topped plastic (specimen) container with an attached spoon to use.

Try not to collect urine or water from the toilet with the stool sample, but don't worry if you do. If you need to urinate, do this first before collecting the stool sample.

To collect a stool sample:

- label the container with your name, date of birth and the date
- place something in the toilet to catch the stool, such as a clean potty or a clean empty plastic food container (e.g ice cream tub), or spread clean newspaper or plastic wrap over the rim of the toilet
- make sure the sample doesn't touch the inside of the toilet
- use the spoon or spatula that comes with the container to place the sample in a clean screw-top container and screw the lid shut
- if you've been given a container, aim to fill around a third of it – that's about the size of a walnut if you're using your own container
- put anything you used to collect the sample in a plastic bag, tie it up and put it the bin
- wash your hands thoroughly with soap and warm running water

Follow any other instructions your doctor gives you.

https://www.nhs.uk/common-health-questions/infections/how-should-i-collect-and-store-a-stool-faeces-sample/

Appendix D: Self Collected Vaginal Swab Guide

Patient Instructions

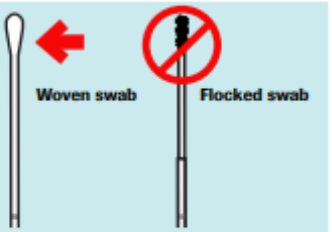
Roche

Vaginal Swab Specimen Collection Guide

Self-collection in a clinical setting with the cobas® PCR Media Dual Swab Sample Kit

HANDLING PRECAUTIONS:

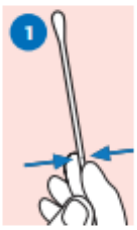
- Handle the collection tube carefully, as tube media can cause irritation if contacted with skin or other body parts.
- If the tube contents are spilled on your skin, wash the affected area with soap and water. If contents splash into your eyes, flush them with water immediately.
- Do not attempt to clean up any spilled contents of the tube.
- If any of these events occur, always notify your healthcare provider.

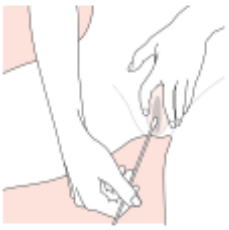


PREPARING FOR SAMPLE COLLECTION:

- Wash hands prior to collection. Undress to expose the vaginal area. Put yourself in a comfortable position.
- Remove the collection tube and the woven swab from the collection kit. Discard the flocked swab, as it is not needed for this procedure. **Use only the woven swab.**

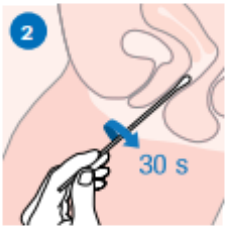
NOTE: ***Do not*** pre-wet the swab in **cobas®** PCR Media before collection.





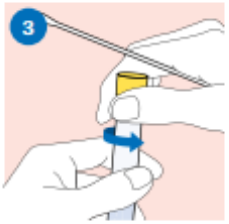
POSITION:

- In one hand, hold the woven swab with the scoreline above your hand and with the other hand separate the folds of skin around the vaginal opening (labia).



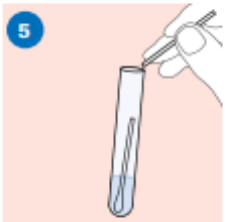
COLLECT:

- Insert the swab about 5 cm (2 inches) into the vaginal opening.
- Gently turn the swab for about 30 seconds while rubbing the swab against the wall of the vagina.
- Remove the swab carefully.
- Do not touch the swab to any surface before placing it into the collection tube.



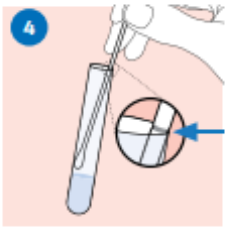
OPEN TUBE:

- While holding the swab in the same hand, remove the cap from the tube as shown in the diagram.



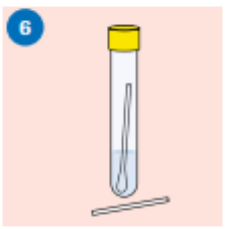
BREAK:

- Carefully lean the swab against the tube rim to break the swab shaft at the scoreline.



ALIGN:

- Lower the swab into the tube until the visible scoreline on the swab shaft is lined up with the tube rim.
- The bud of the swab should not be submerged into the liquid prior to breaking the shaft.



CLOSE:

- Tightly close the cobas® PCR Media Tube.
- Return the sample to your healthcare provider as instructed.
- Discard the top portion of the swab.

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Appendix E: Clinical Collected Endocervical Swab Guide

Clinician Instructions

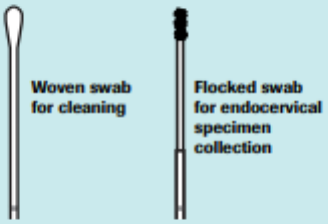
Roche

Endocervical Swab Specimen Collection Guide

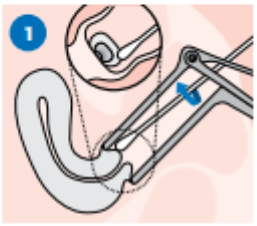
Clinician-collection with the cobas® PCR Media Dual Swab Sample Kit

PROCEDURAL NOTES:

- Vaginal lubricants, speculum jellies, creams, and gels containing carbomer(s) may interfere with the test and should not be used during or prior to sample collection.
- If the collected specimen contains excess blood (specimen has a red or brown color), it should be discarded and not used for testing.
- Avoid contact of the **cobas®** PCR Media with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water.



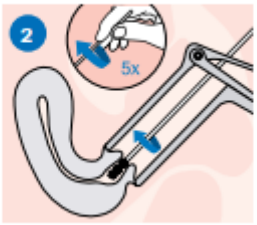
NOTE: ***Do not*** pre-wet the swab in **cobas®** PCR Media before collection.



CLEAN:

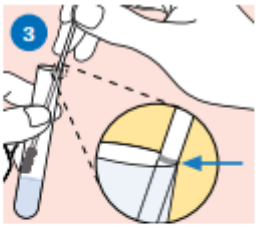
- **Using the woven swab, remove excess mucus from the cervical os and surrounding mucosa.**
- **Discard the swab after cleaning.**

NOTE: **Cleaning excess mucus from the cervical os is required to ensure an adequate sample is obtained for processing.**



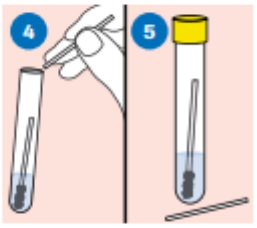
COLLECT:

- **To collect the specimen, hold flocked swab with the scoreline above your hand and insert into the endocervical canal.**
- **Gently rotate the swab 5 times in one direction in the endocervical canal. Do not over-rotate.**
- **Carefully withdraw the swab, avoiding any contact with the vaginal mucosa.**



ALIGN:

- **Remove the cap from the cobas® PCR Media Tube.**
- **Lower the swab specimen into the tube until the visible scoreline on the swab shaft is aligned with the tube rim.**
- **The bud of the swab should not be submerged into the liquid prior to breaking the shaft.**



BREAK:

- **Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.**

CLOSE:

- **Tightly close the cobas® PCR Media Tube. The specimen is now ready for transport.**
- **Discard the top portion of the swab.**

SPECIMEN TRANSPORT AND STORAGE:

- Transport and store the **cobas®** PCR Media Tube containing the collection swab at 2°C to 30°C.
- The specimen should only contain 1 flocked swab and may be rejected if the tube contains no swab or 2 swabs.

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Appendix F: Self Collected Roche CT/GC Urine Guide

cobas® PCR Urine Sample Kit

For *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Short guide[1] for urine sample collection

URINE SAMPLE

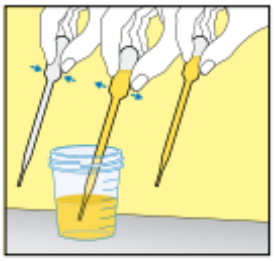
FOR THE PATIENT

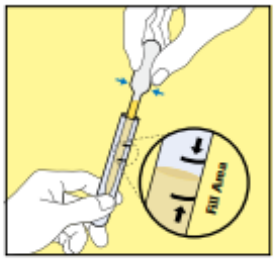
1. COLLECT:
Prior to sampling, the patient should not have urinated for **at least one hour** and **female patient must not clean** the labial area.

Ask the patient to provide the **first catch urine** (10-50mL) into a urine collection cup.

FOR HEALTHCARE PROFESSIONALS

2. PIPET:
Use the provided disposable pipet **to transfer** (within 24 hours) the **urine** into the **cobas®** PCR Media tube. **The correct volume of urine has been added when the fluid level is between the two black lines on the tube label.**



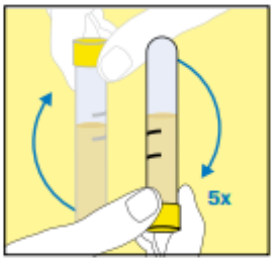


3. CLOSE:
Tightly re-cap the tube.

HANDLING PRECAUTIONS:
Use care to avoid splashing of contents.

4. MIX:
Invert the tube 5 times.

The specimen is now ready for transport.



Transport: 2°C to 30°C • **Stability of sample:** 12 months

THE SPECIMEN WILL BE REJECTED IF THERE IS:
- Inadequate volume (insufficient or excess)
-Excess of blood (>0.35%)
-Replens® vaginal moisturizer

Roche

cobas®

[1] Individuals collecting or directing the collection of samples should consult the complete instructions for use.