# Aims

To describe the repertoire of tests available in the AHC-COMRU microbiology laboratory.

To assist clinical staff in selecting the correct microbiology test and collecting appropriate high quality specimen(s):

* To minimise the number of clinically unhelpful results generated by the laboratory.
* To enhance the efficiency and cost-effectiveness of the laboratory.

# Principle

To ensure the best use of the laboratory, clinical staff should select the correct test and collect an appropriate sample for given clinical problem.

Submission of poor quality specimens with inadequate clinical information often generates laboratory results which do not inform patient management. Submission of such specimens also places strain on limited laboratory resources.

Inclusion of a suspected diagnosis or relevant history/examination findings always helps the laboratory staff process specimens in the best way. Absence of clinical information on the specimen request form may result in certain culture results being incorrectly labelled as “No significant growth”.

Specimen collection should be done only by trained staff, using appropriate personal protective equipment (gloves +/- mask, gown, and googles depending on the patient, specimen and suspected diagnosis). An aseptic / “no-touch” technique should be used to minimise specimen contamination.

# Specimen repertoire

**Note:** requirements for unusual specimens not included on this list should be discussed with the microbiology laboratory staff before collection to avoid problems in processing or interpretation.

| **Specimen type** | **Laboratory process** | **Comments** |
| --- | --- | --- |
| **Sterile site specimens: any growth potentially significant\*** | | |
| Blood | Culture for 7 days  Daily inspection  Manual sub-culture on days 1 & 7 | ***The volume of blood cultured is important:*** always send >1ml |
| Cerebrospinal fluid (CSF) | Cell count  Gram stain  Culture for 48 hours  ZN stain & TB GeneXpert ***if requested***  India ink stain ***if requested*** | If TB is suspected a large volume of CSF is required (see below): ***TB investigation should not be requested routinely on all specimens*** |
| Joint fluid and other normally sterile body fluids | Cell count  Gram stain  Culture for 48 hours  ZN stain & TB GeneXpert ***if requested***  Melioid culture ***if requested*** |  |
| Pus | Gram stain  Culture for 48 hours  ZN stain/ wet prep ***if requested***  Melioid culture ***if requested*** | Pus sent in a sterile container is ***always*** preferred to a swab |

*\*Collect all specimens before start of antimicrobial treatment if possible*

| **Specimen type** | **Laboratory process** | **Comments** |
| --- | --- | --- |
| **Non-sterile site specimens: may be contaminated by colonising organisms** | | |
| **1. Eye / Respiratory** | | |
| Ear swab\*\* | Gram stain  Culture for 48 hours | These specimens ***will not be processed*** without adequate clinical treatment information |
| Eye swab\*\* | Gram stain  Culture for 48 hours | If from a neonate, culture for *Neisseria gonorrhoeae* is also done |
| Gastric aspirates / sputum for TB | ZN stain (days 1, 2, & 3)  TB GeneXpert days 1 & 2 only | Young children cannot produce good quality sputum  GeneXpert cannot be used for TB treatment monitoring |
| Sputum / ETT aspirate\*\* | Gram stain  Culture for 48 hours  ZN stain & TB GeneXpert ***if requested***  Melioid culture ***if requested*** | ***These specimens usually have little clinical value*** as they are often heavily contaminated by colonising organisms |
| Throat swab | No Gram stain  Culture for 24 hours  Melioid culture ***if requested*** | Routine swabs only report growth of beta-haemolytic streptococci  Culture for *N. gonorrhoeae* is done if flagged as a suspected sexual abuse case  Culture for *Candida* sp., if flagged as an HIV or other immunocompromise case |
| **Gastro-intestinal tract** | | |
| Faeces | No Gram stain  Wet prep / ZN stain for parasites  Culture for 48 hours ***if requested*** | Culture will identify *Salmonella* sp. and *Shigella* sp. |
| **Skin / soft tissue** | | |
| Skin / pus / wound swab\*\* | Gram stain  Culture for 48 hours  Melioid culture ***if requested*** | Pus in a sterile pot is always preferred to a swab |
| **Uro-genital tract** | | |
| Penile / rectal swab | Culture for *Neisseria gonorrhoeae* only | Only cultured if flagged as from a suspected sexual abuse case |
| Urine | No Gram stain  Culture for 24 hours  Melioid culture ***if requested*** | Clean catch preferred to bag specimen |
| Urine for gonococcal infection | No Gram stain or culture: CT / GC GeneXpert only | Male suspected sexual abuse cases only |
| Vaginal swab | Gram stain  Culture for 48 hours  CT / GC GeneXpert ***if requested*** | Please label if from a suspected sexual abuse case |

\*\**For these specimens, consider treating empirically first and then only sending culture in case of empiric treatment failure*

# Further details on specific specimen types

## Sterile site specimens

Blood, CSF, and fluid from body compartments should be sterile: any growth is considered significant with a few exceptions. The laboratory will identify all organisms to species level where possible and do antimicrobial susceptibility tests on all significant isolates.

### Blood cultures

Thorough cleaning of the skin using chlorhexidine-alcohol (with prior washing with soap and water if the skin is visibly dirty), allowing the skin to dry after cleaning, and no palpation of the vein following cleaning will minimise the chance of introducing contaminating skin organisms into the blood culture bottle.

Following removal of the metal cap from the blood culture bottle, the rubber septum should be cleaned with 70% alcohol and allowed to dry.

The likelihood of detecting a bacteraemia is directly related to the volume of blood inoculated into the culture bottle. A blood to broth ratio of 1:5 to 1:10 is optimal. For the 20ml bottles used at AHC, this translates to 2 – 4 ml of blood per blood culture bottle. Inoculating <1ml of blood is highly likely to result in false negative results. The blood culture bottle should be inoculated beforefilling CBC, serum, or other blood tubes (to ensure enough blood is inoculated and to reduce the possibility of contamination).

Blood cultures should be transported to the laboratory as soon as possible after collection. If delays occur, the bottle should be kept at room temperature and not put in the fridge.

The laboratory will report all organisms identified in the culture. It usually takes 24 – 48 hours to issue a final report once a blood culture is recognised as positive, although certain organisms may take longer to fully identify.

Final culture results will be released after the day 7 sub-culture (i.e. on day 9, since the sub-culture takes 48 hours before being classed as negative) or as soon as a positive organism is fully identified and antimicrobial susceptibility testing is complete. Interim reports will be issued for the day 1 sub-culture result, day 2 inspection for growth, and as soon as a culture is positive.

All isolates will be considered significant and reported along with antimicrobial susceptibilities with the following exceptions:

* Coagulase negative staphylococci (non-neonates) and most Gram positive bacilli (e.g. *Corynebacterium* spp. and *Bacillus* spp.) will be reported as “probable contaminants: and antimicrobial susceptibilities will not be done.
* Coagulase negative staphylococci, environmental Gram negative organisms (e.g. *Acinetobacter* spp.), and yeasts will be reported as “uncertain significance”. In these cases, antimicrobial susceptibilities will be available on request if the organism is judged to be clinically significant(usually impossible to say without a repeat blood culture).

Common significant isolates at AHC include: *Salmonella* Typhi, *Streptococcus pneumoniae*, *Staphylococcus aureus,* coliforms (e.g. *Escherichia coli*, *Klebsiella pneumoniae*), *Burkholderia pseudomallei*, and beta-haemolytic streptococci (e.g. Group A Streptococcus).

### CSF cultures

The correct volume to send to the laboratory is noted in the table below. In all cases, at least 1ml of CSF, and preferably more, should be collected into three sterile containers following thorough decontamination of the skin. A blood glucose measurement should be obtained at the same time as the lumbar puncture.

The volume of CSF collected is important: larger volumes of CSF allow all lab tests to be done as well as possible (protein, glucose, Gram stain, culture, +/- JEV surveillance). It is particularly important to take large volume of CSF if TB is suspected: it may be necessary to repeat the LP if TB becomes a likely diagnosis only after the first specimen is collected. It is safe to take >1ml of CSF in all age groups(Thwaites et al. Journal of Infection (2009) 59, 167-187; see table below).

|  |  |  |  |
| --- | --- | --- | --- |
| **Age group** | **Routine CSF volume (ml)** | **Safe CSF volume (ml)** | **Safe CSF volume (drops)**  **[1 drop ~ 50-60 µl]** |
| Term neonate <1 month | 1-2 | 2-4 | 50 drops |
| Infant 1 month-1 year | 3 | 6-9 | 100-150 drops |
| >1 year-old | 4 | 8-11 | 130-180 drops |

CSF should be transported to the laboratory as soon as possible after collection, to ensure specimen integrity.

Routinely the CSF specimen will undergo glucose and protein estimation (main lab), Gram stain, and culture. ZN stain and TB GeneXpert are available on request, but the chance of these being positive is very dependent on the volume of CSF collected: these tests should not be requested routinely on all CSF specimens.

All organisms will be identified. Important pathogens are age-specific:

* **<2 months of age**
  + Group B streptococcus
  + *Escherichia coli*
  + *O*ther coliforms (e.g. *Klebsiella pneumoniae)*
  + *Listeria monocytogenes*
* **≥2 months**
  + *Haemophilus influenzae* (type B)
  + *Neisseria meningitidis*
  + *Streptococcus pneumoniae*
  + *Streptococcus suis*
* **Others**
  + *Cryptococcus neoformans*
  + *Mycobacterium tuberculosis*

### Sterile fluid / pus specimens

Fluid or pus collected into a sterile specimen container is always preferred to a swab specimen: there is a much higher chance of identifying significant infections from good volume pus specimens than if a swab is sent.

Clinical details help greatly in processing these specimens: clear information regarding suspected diagnosis and location of specimen (e.g. “pus from ruptured appendix” is much more helpful than “abdominal pus”).

Pus should be sent to the laboratory as soon as possible after collection, to ensure specimen integrity.

In general, all organisms isolated will be fully identified and antimicrobial susceptibilities performed.

TB GeneXpert can be requested on these specimens if the diagnosis is strongly suspected; however, this assay is not well validated on non-pulmonary specimens.

See also section 4.2.5, regarding suspected melioidosis.

## Non-sterile site specimens

In many cases, these specimens (in particular swabs) do not aid clinical management. Careful consideration should be given before collecting the following:

* Eye swab in non-neonates
* Ear swabs in all ages
* Endotracheal tube aspirates
* Skin swabs

### Eye / Respiratory

#### Eye swabs

Neonatal eye swabs are cultured to identify *Neisseria gonorrhoeae* in addition to routine pathogens (*Staphylococcus aureus*, beta-haemolytic streptococci, respiratory organisms (e.g. *Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae*,coliforms, *Pseudomonas aeruginosa).*

#### Ear swabs

Ear swabs are seldom helpful. Almost all ear swabs will grow a potentially pathogenic organism: an ear swab will not distinguish between otitis media and otitis externa.

* Otitis externa tends to be associated with *Staphylococcus aureus, Pseudomonas aeruginosa,* coliforms, yeasts and other fungi
* Otitis media is usually caused by *Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pneumoniae.*

The laboratory will reject ear swabs sent without clinical details and will only process specimens from OPD that indicate empiric treatment has already been tried.

#### Sputum / ETT aspirates

Young children cannot produce sputum that is not contaminated by upper respiratory tract organisms: it is rarely useful to send a sputum sample in children with suspected pneumonia.

Endotracheal tube aspirates (ETT aspirates) frequently grow colonising organisms are often of limited value in predicting the cause of ventilator-associated pneumonia.

See also section 4.2.5, regarding suspected melioidosis.

#### Sputum / Gastric aspirates for TB

Investigation of suspected pulmonary TB requires a combination of clinical, radiological, and laboratory tests. Gastric aspirates are used as a proxy for sputum in children too you to cough up good quality sputum specimens (this age group swallows sputum rather than expectorating it).

Daily early morning sputum or gastric aspirate specimens should be collected on three consecutive days in leak proof wide mouthed specimen containers.

The microbiology laboratory identifies acid-fast bacilli presence in the specimens by Ziehl-Neelson (ZN) staining. Specimens for days 1 and 2 (or 3 if one of the earlier specimens is insufficient) will also undergo MTB/RIF testing using the Cepheid GeneXpert system. This PCR-based system identifies the presence of *Mycobacterium tuberculosis* complex DNA and the presence of rifampicin resistance (a marker for multi-drug resistant [MDR] TB).

For monitoring of patients on TB treatment, sputum or gastric aspirate specimens will be investigated using ZN staining only. It is not possible to monitor treatment using the GeneXpert system.

#### Throat swabs

Throat swabs are routinely cultured to identify beta-haemolytic streptococci only (i.e. Groups A, C, and G streptococcus). If the specimen request form is appropriately labelled, swabs from:

* Suspected sexual abuse cases are also cultured to identify *Neisseria gonorrhoeae* infection.
* HIV cases or suspected candida infection are cultured to identify yeast infections.

See also section 4.2.5, regarding suspected melioidosis.

### Gastro-intestinal tract

#### Faeces

Faecal specimens should be collected into a sterile, leak-proof plastic container, taking care not to overfill the container*.*

Intestinal parasites will be identified by microscopy of stool specimens: non-pathogenic organisms (e.g. *Entamoeba coli*) will not be reported.

Stool specimens will be cultured to identify *Salmonella* spp. and *Shigella* spp. if requested. Antimicrobial susceptibilities will be reported routinely if these organisms are identified.

### Skin / Soft tissue

#### Skin / Pus / Wound swabs

Skin swabs for simple erysipelas, cellulitis, or boils frequently do not yield clinically useful information: these infections are usually caused by beta-haemolytic streptococci or *Staphylococcus aureus* and will respond to the empiric treatments outlined in the AHC antibiotic guidelines.

Swabs from complicated, unusual or treatment-resistant presentations should be collected using an aseptic technique and transferred to the laboratory without delay.

Clear clinical details should be included on the specimen request form, since the range of potential pathogens is large and varies by mechanism (e.g. bite wound or burn or chronic ulcer).

All potential pathogens (e.g. *Staphylococcus aureus* or Group A Streptococcus) will be reported along with antimicrobial sensitivities. Certain organism groups, e.g. coliforms such as *Escherichia coli*, will only be reported if growth is pure or heavy.

### Uro-genital tract

#### Urine

The preferred urine specimens are clean catch (young children) or mid-stream (older children): these specimens are less likely to be contaminated by skin organisms than bag specimens.

The laboratory processes the specimens in a quantitative manner, to enable significant growth to be detected. Urine culture results are reported as summarised in the table below.

See also section 4.2.5, regarding suspected melioidosis.

| **Culture result** | **Report** | **Interpretation** |
| --- | --- | --- |
| ***No bacterial growth*** | No growth | No UTI |
| ***Single organism*** |  |  |
| <104 CFU/ml | No significant growth | No UTI |
| 104 -105 CFU/ml | Growth of 104 -105 cfu/ml of…  *Antimicrobial sensitivities reported* | Possible UTI |
| >105 CFU/ml | Growth of >105 cfu/ml of…  *Antimicrobial sensitivities reported* | UTI |
| ***Two organisms*** |  |  |
| Both <105 CFU/ml | No significant growth | No UTI |
| One >105 CFU/ml | Mixed growth incl. >105cfu/ml of...  *Antimicrobial sensitivities reported for the dominant organism only* | Possible UTI or contaminated specimen |
| Both >105 CFU/ml | Mixed growth of >105cfu/ml of...  *All antimicrobial sensitivities reported* | Possible UTI or contaminated specimen |
| ***>2 organisms*** | Mixed growth of >2 organisms  *Antimicrobial sensitivities not reported* | Contaminated specimen |

#### Investigation of suspected sexual abuse

For males, a urethral and rectal swab (if anal penetration is suspected) should be sent for *Neisseria gonorrhoeae* culture. Other organisms will not be identified. A urine specimen may also be sent for *N. gonorrhoeae* and *Chlamydia trachomatis* testing by GeneXpert PCR.

For females, a vaginal swab should be sent for culture. In addition to *N. gonorrhoeae*, the laboratory will report the presence of other potential causes of discharge such as *Candida* spp. If other organisms are identified in heavy or pure culture (e.g. Group A Streptococcus, *Staphylococcus aureus*, respiratory organisms), they will be reported along with antimicrobial susceptibility results. A second vaginal swab may be submitted for *N. gonorrhoeae* and *Chlamydia trachomatis* testing by GeneXpert PCR.

### Melioidosis

In addition to standard bacterial culture, *Burkholderia pseudomallei* selective cultures can be set up on request for the following specimens: throat swabs, urine, pus samples. This additional culture work may take up to five days to yield a positive result.

Addition of a throat swab may significantly increase the diagnostic yield in suspected melioidosis cases.