1.0 GENERAL

Bacteriology is one branch of microbiology study for isolation and identification of causative agents as well as monitoring of disease.

2.0 SPECIMEN COLLECTION

Properly collected specimens should be sent to the lab as soon as possible without delay in order to avoid overgrowth of commensal or loss viability of pathogen.

Collect specimens before giving antibiotics. If antibiotics are given, please indicate in the request form.

Each specimen should be accompanied by fully completed PER-PAT 301 request form (except for test required special request form)

Specimen must be collected from actual infection site and must be collected with minimum contamination from surrounding material

2.1 Collection of Cerebrospinal Fluid (CSF) for C&S or Bacterial Antigen Detection.

i. Disinfect the skin over the lumbar spine with 2% iodine followed by 70% alcohol.
ii. Observing aseptic techniques perform a lumbar puncture and collect about 3 – 4 ml of CSF directly into sterile Bijou/sterile container.
iii. Send the specimen immediately to the laboratory. DO NOT STORE IN REFRIGERATOR, as organisms causing meningitis are usually very sensitive to cold.

2.2 Other Body Fluids for C&S

i. Transfer pleural, pericardial, peritoneal and synovial fluids aspirated aseptically to a sterile bottle and send to the lab without delay.

2.3 Eye Swab

i. Collect the exudates with a sterile swab.
ii. Dip the swab into Amies transport media and send it to the laboratory immediately.

For *Neiserria gonnorrhea*, please refer 2.18

iii. For corneal scrapping, directly inoculate on Blood Agar, Chocolate Agar, Mac Conkey Agar and fungal media (on request), send to the laboratory immediately.

2.4 Ear swab

i. Clean the areas with sterile swab moisten with sterile saline.
ii. Collect the exudates with a sterile swab.
iii. Dip the swab into Amies transport media and send it to the laboratory immediately.

2.5 Tissue, Pus and Swab

Send tissue and pus in sterile container.

Tissue is the preferable specimen for wound infection. Collect tissue and aseptically transfer into a sterile container. **DO NOT ADD FORMALIN SOLUTION**.

For swab:
i. Clean the skin over infected area by wiping with sterile saline or sterile distilled water
ii. Pass swab deep into lesion
iii. Dip the swab into Amies transport media and send it to the laboratory immediately.

2.6 Collection of Pus Aspirate

i. Disinfect the skin over the inflamed area with 2% iodine followed by 70% alcohol.
ii. With a sterile syringe, aspirate the pus or exudates and transfer the pus into a sterile universal bottle.
iii. Send the specimen immediately to the laboratory.

2.7 Stool

Note: Bedpans for collecting specimens for culture should be clean.

2.7.1 Fresh stool

i. Using a scoop/swab, collect a small amount of stool, taking care to include materials containing pus, mucus or blood if present and transfer into a clean container.

2.7.2 Rectal Swab

Note: Should only be taken if a stool specimen is not available. It is a less satisfactory specimen than stool.

i. Insert a swab deep into the anus so that the swab may come into contact with some faecal material. A satisfactory rectal swab should show some faecal material.
ii. Dip the rectal swab into Selenite F. Dip the rectal swab in Alkaline Peptone Water for suspected cholera cases.

2.8 Sputum for culture & sensitivity

i. Early morning sputum is the preferable specimen.
ii. Rinse the mouth.
iii. Gargle with water.
iv. Ask the patient to spit directly into a sterile container. Ensure that the expectorant is sputum and not saliva.
v. Send the specimen immediately to the laboratory.

Note: Send nasopharyngeal aspirate, tracheal aspirate or lung aspirate whenever possible or indicated. These specimens are more representative of the lower respiratory tract specimen.

2.9 Blood for culture & sensitivity

Collection procedure: Ensure aseptic technique.

i. Check medium for blood culture for gross contamination before use. Bottles with gross turbidity should be returned to the lab. Do not open cap of the culture bottle.
ii. Clean venepuncture site with 2% povidone followed by 70% alcohol applying in concentric fashion.

Note: Do not touch venepuncture site after disinfections unless the finger to be used for palpation has been similarly disinfected.

iii. Disinfected the top of the cap of culture bottle with 70% alcohol.
iv. Withdraw 8 – 10 ml of blood (for adults) or 3-5 ml of blood (for paediatric), then inject the blood directly into the bottle through the perforation in the bottle cap. Gently swirl to mix.
v. Send the specimen to the laboratory immediately.

vi. Obtained 3 blood culture sets at 3 different venepuncture sites within 24 hours with 1 hour interval for suspected infective endocarditis.

    Only one blood culture bottle should be inoculated at one time from one venepuncture site.

2.10 Nasal Swab for MRSA

i. The swab needs to be moisten with sterile saline before sampling.
ii. Rub the swab over the mucosa of the nasal cavity.
iii. Dip the swab in Amies transport media and send the specimen to the laboratory immediately.

2.11 Throat Swab for C&S

i. Insert swab carefully through the mouth with the tongue depressor.
ii. Rub swab over each tonsillar area and the posterior pharynx. Any area with exudates should be touched.
iii. Do not allow swab to touch tongue or lips.
iv. Dip the swab in Amies transport media immediately.

If diptheria is suspected, please call the laboratory.

i. Lift edge of the membrane and swab under it to search for deeply located lesion.
ii. Obtain at least two swabs and send to the lab immediately.
iii. Please note in the request form.

2.12 Nasopharyngeal Swab for C&S

i. This is especially useful for the diagnosis of whooping cough.
ii. A special swab mounted on a soft flexible wire is used (can be obtained from lab).
iii. Pass the swab softly through the nostril and along the floor of nasal cavity into the nasopharynx, rotate it and withdraw.
iv. For *Bordetella pertussis* PCR, after step (iii) insert swab into transport media immediately and send swab to the laboratory or store it at room temperature if it cannot be sent immediately to the lab (less than 24 hours).

2.13 Tracheal aspirate, bronchoalveolar lavage (BAL), bronchial washing

i. Aseptically transfer aspirate or washing into sterile container
ii. Send to lab as soon as possible.

2.14 Urine for cuture & sensitivity

i. Collect 5 – 10 ml midstream urine in a sterile container. Early morning urine is preferable to enable the organism to multiply in the bladder before collection. If not possible a period of 3 hours elapses after the last urination.
ii. Male patients should clean the gland penis with plain soap and water after retracting the foreskin.
iii. Female patients should similarly cleanse the labial folds and vulva.
iv. Discard the first portion of voided urine and collect the midstream directly into a sterile bottle.
v. For patient on urinary catheter, remove the catheter, clean the genital area, insert the new catheter and collect the midstream urine into a sterile bottle, **DO NOT COLLECT URINE FROM URINARY BAG.**

Note: The specimen should reach the laboratory within 2 hour after collection.

2.15 Endocervical swab

i. Clean cervical area from mucus and secretion with swab (use speculum without lubricant to visualize the cervix).
ii. Using new sterile swab, collect endocervical sample and dip the swab in Amies transport media and send the specimen to the laboratory immediately.
iii. For isolation of *Neiserria gonnorrhea*, please refer 2.18

2.16 High Vaginal Swab (HVS)

i. Clean the area from excess secretion or discharge.
ii. Obtain sample from mucosal membrane of vaginal with sterile swab.
iii. Place the swab in transport media and sent to the lab immediately.

2.17 Urethral swab

i. Insert swab into the anterior urethra.
ii. Leave the swab in place for few seconds .
iii. Place the swab in Amies transport media and sent to lab immediately.
iv. Proceed as 2.18 for gonococci.

2.18 Culture for Gonococci

i. Allow GC+LCAT agar (can be obtained from lab) to reach room temperature
ii. Collect the exudates from relevant sites with a sterile swab.
iii. Streak immediately onto GC+LCAT agar (can be obtained from lab)
iv. Send to the lab immediately.
v. Above specimen is to be accompanied by slide smear for gram stain.

2.19 Catheter tips

i. Clean the infected skin area with 70% alcohol
ii. Aseptically remove catheter and cut 3-5 cm of the tip into sterile container
iii. Send to the lab as soon as possible.

* This specimen should only be sent if suspected catheter-related infection upon request.

2.20 Tuberculosis

(Direct smear, culture or PCR detection)

2.20.1 Sputum

i. Sputum is preferably collected when the patient first wakes up in the morning. Rinse the mouth (gargle with water)
ii. Ask the patient to spit directly into a sterile universal bottle. **ENSURE THAT THE EXPECTORANT IS SPUTUM AND NOT SALIVA**.
iii. Send the specimen immediately to the laboratory. If delay is unavoidable, store at 2 - 8˚C.
iv. For sputum AFB, only 2 specimens collected at separate occasions are required.
v. Use gazetted AFB form

2.20.2 Other specimen

i. Collect aseptically and transfer into sterile container
ii. Send to the lab immediately. If delay is unavoidable, store at 2 - 8˚C.

2.20.3 Blood/bone marrow aspirate for TB

i. Collect 3-5 ml specimen aseptically as describe in 2.9
ii. Transfer the specimen into BACTEC MYCO F/LYTIC blood culture bottle (red cap).
iii. Send the specimen to the laboratory immediately. If delay is unavoidable keep the blood culture at room temperature.

2.21 *Clostridium difficile* toxin detection in stool

i. Collect fresh stool (approx. peanut-sized) in a sterile bottle.
ii. Send to the lab immediately.

* Please include the relevant clinical summary for this test as follow:
    a. Antibiotic-associated diarrhoea
    b. Colitis
    c. Pseudomonas colitis

2.22 Blood film for malaria parasites/filarial parasites

i. Disinfect the venepuncture site as explain in 2.9
ii. Collect 3-5 ml of blood in an EDTA tube
iii. Prepare a thick smear
    a. Clean new glass slide with alcohol.
    b. Air dry the slide
    c. Put a single drop of blood onto slide
    d. Using another glass slide as spreader, quickly spread the blood to make even thick film size of 1.5cm in diameter
iv. Prepare thin smear
    a) On the same slide, about 8 mm form the thick smear, drop one small amount of blood.
    b) Put the slide on flat surface, using another slide as spreader, touch the blood and allow the blood to run along its edge.
    c) Without removing the spreader, tilt it 45° and firmly push the spreader forward.

v. Air dry the slide and label it properly before place into plastic or container
vi. Send slide together with EDTA blood tube to the laboratory.

* Note:
a. Place the slide on printed paper. If the printed word can be read, the slide is suitable for processing.
a. If necessary, select the third finger or big toe for infants, clean it with 70% alcohol and dry with sterile cotton. Using sterile lancet, prick the finger or toe. Gently press and express first drop and wipe it away. Use the second drop as sample.
b. Sample is best taken when patient is having fever episode.

2.23 Blood film for filarial parasites

Sample is best taken at night (10pm – 12am). Send 3 – 5 ml blood in EDTA tube.

2.24 In-use Test For Disinfectants

Using aseptic technique, transfer 5 ml disinfectants into sterile screw cap container

2.25 Sterility test

Using aseptic technique, transfer 5 ml tested liquid into sterile screw cap container

2.26 Biological Indicator for Autoclave

i. Send Attest indicator that has gone through the autoclaving cycle together with an untreated Attest (control) to the lab.
ii. Ensure that both test and control Attest is from the same batch.

2.27 Environmental sampling

The test for environmental sampling must be requested through Infection Control team for outbreak investigation.

2.28 Urease test for *Helicobacter pylori* organism

i. Place tiny piece of tissue into urease medium (can be obtained from the lab)
ii. Positive: Read after 5 minute, medium turn to pink in colour
iii. Test is performed in endoscopy room

2.29 Mycology specimen

i. Hair: include affected hair with hair shafts intact
ii. Skin scrapping: After clean the affected area with alcohol swab, using blunt sterile scalpel, scrap the border of the lesion to obtain specimen
iii. Nail: Clip the nail and scrap the excess keratin produced beneath the nail
iv. Place specimen in a clean envelope (preferably black) or sterile container and send it to the lab. Store at room temperature if required.

2.30 Respiratory viruses for PCR

i. Obtain the collection ice box from lab
ii. Aseptically collect specimen from throat or nasal swab into viral transport media (VTM) using dacron swab or collect nasopharyngeal aspirate or tracheal aspirate into sterile container. Label the tubes properly.
iii. Seal the VTM with paraflim and place the sample into 3 layer of packaging with ice (can be obtained from lab) and seal the box. Attached 2 copies of request form outside the box and send to the lab immediately.

* Please refer to list of test for special request form for PCR test.

3.0 WORK SCHEDULE

24 hours working time

4.0 REPORTING OF CRITICAL RESULT

Critical result will be notified to the requester immediately. The following result is considered as critical result;

1. Gram stain positive blood culture
2. Blood Culture Positive
3. CSF/ Sterile body fluid positive
4. Multiresistant Organism eg. MRSA/ ESBL/ CRE positive
5. BFMP positive
6. AFB Positive smear

5.0 COMMUNICATION

i. Clinical consultation/ enquiries can be directed to the:

a. Clinical Microbiologist
Dr.Siti Hawa Hamzah ext. 157

b. Microbiologist
Puan Kamala Devi A/P Subramaniam ext. 166
Puan Nurizal Mat Zaid
Puan Hasanatunnur Azmi
Puan Tan Chwee Ming

ii. Results can be traced at ext. 166

6.0 SUMMARY OF SAMPLE TAKING AND PREPARATION

Name of specimen	Test	Container	Container obtained from	Specification/Comments
Blood	Blood/ Fungal/ AFB blood culture	BACTEC blood culture bottle	Microb ext 166	Adult : 8-10 ml of blood Paeds : 1-3 ml of blood
Body Fluid aspirate	Culture	Sterile container	Pathology counter	-
CSF	Culture/ AFB Culture	Sterile bijou bottle	Microb ext 166	-
Corneal Scrapping	Culture/ Fungal	Agar Plate (BA, Mac, Choc,fungal media	Pathology counter	-
High Vaginal swab	Culture	Amies	Microb ext 166	-
Pus, wound and swab	Culture	Amies	Microb ext 166	-
Respiratory specimen, Nose &Throat swab	Culture	Amies	Microb ext 166	-
Skin Scrapping, Nail, Hair	Culture/ Fungal Culture	Clean black paper/ Sterile container	Pathology counter	-
Rectal Swab	Stool Culture	- Selenite F - APW	Microb ext 166	-
Stool	Ova and Cysts, Occult blood, AFB	Clean container	Pathology counter	Send specimen immediately to lab
Tissue	Culture, Fungus culture, AFB	Sterile container	Pathology counter	-
Urethral, cervical & neonatal eye	Gonococci Culture	Smear onto GC agar	Microb ext 166	Send specimen immediately to lab
Urine	Culture	Sterile container	Pathology counter	-
Semen Fluid	Semen analysis	Sterile container	Pathology counter	Send within 30minutes of collection
CSF	Virus Isolation	Sterile bijou bottle	Microb Lab ext 166	Send specimen immediately to lab
Rectal swab	Enterovirus	VTM and swab	Microb Lab ext 166	Soaked sample swab in medium & shake swab briefly. Cut shaft and tighten cap. Send immediately
Respiratory swab , throat swab , nasopharyngeal swab, vesicle swab	Virus isolation	VTM and swab	Microb Lab ext 166	
Chlamydiae genital, eye	Chlamydiae antigen –IF method	Chlamydiae collection kit	Microb Lab ext 166	Send specimen immediately after fixation

* VTM: Viral transport media