PATHOLOGY USER GUIDE – MICROBIOLOGY ESNEFT

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Summary of Changes:
• New document

Contents

1 MICROBIOLOGY PERSONNEL

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2 DATA PROTECTION AND PATIENT CONFIDENTIALITY

The EU General Data Protection Regulation (GDPR) is a pan-European data protection law, which superseded the EU's 1995 Data Protection Directive and all member state law based on it, including the UK's DPA 1998 (Data Protection Act 1998), on 25 May 2018.

The GDPR extends the data rights of individuals (data subjects), and places a range of new obligations on organisations that process EU residents' personal data.

3 ACCREDITATION

The service is actively working towards achieving accreditation from UKAS under the BS EN ISO 15189-2022 standard.

4 COMPLAINTS

To make a complaint about the performance of the laboratory please contact the Trust Patient and Liaison Service (PALS) as follows:

By Phone
PALS can be contacted by telephone from 9am to 4pm, Monday to Friday
(Confidential answerphone out of hours)
Free phone 0800 783 7328
Direct line 01206 742683 or 746448

If your call is urgent and you require assistance outside these hours please dial 01206 747474 and ask to speak to the Duty Matron for Colchester. For Ipswich dial 01473 712233 and ask to speak to the Duty Matron

In Writing
Patient Advice and Liaison Service
East Suffolk North Essex NHS Foundation Trust
Colchester General Hospital
Turner Road
Colchester
Essex
CO4 5JL

By email
PALS@esneft.nhs.uk

5 LOCATION OF THE MICROBIOLOGY DEPARTMENT TO WHICH ALL SAMPLES AND ENQUIRIES ARE REFERRED

Ipswich Postal Address: Microbiology and Molecular Department Pathology Directorate East Suffolk North Essex NHS Foundation Trust Ipswich Hospital Heath Road Ipswich IP4 5PD Microbiology Tel: 01473 703750 or internal extension 5750 Molecular Tel: 01473070459 (ex 8459)	Dx address for samples: Microbiology Department DX 6050200 Ex Ipswich 90 IP
Colchester Postal Address: Microbiology Department 214 Turner Road Colchester CO4 5JR	Dx address for samples: Microbiology DX 6030802

Tel: 01206 747374 or internal extension 7374	

The Ipswich Laboratory is situated at the back of the Hospital, off the main street. From the Main outpatient entrance at the front of the Hospital, follow the signs to Pathology reception and ask the reception staff for the Microbiology Department. The Molecular Laboratory is situated at the back of the Pathology Block.

The Colchester Laboratory is a standalone building on Turner Road away from the hospital, adjacent to the Highwoods Country Park.

Full service for analysis of samples and for consultation is available from 09:00 - 17:30. A reduced service is available 17:30 - 09:00. Details of this service are detailed in the Out of Hours area of this document (below).

6 CLINICAL ADVISORY SERVICE

During office hours the duty Consultant Microbiologist can be contacted on 01473 703012 (internal extension 5012) for Ipswich, or 01206747374 for Colchester. For molecular advice contact Dr Husain on the above no or 01473703012 (5012)

For urgent clinical advice out of hours the duty Consultant Microbiologist is available via the Hospital Switchboard.

Microbiologists assist in the interpretation of laboratory results and can offer further advice if required on specimen conditions for infectious conditions and patient therapy. Therapeutic advice may be based on culture results (definitive) or clinical suspicion (empirical).

Advice and guidelines for the use of Antimicrobials can be found on the Trust Intranet by following the link.

https://intranet.esneft.nhs.uk/intranet/documents/283/10085/

Infection Control is an integral part of the work of the Microbiology Department.

For Infection Prevention & Control Advice Monday to Friday 8am to 5pm contact ext 4268. for Ipswich and Ext 712233 then 5742 for Colchester and through the link;-

https://intranet.esneft.nhs.uk/pages/infection-prevention-control

For acute or urgent issues the infection prevention team can be bleeped via switchboard. Further contact information for the team can be found by following the link above. Out of hours and weekends Infection Prevention advice is provided by the Duty Medical Microbiologist. See contact numbers in General Information.

7 NON-CLINICAL ADVICE

Senior and Specialist Biomedical Scientists are available to give advice on technical matters including the transport of specimens, the correct containers and transport media to use, and the requirements for acceptance criteria. Please use the telephone numbers listed in section 5.

8 EMERGENCY AND ON-CALL INVESTIGATIONS

The Laboratory operates a 24-hour service. Full routine and emergency services operate between 09:00 and 17:30 Monday to Friday and from 09:00 to 12:30 on Saturday mornings.

In Ipswich, specimens can be delivered to the Blood Sciences reception (opposite Microbiology reception) at any time.
Out of normal laboratory hours, urgent Ipswich samples can be examined by contacting the on-call Biomedical Scientist via **bleep 907** or the hospital switchboard if no reply.

In Colchester, they can be contacted via the switchboard. Every effort will be made to examine samples urgently in cases where results are likely to influence treatment or when samples would deteriorate if delayed.

Such requests include:

1. CSF
2. Sputum / Bronchial Lavage
3. Urine
4. Wound swabs /tissues/fluids
5. Blood cultures are checked out of hours once a night and positives telephoned

After midnight on-call work is restricted to CSF samples only. Other samples are processed by arrangement between the patient's consultant and the duty Microbiology consultant only.

8.1 Treatment

Advice on empirical and specific treatment of infections is located in the Antibiotic pages of the Trust's intranet. Please consult these sources first.

https://intranet.esneft.nhs.uk/intranet/documents/#list/283

8.2 Urgent Requests

Notification that urgent samples are being sent to the lab must be arranged at all times.

For Ipswich, during normal laboratory hours, phone the laboratory on hospital extension 5750 (outside line 01473 703750). Out of hours contact the on-call system.

For Colchester, during normal working hours please call extension 7374 (outside line 01206747374). Out of hours staff can be contracted via switchboard.

All Emergency Department (A&E) requests where there is a likelihood of 4 hour breach must be notified by telephone.

8.3 Turn-around times

For urgent Microbiology specimens sent with prior notification to the microbiology laboratory, it is expected that microscopy results would be available within 2 hours of receipt.

For urgent rapid respiratory molecular work the expected turnaround time will be 4 hours.

8.4 Out of Hours Work

It is the responsibility of Health Care Professionals taking samples to make sure the samples reach the laboratory, either using the portering services or air tube, where available. In Colchester, internal transport of samples can be requested by calling the Estates and Facilities helpdesk on extension 7676.

With urgent or emergency work, please do not telephone the laboratory to ask if the results are available. All results will be available on the relevant ICE system for each site.

Where tests other than those mentioned above are required, and it is ESSENTIAL that they are taken during the out of hours period, send them to the laboratory and they will be handled and preserved to ensure that valid analytical results will be obtained when the sample is analysed.

9 SPECIMEN COLLECTION

Each specimen must be clearly labelled by hand or using pre-printed patient labels which meets the following criteria (Path-ALL-GP-26).

The patient details on the specimen must match those on the provided request form. A minimum of 4 matching identifiers are required to enable positive identification of patient.

The following ***must*** be present on the sample, with matching identifiers on both the sample ***and*** the request form:

- Surname
- Forename (initials/abbreviations are not acceptable)
- Date of birth
- NHS number or Hospital Number

FAILURE TO MEET THESE LABELLING CRITERIA WILL RESULT IN THE SPECIMEN BEING REJECTED

Each specimen must be appropriately placed in a sealed transport bag for transport to the laboratory.

Specimens that have leaked or are not adequately labelled will not be analysed.

High Risk samples from patients with (or suspected to have) Creutzfeldt-Jacob Disease (CJD), Transmissible Spongiform Encephalitis (TSE), Ebola, or Rabies must be labelled clearly with 'Danger of Infection' on the request form and sample bags. The Laboratory must be notified that the samples are coming prior to collection and the request must be discussed with the relevant Consultant.

For Viral Haemorrhagic Fever, please refer to guidance in the Viral Haemorrhagic Fever (VHF) Policy on the Trust Intranet (see section 5.5) and SOP PATH-ALL-GP-33 VHF Patient Pathways.

9.1 Leaking Samples

Leaking samples will not be processed by the laboratory unless they precious or unrepeatable when they will be processed if doing so, does not put lab staff at risk. Samples that are processed will have a comment added to the report noting that this was a leaking sample.

9.2 Patient consent

East Suffolk and North Essex NHS Foundation Trust (ESNEFT) is committed to ensuring that all staff involved in the patient consent process adhere to the Department of Health guidance on consent to examination and treatment. Patients have a fundamental legal and ethical right to determine what happens to their own bodies. Valid consent must be obtained before starting treatment or physical investigation, or providing personal care, for a person, and is a fundamental part of good practice. A healthcare professional (or other healthcare staff) who does not respect this principle may be liable both to legal action by the patient and to action by their professional body. Consent is presumed with regards blood tests when the sample and request form – containing the requestor details – arrives in the laboratory. Phlebotomy presume consent when the patient arrives for the phlebotomy appointment with a completed request form and presents for venepuncture.

9.3 General principles (applies to every specimen collected)

Successful laboratory diagnosis depends on the collection of specimens at the appropriate time, using the correct technique and equipment and transporting items safely to the relevant laboratory.

- Confirm the identity of the patient.
- Verify that the patient meets pre-examination requirements [e.g. medication status (time of last dose, cessation), sample collection at predetermined time or time intervals, etc.];
- Ensure specimens are collected in the appropriate containers and the lid securely tightened.
- Ensure that there is sufficient Volume of sample to perform the tests required. If sample is small and more than one test is required, state preferred test priority. If no priority stated, the laboratory will decide test priority.
- Ensure that, wherever possible, specimens are taken prior to the commencement of antimicrobials. If these have already started, the agent should be noted on the request card.
- Ensure that date and time of collection of the specimen is completed on the request card/form, as well as on the specimen container. *This is particularly important when request forms have been prepared in advance.*
- Ensure the identity of the person collecting the primary sample is included on the request form.
- In situations where the primary sample is collected as part of clinical practice, information and instructions regarding primary sample containers, any necessary additives and any necessary processing and sample transport conditions shall be determined and communicated to the appropriate clinical staff.
- For patient-taken samples, it is important to supply the patient with the correct sample containers and to explain fully haw the sample is to be collected. Date and time of sample collection should be added to sample and request form by the patient.

If a large and/or complex set of investigations are to be requested, especially on a specimen of limited quantity, then it is good practice to contact the laboratory in advance. In this way, priorities can be established and the lab will be able to analyse the sample in the most productive way.

Where the user requires deviations and exclusions from, or additions to, the documented collection procedure, please notify the laboratory so that these can be recorded and included in all documents containing examination results and be communicated to the appropriate personnel.

9.4 Forensic and medico-legal specimens

The department has capabilities for testing of medico-legal specimens. All requests for forensic tests must be discussed with the duty Consultant Microbiologist prior to sending the specimen to the laboratory.

10 REQUEST FORM

Electronic request forms (ICE) should be used whenever possible. It is essential that the request form bears - as an absolute minimum - the patient's name, case number (whenever this is available, or address if the hospital number is not known), time of sampling, date, the signature of the person making the request and the location to which the report is to be sent. All requests sent to the laboratory are considered a service agreement between the requester and the laboratory to undertake the analysis of the sample for the tests requested. The laboratory may refer the sample to another laboratory for specialised or confirmatory analysis in order to provide the results.

All antenatal screening requests must be accompanied with a fully completed family origin questionnaire. This must contain the estimated delivery date.

11 REVIEW HISTORY

This is a new document, so first revision.

12 INTERFERENCE

In laboratory testing there are potential "uncertainties" that may affect test results (for example, specimen not collected correctly, presence of antimicrobials, biological variation). Additionally, factors within the laboratory may lead to variation (for example, incubation times, time to process). The Microbiology laboratory has measures in place to minimize the level of uncertainty and this is reflected by the Quality Assurance processes in place. Results provided by the laboratory are representative of the sample tested and must be considered against clinical presentation. There are a number of factors that may affect the quality and validity of a result that are outside of these that may affect the quality and validity of a result that are outside of the laboratories control.

12.1 Uncertainty

Performance of tests and interpretation of results in general is dependent upon correct pre-analytical stages

- specimen collection
- completion of request form
- specimen transport

Also,

- the timing of the collection of the specimen in relation to anti-microbial therapy
- variables within the laboratory that may affect analysis of the specimen

Information on uncertainty of measurement for specific tests that are reported numerically may be requested from the laboratory.

12.1.1 Delays

The time elapsed from taking (collecting) the sample to its reaching the laboratory may be significant if the specimen is not kept at the required temperature prior to dispatch or testing.

- Delays may cause changes that could radically alter the result. The laboratory count of bacteria in a delayed specimen could be significantly different from that of the specimen when it was collected. Urine in the bladder for example is normally sterile, whilst the urethra is not sterile and even a carefully taken urine specimen may contain a few microbes that will multiply and provide misleading results.

If specimens cannot be sent to the laboratory immediately, they should be stored as follows:

- Blood culture samples at ambient temperature.
- All other specimens for culture in a specimen refrigerator at 4°C overnight, except for genital culture swabs, which should be stored at room temperature.
- Blood samples for serology will be stable at 4°C overnight or over a weekend.

12.1.2 Virology

In many cases of suspected acute viral illness, the date of onset of clinical signs and symptoms will be relevant to the choice of specimen type and test requested. For example,

- Swab samples should be taken as soon as possible or within 5 days of onset of symptoms.
- Faecal samples in cases of viral gastroenteritis should be collected within 48 hours and should be diarrhoeal with a Bristol Stool score of 6 or 7.

The date of onset must be supplied with such requests and date(s) of contact with, for example, infectious rashes, should be provided where tests for immunity or susceptibility are requested, or infection is suspected, to allow interpretation of results.

12.1.3 Serology & antigen detection

In general, for blood and serum or plasma samples, the following conditions would render the specimen unsuitable for testing:

- Overt bacterial contamination
- Gross lipaemia
- Gross haemolysis
- Multiply frozen/thawed
- Containing particulate matter

12.2 Molecular

Samples for PCR testing where bound rather than flocked swab is used, can reduce the sensitivity of any PCR assay especially at low viral loads. Negative results where this swab type was used should be interpreted with caution. Samples taken with a bound swab should be repeated with a flocked swab and/or discussed with Consultant Virologist.

Molecular samples for routine PCR received in Roche media are stable for up to 12 months from the time of the swab being placed into the media.

12.3 Measurement of Uncertainty (MoU)

MoU values are available on request from the laboratory and may explain why tests near a numerical cut-off may be reported as both positive and negative on different samples.

For tests that have a measured quantity the laboratory have calculated the expanded measurement of uncertainty, expressed as plus or minus the uncertainty at a 95% confidence level

Eg. for measuring the cut off control for rubella immunity of 10 IU/ml the MoU was found to be 1 IU/ml

Expanded uncertainty is 10 IU/ml ± 1.0 IU/ml at a 95% confidence level.

This shows that 19 times out of 20 the probability is that if a patient has a level of 10 IU/ml the result will be within the range 9 to 11 IU/ml ie they may get reported as both detected and not detected on different samples. If the patient's level is 11 IU/ml then they will probably be reported as Detected Immune. This allows us to report some tests as equivocal as the results are within the uncertainty of the test method and cannot with surety be reported as either positive or negative.

12.4 Performance characteristics of tests and assays

Please contact the laboratory for details of individual examination procedures and tests, such as:

- Principles of tests and methods
- Units of measurement
- Specific interfering factors
- Uncertainty of measurement (for results in measured quantity values)
- Biological reference intervals
- Clinical decision values
- Laboratory clinical interpretation
- Alert/critical values

- Potential sources of variation
- References

13 INTERPRETATION OF RESULTS

The interpretation of test results produced by the laboratory will be based wherever possible on evidence based criteria, this will be based on information from a number of sources including;

- Information for use (IFU's) published by the assay manufacturer which has been confirmed by local verifications
- Published Standard Microbiology Investigations (SMI's) guidance produced by UKHSA
- Published Guidance from specialist bodies including; British National Formulary (BNF), British Association for Sexual Health and HIV (BASHH), European Committee on Antimicrobial Susceptibility Testing (EUCAST), Infectious Diseases in Pregnancy (IDPS) UKSHA guidance.

Technical interpretation of results will be performed by suitably qualified Biomedical Scientists who are HCPC registered and are deemed competent in the tasks they are undertaking by the Laboratory Manager.

Clinical Microbiologists who either hold Royal Collage of Pathologists qualifications or are working or the supervision of a Consultant Microbiologist will perform clinical Interpretation.

The interpretation of results produced by external laboratories will be indicated on the results authorised by the laboratory.

In situations where national guidance is not available, advice will be based on the clinical expertise and experience of qualified Consultant staff.

14 MICROBIOLOGY SERVICE

Full information of the scope of service, sample types, containers used, clinical indications, Reference Laboratories used and expected turnaround times is provided on the Trust Intra net and in MM-ALL-INFO-28.

15 SPECIFIC BACTERIOLOGY SAMPLE REQUIREMENTS AND INFORMATION

15.1 Blood Cultures

15.1.1 Instructions to clinicians

The routine blood culture set consists of an aerobic bottle and anaerobic bottle. Both should be inoculated with a minimum volume of 9 mL of blood for adult patients For paediatric patients the one Paediatric bottle should be inoculated with a minimum volume of 1ml of blood Paediatric aerobic bottles are available from pathology website on request. Failure to

fill bottles to the minimum levels will have negative impact on the isolation yields from any samples and in turn the treatment of the patients.

15.1.2 Monovette Guidelines

1 Wash hands, Wear gloves
2 Assemble equipment needed; one Monovette safety needle or Safety Multifly needle, one blood culture adaptor, skin cleansing Chloroprep Frepp sponge, any monovette tubes needed for other blood samples and Sani-cloth chlorhexidine wipes for the blood culture bottles.
3 Apply tourniquet and identify the vein to be used, Release tourniquet and prepare skin site with Frepp sponge for 30 seconds – allow to air dry.
4 Remove the covers from the tops of the blood culture bottles and swab the septum with the sani-cloth chlorhexidine wipe. Allow to Air dry.
5 Attach the safety needle or multifly needle to the blood culture adaptor.
6 Re apply tourniquet (not to be left on longer than 60 seconds) and without re touching the skin site puncture the vein with the needle bevel facing upwards.
7 Insert Aerobic or paediatric bottle first into the adaptor followed by the Anaerobic bottle. Keep bottles below puncture wound and allow minimum of 9ml of blood to flow into each bottle except the paediatric bottle that requires a minimum of 1 ml.
8 If further blood tubes are required remove the blood culture adaptor and click the blood tubes onto the safety needle according to the standard procedure.
9 When finished release the tourniquet, withdraw the safety needle from the vein and if using the multifly needle use your thumb to slide the tubing up over the needle until locked in place.
10 Dispose of needle and adaptor into sharps bin.
11 Label the blood culture bottles and all other tubes used with: Hospital number, NHS number, name, DoB, Ward and date /time collected. If electronic order comms (OCS) are used e.g.ICE then these labels can be used except on transfusion tubes. Do not place labels over blood culture bottle barcodes. Do not remove the barcodes; these are needed by the laboratory.
12 Ensure the OCS or manual request form has been completed correctly.
13 Put Blood cultures into bag and request form into marsupial sleeve.
14 Arrange immediate transport to the laboratory. Do not refrigerate. The pneumatic tube system can be used, but ensure that the carrier used is chipped to Microbiology.
15 Blood cultures are monitored continuously for at least 5 days with an interim negative report sent out after 48 hours incubation for adults and 36 hours incubation for paediatrics.
16 Clinically significant findings are telephoned on the day of detection, with a full report following, once the organism has been identified and sensitivities are available.
17 Microscopy results on bottles that flag as positive are interim reported following assessment of the Gram stained slide.

Please do not:

- Refrigerate
- Cover the barcode with labels
- Remove barcode labels from bottles

NOTE: If blood for other tests such as blood gases or ESR is to be taken at the same venepuncture, the blood culture bottles should be inoculated first to avoid contamination. It is preferable to take blood for culture by a separate stab.

NOTE: Blood culture vials that are potentially contaminated (media is cloudy) or beyond expiry dates must not be issued from the laboratory or used for specimen collection.

15.1.3 Service Details

Blood cultures are continuously monitored for at least five days after receipt. Clinically significant interim findings are telephoned on the day of detection to the medical team caring for the patient.

15.2 Fluids and Tissues

15.2.1 CSF

- An adequate volume is essential to obtain a reliable and sensitive result. Always collect and clearly number at least two specimens of CSF in sterile 28ml universals for Microbiology. From adults collect a first sample of 1ml and a second sample of as near 5ml as possible into sterile 28ml universal bottles. Collect a third sample of 0.5ml into a yellow topped fluoride EDTA tube
- If the first sample is bloodstained and/or subarachnoid haemorrhage (SAH) is suspected, collect a first sample of 1ml, a second of about 4ml and a third of about 1ml before collecting the final 0.5ml fluoride sample. Send bottles 1 and 2 to Microbiology and the fluoride tube (for glucose/protein) should be sent with bottle 3 xanthochromia to Biochemistry if requested. The pigments that cause xanthochromia are sensitive to light; please wrap the third bottle in paper before sending to Biochemistry.
- Do not use the pneumatic tube delivery system for CSF samples as the system may affect the integrity of the cells in the sample leading to incorrect test results.
- If infection is part of the differential diagnosis, send samples to Microbiology for microscopy and culture, together with the 0.5ml CSF fluoride tube (for Glucose and Protein) and a blood for glucose estimation taken at the same time to Biochemistry. All bottles must be clearly labelled in order that they are taken, as this is important in the clinical interpretation of the results.
- Microscopy test results will be telephoned immediately and culture results telephoned when positive. All negative results will be available after 48 hours of culture.

- If meningeal leukaemia or other malignancy is suspected and intracranial sepsis is not part of the differential diagnosis send CSF to the Haematology or Histopathology laboratory as appropriate.

- Normal CSF Cell counts

Leucocytes	Neonates	0-30 cells x 10^6/l
	1-4yr old	0-20 cells x 10^6/l
	5yr-puberty	0-10 cells x 10^6/l
	Adults	0-5 cells x 10^6/l
Erythrocytes	Newborn	0-675 cells x 10^6/l
	Adults	0-10 cells x 10^6/l
	Bloody tap	WBC to RBC ratio 1:500 to 1:1000

15.2.2 Pus and Body fluids (other than CSF)

1. **Peritoneal dialysates** - should be sent as complete dialysis bags.
2. **Pleural aspirate/Joint aspirates** - should be sent in sterile plastic universal container available from laboratory.
3. **Pus and other fluids** - send up to 20 ml in a sterile container.

15.2.3 Tissue Samples

1. **Bronchial biopsy specimens** - see Respiratory Section.
2. **Gastric biopsy** (Helicobacter pylori) - these should be sent in sterile saline (available from the laboratory) to the laboratory without delay. Samples should only be sent with prior arrangement with the laboratory
3. **Post-mortem material** - in sterile dry containers. These specimens should be taken as a separate procedure from those to be fixed for histological examination.
4. **Lymph nodes and other tissues** - send in sterile dry container.

15.3 Urine samples

15.3.1 General

- Urine containers are provided by the laboratory. These contain boric acid as a preservative. Urine containers should be filled to the mark on the container. The specimen will remain useful for up to 48 hours at room temperature.
- Small volumes of urine should be collected into Paediatric Urine bottles, which are available from the Pathology Consumables websites.

15.3.2 Collection of mid-stream urine

Instructions to the patients should be as follows:

1 Carefully wash the external genitalia and dry with a clean towel.
2 Unscrew the top of the sterile container, avoiding touching the inside of the bottle or lid.
3 Start passing urine, allowing the fisrt part to flow into the pan.
4 Collect the next part of the specimen straight into the container (women should separate the labia with the fingers of the hand which is not holding the sterile container). The bottle should be filled to the fill line.
5 Screw the lid on the container firmly. Wipe and dry the outside of the container and write your name and date on the label.

15.3.3 Collection of urine from infants

Bag Urine: External genitalia should be washed and dried before collection. Specimen should be decanted into a sterile plain universal.

Supra Pubic Aspirate: These should be decanted into a sterile plain universal - these are treated as sterile fluids and should be sent to the laboratory immediately

15.4 Incontinent Patients

Pads: Samples collected from incontinence pads are not acceptable sample, all samples should be repeated with a fresh sample. If it not possible to collect a fresh sample then clinical decisions should be made on symptoms/ assessments.

Indwelling catheter samples

- Urine should be aspirated with needle and syringe from the catheter sampling port after disinfection of the port with alcohol.

Urine for suspected tuberculosis

- At least 3 full early morning urine samples should be collected into large containers (do not use small boric acid pots).

15.5 Enteric samples

15.5.1 Faeces

- Clinical details should include history of food poisoning, foreign travel and recent/current antibiotics. If the sample is from an “in-patient” please state clearly whether the patient was admitted with diarrhoea or it developed after admission.
- Helicobacter pylori faecal antigen testing now performed - see below for details
- A “plum sized” portion of faeces or 5-10ml of liquid should be sent in the appropriate container.
- Stool specimens can readily be collected in an ordinary toilet if, after micturition and flushing of the pan, 6 pieces of non-absorbent toilet paper (not the soft type) or sheets made from newspaper are placed on the surface of the water before opening the bowels. Ample faeces should be found floating on the paper and a specimen may be lifted out using the plastic spoon provided with the container and placed in the clean container provided by the laboratory.

15.5.2 Examination for threadworm

- Sellotape slide impressions are required for the exclusion of threadworm infections.
- Specimens are best collected on waking. Separate buttocks and place a strip of sellotape across the anus and press firmly. Stick sellotape on microscope slide and transport to laboratory in a plastic slide container.

15.5.3 H. pylori faecal antigen testing

- Faecal samples are collected as above and sent to the laboratory. Do not request stool antigen tests within 2 weeks of using Proton Pump Inhibitors or within 4 weeks of using antibiotics as these drugs may suppress the bacteria and lead to false negative test results.

15.6 Respiratory samples

15.6.1 Sputum

- Deep expectorated sputum should be sent for examination in a sterile 60ml sputum container.
- Care must be taken to remove food debris from mouth before collection.
- All specimens are assessed before culture. Specimens received more than 48 hours old are normally not cultured. Examination for AAFB is performed generally only when requested or if clinical details are suggestive.

- Induced sputum for examination of *Pneumocystis jiroveci* (formerly *P.carinii*) PCR assays should be sent in 60ml Sputum container

15.6.2 Bronchial Washings/Biopsy

- These specimens should be collected in plain sterile containers (NO formalin). Biopsies for microbiology should be performed before those for histology.
- These should be sent immediately to the laboratory.

15.6.3 Pleural Fluids

- Samples should be collected aseptically into sterile universals
- These should be sent immediately to the laboratory.

15.6.4 Specimens for Tuberculosis and other Mycobacteria

- Tissue is preferred to swabs for TB examination. Specimens should be collected aseptically and placed in a CE Marked leak proof container without preservatives in a sealed plastic bag, and sterile distilled water added to prevent desiccation. A caseous portion should be selected if possible: the majority of organisms will be found in the periphery of a caseous lesion. Tissue biopsy specimens received in formalin are unacceptable and will not be processed
- Sputum specimens should be relatively fresh (less than 1 day old) to minimise contamination. Purulent specimens are best. Two to three samples of ≥5mL should be collected approximately 8-24 hours apart with at least one from early morning. Samples taken shortly after patient waking have the greatest yield. When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline ('sputum induction') before expectoration may be helpful. Note: Decontaminated and neutralised samples are not recommended as they may lose viability during transit to the laboratory. Bronchoalveolar lavage/bronchial washings may be sent if spontaneous or induced sputum is unavailable or if such specimens are AFB smear negative. Note: Contamination of the bronchoscope with tap water, which may contain environmental Mycobacterium species, should be avoided. Minimum sample size is preferably 5mL.
- Sterile site body fluids (CSF, pleural fluid, etc): Collect aseptically as much (for example >6mL in adults) CSF sample as possible into a CE Marked leak proof container in a sealed plastic bag. If only a small volume is available after initial lumbar puncture, and the findings of cell counts and protein suggest TB meningitis, a second procedure should be considered to obtain a larger volume to improve chances of achieving positive cultures. It should be noted that pleural or pericardial fluids are not very sensitive samples for the detection of M. tuberculosis, and that a concurrent pleural or pericardial biopsy taken with the fluid is more useful. A negative result on these fluids does not rule out the diagnosis.

- Urine specimens should be collected in the early morning on three consecutive days in a CE marked leak proof container (that does not contain boric acid), and placed in a sealed plastic bag. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.
- Pus or pus swabs should be collected aseptically, and the largest practical sample submitted in CE marked leak-proof container in a sealed plastic bag. Pus is the sample type of choice. Swabs are less preferable as mycobacteria, if present, may adhere to the swab rather than be transferred successfully to the culture media.
- Microscopy for Mycobacteria is performed on all specimens for Mycobacterial culture except urine and reported on the day of processing. Positive microscopy is telephoned immediately.
- Cultures are continued for 6 weeks, and occasionally up to 12 weeks depending on clinical details and specimen type. Positive cultures are telephoned immediately.
- All Mycobacterial isolates are sent to the Reference Laboratory for confirmation and, where appropriate, sensitivity testing.
- In agreement with the duty Consultant Microbiologist samples can be sent to the reference laboratory for PCR to detect *M tuberculosis* and resistance to some anti TB drugs in urgent cases.

15.6.5 Pernasal swabs

- Flexible Pernasal swabs with transport medium are recommended for patients with suspected whooping cough. These are available on request from the laboratory during routine hours.
- Patients with suspected whooping cough can be referred to the department by appointment to have swabs taken by medical staff. The use of “cough plates” may be considered where difficulty is encountered in obtaining a pernasal sample, please contact the laboratory for advice.
- Cultures for whooping cough are continued for five days.

15.6.6 Specimens for T Spot Test

- The testing laboratory is performed by Oxford ImmunoTec. This laboratory does not require prior booking and will accept samples sent Monday to Thursday.
- The required blood sample for adults consists of two 7.5ml Lithium heparin tubes (Orange top).
- The requestor must make arrangements to take the sample either on the ward, or in our phlebotomy area. The samples must be received by the Ipswich laboratory before 13:00 to allow overnight courier to Oxford. The request form must include the NHS number of the patient and the date and time the sample was taken.
- This service is available during weekends and Bank Holidays.

15.7 Swabs

15.7.1 Throat swabs

- The tonsils or tonsillar fossae should be swabbed. Take care not to touch mouth. Specimens should be sent in the normal charcoal transport medium for bacteriological investigation.
- Please record any foreign travel or suspected Diphtheria.
- For respiratory viral investigations use a Viral transport media swab.

15.7.2 Nose swabs

- The swab should be moistened with sterile water. Swab both anterior nares vigorously.
- Specimens should be sent in the normal charcoal transport medium for bacteriological investigation including MRSA carriage.
- For respiratory viral investigations use a Viral transport media swabs.

15.7.3 Swabs from Skin

- If area to be swabbed is dry, moisten swab with sterile water before sampling. Charcoal transport swabs should be sent in transport medium.
- NOTE: Swabs are inappropriate for dermatophyte examination, submit skin scrapings, tissue or nail using the mycology packs (black card envelopes) available from the Pathology Consumables Website.
- If swabbing vesicles for viral PCR please use a flocked swab and place the sample virus transport media swab.

15.7.4 Skin Ulcers

- Swabs should be sent in charcoal transport medium.
- Swabs should be taken from under the leading edge of ulcer skin, not from the centre of the ulcer.
- Antibiotic sensitivities are normally reported on primary pathogens - e.g. *Staph. aureus*, pyogenic streptococci, anaerobic bacteria and sometimes on pure growths of other bacteria where it appears appropriate. Topical antibiotics are not normally reported.

15.7.5 Ear Swabs

- Swabs should be sent in charcoal transport medium.
- If the ear is dry, moisten the swab with sterile water before sampling and send in transport medium.

15.7.6 Eye Specimens

- Examination for pyogenic infection – a charcoal bacterial swab is required.
- Examination for Chlamydia – Pus or exudate should be removed and the conjunctiva swabbed vigorously enough to remove epithelial cells. The correct chlamydia swab and transport medium must be used.
- Operative material/corneal scrapes should be sent by arrangement.
- Examination for Ancanthomoeba – The contact lenses and container should be sent by arrangement or a corneal scrape in saline.
- Examination for viral agents – a viral transport media swab is required.

15.7.7 Wound Swabs

- When available pus or fluid in a sterile dry container is always preferable to a swab. Clinical details **must** include anatomical site so that appropriate investigation can be performed. Swabs should be sent in charcoal bacteriological transport medium.

15.8 Genital samples

15.8.1 Genital Tract Specimens

- Swabs for bacteriology must be sent in charcoal transport medium.

15.8.2 Examination for Gonorrhoea by culture

- High vaginal swabs are examined for *N. gonorrhoeae* although endocervical and urethral swabs are required for the exclusion of *N. gonorrhoea.*
- Urethral swabs for *N. gonorrhoeae* should be sent in charcoal bacteriological transport medium.
- For oral investigation send a throat swab in charcoal bacteriological transport medium.
- For rectal investigation send a rectal swab in charcoal bacteriological transport medium.

15.8.3 Molecular examination for Chlamydia / N.gonorrhoeae

- An endocervical swab from females and a urethral swab from males should be taken from the patient using the special female or male Chlamydia / N.gonorrhoeae sample collection packs. The collected swabs must then be inserted to the bottom of the Chlamydia sample tube, the swab should be snapped off at the score line and the lid tightened securely.

- For females use the cleaning swab supplied in the sample collection pack to remove mucus or pus from the endocervical canal before the collection swab is inserted into the cervical canal and rotated for 15 seconds.
- For males insert the collection swab into the urethra and rotate for 5 seconds.
- The swabs must remove epithelial cells.
- Where the facilities to take a Cx swab are not available, a dry transport swab specifically for chlamydia is available (Self taken LVS/HVS swabs).
- First catch urines samples can be sent in either plain urine tubes or if large numbers are to be sent, urine preservation tubes can be used and are available from the laboratory.

The specific swabs and transport media available from the laboratory MUST be used for chlamydia investigations.

15.8.4 Examination of Vaginal Discharge

- High vaginal swabs collected under vision from the posterior fornix or cervical swabs should be sent in bacteriological transport medium. Swabs will be examined microscopically for the presence of pus, yeasts and "abnormal flora" indicating bacterial vaginosis, candida and bacterial cultures will be performed on all samples and *Trichomonas vaginalis* culture on selected samples.
- Pre-pubertal discharge-low vaginal swabs should be taken. These swabs are examined for *Neisseria gonorrhoeae* and other pathogens. Consider threadworms as a cause of symptoms.

15.8.5 Examination of IUCD for Actinomyces

- The IUCD if available in a sterile container should be sent with appropriate clinical details. Routine removal of IUCD's do not require to be sent and will not be processed if received.

15.8.6 Bartholin’s Abscess

- Send swabs in charcoal bacteriological transport medium.

15.8.7 Pouch of Douglas Fluid

- Send FLUID in a sterile universal container with relevant clinical details.

15.9 Vascular Cannulae

- Send cannula tip when phlebitis or other line infection suspected.
- Take a bacteriological swab from the cannula insertion site.

- Create a sterile field and clean exit site with alcohol - allow to dry. Remove cannula with sterile precautions. Do not allow the cannula to touch anything.
- Send the distal 5cm of line and proximal 5cm lying in the vein/artery in separate sterile labelled containers to the laboratory.
- When the specimens are collected with these precautions, a quantitative estimation of colonisation will be performed.
- If appropriate send the distal 5cm of drain to the laboratory in a sterile container.

15.10 Dermatophytes

Specimens usually consist of skin, nail or hair from infected areas. Ideally a black card mycology transport pack is used for transportation to the laboratory. These are available from the laboratory. If not, specimens should be placed in sputum pots.

Skin - First swab the infected area with an alcohol swab. Scrapings should be taken from the edge of a lesion with a blunt scalpel.

Nail - Send full thickness clippings and debris from beneath the nails.

Hair - Send hairs that fluoresce a greenish colour under Wood's light. Otherwise send lustreless hairs of those broken off at follicle mouths; extract affected hairs with forceps and any intrafollicular fragments with a Hagedorn needle.

Other - For other specimens such as sputum, pus, urine, blood, serum or biopsy material, standard methods of transmission are suitable. When delay in transmission of the specimen is inevitable consult the laboratory about the methods for the prevention of bacterial growth.

Microscopy results are reported on day of processing. Culture results follow in up to 3 weeks.

16 SPECIMEN CONTAINERS

16.1 Transport of Specimens

Regulations for external transport and local policies for internal transport must be followed.

16.2 Delivery of Specimens to the Laboratory

For Ipswich Hospital, all community specimens are delivered to the Blood sciences reception in pathology. At Colchester Hospital samples can be delivered to Microbiology unless it is outside normal opening hours when they should be left at Blood Sciences reception.

- For urgent samples telephone the laboratory so that the sample will be processed on arrival.

- For urgent samples outside of normal working hours, telephone the on-call Biomedical Scientist (BMS) for Microbiology via switchboard.
- Do not use the pneumatic tube system out of hours for any specimen that needs to be refrigerated, except by prior arrangement with the laboratory.

16.3 Performance characteristics of tests and assays

Please contact the laboratory for details of individual examination procedures and tests, such as:

- Principles of tests and methods
- Units of measurement
- Specific interfering factors
- Uncertainty of measurement (for results in measured quantity values)
- Biological reference intervals
- Clinical decision values
- Laboratory clinical interpretation
- Alert/critical values
- Potential sources of variation
- References

(It is intended to develop an accessible reference document containing these data)

16.4 Repertoire of Tests

Please refer to https: //esneftpathology.nhs.uk

17 QUALITY ASSURANCE

The laboratory adheres to standard principles of internal quality control (IQC), internal quality assurance (IQA) and external quality assessment (EQA).

- IQC uses materials of known characteristics. It relates to tests on the day and show that those assays are in control and that specific results are valid.
- IQA is an overall quality assurance measure that uses materials of unknown content, and shows, through regular repeat testing of a small percentage of samples, that the laboratory is able to obtain consistent results.
- EQA an overall quality assurance measure that uses materials of unknown content. The results are analysed by an external body and compared with national performance.

The laboratory participates in a full range of EQA (sometimes known as “inter-laboratory comparison”, or “proficiency testing”). Where poor or sub-optimal performance has occurred

or is expected, the team ensures action is taken to implement improvements via corrective and preventative actions. Records are kept on the Q-Pulse electronic QMS.

Details are available in the laboratory's Quality Manuals or on request.

18 OTHER SERVICES

18.1 Antibiotic assays

Please note that assays for Gentamicin and Vancomycin are tested in Blood Sciences

Details of expected results from serum monitoring and appropriate dosage modification and re-testing are given on the relevant NHS Trust pharmacy pages of the intranet.

19 TIME LIMITS FOR REQUESTING ADDITIONAL EXAMINATIONS

Bacteriology samples deteriorate after collection and requests for additional testing will only be considered by the Microbiology consultant within 2 days of collection. A freshly collected sample is preferable to repeat testing in most cases.

For further Virology requests please contact the laboratory directly or through the Pathology Helpdesk.