1 Abbreviations

ACORN	A Clinically-Oriented Antimicrobial Resistance Network
AMR	Antimicrobial Resistance
BAL	Broncho-Alveolar Lavage
CAI	Community-Acquired Infection
CBC	Complete Blood Count
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
CXR	Chest X-Ray
GCS	Glasgow Coma Score
GLASS	Global Antimicrobial Surveillance System
HAI	Hospital-Acquired Infection
IgM	Immunoglobulin M
JEV	Japanese Encephalitis Virus
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
qSOFA	Quick Sepsis-related Organ failure Assessment
TB	Tuberculosis
UTI	Urinary Tract Infection
WHO	World Health Organization

2 Purpose

To summarise the key clinical / pre-analytic elements of diagnostic stewardship for antimicrobial resistance (AMR) surveillance in hospitalised patients. The laboratory aspects of diagnostic stewardship will be covered during the ACORN laboratory assessment and on-going monitoring.

The endpoint of diagnostic stewardship is to ensure that the right patients have the right tests at the right time and that the results of these tests are used to ensure that they receive the right treatment. Systematic testing of patients with suspected infection will result in data that can be used to formulate local treatment guidelines as well as be used for AMR surveillance activities.

3 Investigation of patients with suspected infection

Wherever possible, microbiologic specimens should be collected prior to starting antibiotics. Specimens for microbiologic testing should be collected by trained staff (see section 5) and should be sent to the laboratory for processing accompanied by a completed specimen request form (see section 6 for an example). Certain suspected aetiologies, e.g. melioidosis, may require additional diagnostic tests and notification of the laboratory for confirmation.

In the context of ACORN AMR surveillance, the simplest diagnostic stewardship advice for clinicians is to collect a blood culture in all patients in whom intravenous antibiotics are started for an acute illness. The blood culture should be taken before the first dose of antibiotics. Additional specimens should be collected on the basis of suspected clinical syndrome, for example:

- Meningitis – collect cerebrospinal fluid (CSF), if safe to do so;
- Pneumonia – consider collecting a sputum sample (adults) or a tracheal aspirate / broncho-alveolar lavage (BAL, intubated patient);
- Skin / soft tissue infection – collect a pus specimen or swab, if not able to collect pus;
- Urinary tract infection / Pyelonephritis – collect a urine specimen.

Specimens should be transported to the laboratory at ambient temperature without delay, ideally within 2 hours of collection. Examples of posters that could be displayed in clinical areas to assist with diagnostic stewardship are included in sections 7 (adult patients) and 8 (paediatric patients).

3.1 Definitions of community-acquired and hospital-acquired infection

Community-acquired infections (CAI) are those symptomatic on admission or within 48 hours of admission. Hospital-acquired infections (HAI) are defined by timing of onset, with Day 1 defined as day of hospital admission: Day 3 of admission onwards OR (Day 1-2 AND patient discharged from acute care hospital in preceding 48 hours) OR (Day 1-2 AND patient has relevant device inserted on this admission prior to onset).

3.2 Recognition and investigation of key infection syndromes

The following sections outline the major acute clinical infection syndromes and operational / clinical case definitions to aid recognition (see section 4 for references).

3.2.1 Sepsis / Severe Febrile Illness

Sepsis is a clinical syndrome defined as life-threatening organ dysfunction caused by a dysregulated host response to infection and is a medical emergency. There is no gold standard diagnostic test.

The original international sepsis-1 definitions included the inadequately specific systemic inflammatory response syndrome (SIRS). A score of two or more was indicative of sepsis:

- Temperature > 38°C or < 36°C
- Heart rate > 90/min
- Respiratory rate > 20/ min or $PaCO_2$ < 32 mm Hg (4.3 kPa)
- White blood cell count > 12,000 /mm^3 or < 4,000 /mm^3 or >10 % immature bands

Adult patients with suspected infection can be rapidly identified as being more likely to have poor outcomes typical of sepsis if they have at least two of the following clinical criteria that together constitute a bedside clinical score termed quick SOFA (qSOFA), which has been incorporated into the current international sepsis-3 definition.

The elements of qSOFA are:

- Respiratory rate of 22/min or greater
- Altered mentation (Glasgow Coma Score [GCS] < 15)
- Systolic blood pressure of 100 mmHg or less

The diagnosis of sepsis remains challenging in children. The UK Sepsis Trust “red flags” can be used to identify children with infection who are at risk of sepsis:

- Abnormal core temperature
  - < 36.0°C / > 38.5°C tympanic OR < 35.5°C / > 38.0°C axillary
- Inappropriate tachycardia
  - < 1y: ≥ 160 /min
  - 1-2y: ≥ 150 /min
  - 3-4y: ≥ 140 /min
  - 5y and above: ≥ 130 /min
- Altered mental state
  - GCS < 15 OR sleepiness, irritability, lethargy, floppiness
- Reduced peripheral perfusion or prolonged capillary refill time
  - Cold feet or hands OR capillary refill time ≥ 3 seconds

Also, for children aged < 5 years, "severe febrile illness" definitions* can also be used to identify children with infection who are at risk of sepsis:

- History of fever (or documented fever [≥ 38.0°C] / hypothermia [< 36.0°C]) **plus** any general danger sign:
  - Unable to drink or breastfeed
  - Vomiting everything
  - Convulsions
  - Lethargic or unconscious
  - Severe malnutrition

(*This definition is a composite of the WHO IB-VPD, IMCI 2014, and the LSTHM Fleming Fund WHO GLASS Road Map)

Sepsis in neonates (infants aged < 28 days old) may be suspected in those presenting with ≥ 1 of:

- Core temperature of > 37.5°C or < 35.5°C
- Respiratory rate of > 60/min
- Respiratory distress (severe chest in-drawing)
- Reduced movement
- Convulsion
- Poor feeding

Sepsis is strongly associated with bacteraemia. All patients suspected to have sepsis should have at least one blood culture taken. Additional investigations should be requested depending on the clinical presentation and availability e.g. chest x-ray (CXR) or other imaging, urine, sputum or wound/pus cultures, serum lactate, complete blood count (CBC), biochemistry, c-reactive protein (CRP), procalcitonin (PCT), echocardiography (if endocarditis suspected). In certain geographic locations testing for additional pathogens (e.g. malaria, dengue) may be recommended.

3.2.2 Pneumonia

Pneumonia is infection of the lung tissue.

Pneumonia should be suspected in adult patients presenting with i) symptoms of an acute lower respiratory tract illness (cough and at least one other lower respiratory tract symptom), ii) new focal chest signs on examination, iii) at least one systemic feature (either a symptom complex of sweating, fevers, shivers, body aches and/or core temperature of ≥ 38°C).

In children aged < 5years, WHO clinical criteria can be used to identify children with suspected severe pneumonia:

- Cough and/or difficulty breathing **plus** ≥ 1 of:
  - Central cyanosis
  - Oxygen saturation < 90% (in air, at sea level)
  - Severe respiratory distress (grunting, indrawing)
  - General danger sign (see "Sepsis / Severe Febrile Illness" section)

In children 5 years and older, pneumonia presents similar to disease in adults.

In all ages, ventilator associated pneumonia is difficult to confirm. Operationally it may be suspected in patients ventilated for ≥48 hours with:

- Increased ventilator requirements **OR** New onset of purulent sputum / Change in character of sputum
  AND
- Fever (> 38°C) / Hypothermia (< 36°C) **OR** Raised (≥ 12,000 /mm^3) / low WBC (≤ 4,000 /mm^3) **OR** New CXR changes
  AND

- Antibiotic started or changed
  AND
- No other likely explanation for deterioration

All patients hospitalised with suspected pneumonia should have a blood culture taken. A CXR is useful to confirm the diagnosis (new radiographic shadowing for which there is no other explanation). A sputum specimen should be submitted for culture in adult patients with a productive cough. In intubated patients, tracheal aspirates or BAL specimens should be cultured if clinically indicated e.g. severe community-acquired pneumonia or suspected ventilator-associated pneumonia. Tuberculosis (TB) and atypical or opportunistic infections should be considered in the differential diagnosis.

3.2.3 Meningitis

Meningitis is inflammation of the meninges surrounding the brain and spinal cord and is a medical emergency.

In adults and older children, meningitis should be suspected in patients with sudden onset fever and signs of meningism:

- Classical signs: Headache, stiff neck, fever and chills, vomiting, photophobia, confusion, seizures, drowsiness
- Less common: Focal neurological signs

In children aged < 5 years, meningitis should be suspected in patients with sudden onset fever (core temperature ≥ 38.0°C) **plus** ≥ 1 of the following:

- Neck stiffness
- Altered consciousness
- Other meningeal sign (e.g. Kernig / Brudzinski sign)

Patients with suspected meningitis should have a blood culture taken. A lumbar puncture to obtain CSF should be performed if no clinical, laboratory or radiographic contraindication is present. A specimen of CSF should be sent to the microbiology laboratory for microscopy and culture. Results should be interpreted with biochemical testing if available (CSF protein and glucose relative to plasma glucose). Further testing on CSF depends on local epidemiology, clinical suspicion and/or availability (e.g. Japanese Encephalitis Virus (JEV) IgM, bacterial or viral PCRs, auramine or Ziehl-Neelsen stain, TB culture, India ink stain, fungal culture). Other blood tests that may be useful include rickettsia and dengue screening, serum cryptococcal antigen (if cryptococcal meningitis suspected).

3.2.4 Urinary tract infection / pyelonephritis

In adults and older children, upper urinary tract infection (UTI, pyelonephritis) presents with

- Supra-pubic/renal angle tenderness **plus** ≥ 1 of:
  - Systemically unwell
  - Fever (temperature ≥ 38.0°C)
  - Dysuria
  - Frequency
  - Haematuria

Urinary tract infections often present non-specifically in young children. Consider urinary tract infection in infants and children with:

- Fever (≥ 38°C) for at least 24 hours without obvious cause
- Vomiting or poor feeding
- Irritability, lethargy, failure to thrive, abdominal pain, jaundice (neonates)

- Specific symptoms such as increased frequency, pain on passing urine, renal angle tenderness

Catheter-associated UTI should be considered in patients with an in-dwelling urinary catheter **AND** symptoms consistent with a UTI.

3.2.5 Skin / soft tissue infection

Skin (e.g. erysipelas, cellulitis) and soft-tissue infections (e.g. abscesses, necrotising fasciitis) present with ill-defined diffuse swelling of the skin and subcutaneous tissues with redness, tenderness, and warmth **plus** ≥ 1 of:

- Systemically unwell
- Fever (temperature ≥ 38.0°C)
- Red streaks or tender lymph nodes
- Spread to involve significant body surface area

Patients with increased risk of severity or complications include:

- Immunosuppressed
- Involvement of genitals, hands, or face
- Very young or very old

4 Useful references

The following were used as source documents for this guideline and contain extra details, which may be useful for implementation.

WHO-GLASS. Diagnostic stewardship. A guide to implementation in antimicrobial resistance surveillance sites (WHO/DGO/AMR/2016.3). World Health Organization; 2016:

- https://apps.who.int/iris/bitstream/handle/10665/251553/WHO-DGO-AMR-2016.3-eng.pdf?sequence=1

AMR Surveillance in low- and middle-income settings: A roadmap for participation in the Global Antimicrobial Surveillance System (GLASS): London School of Hygiene & Tropical Medicine; 2016:

- https://researchonline.lshtm.ac.uk/id/eprint/4574689/.

UK Sepsis Trust:

- https://sepsistrust.org/professional-resources/clinical/

WHO Coordinated Invasive Bacterial Vaccine Preventable Diseases (IB-VPD) Surveillance Network Case Definitions (Updated January 2012): World Health Organization; 2012:

- http://www.who.int/immunization/monitoring_surveillance/resources/IB-VPD_Case_Defs.pdf?ua=1

WHO Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources (2nd edition): World Health Organization; 2013:

- https://www.who.int/maternal_child_adolescent/documents/child_hospital_care/en/

Standards for microbiology investigations (UK SMI):

- https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

5 Appendix A - Specimen collection procedures

These recommendations are intended for staff already familiar with conducting these sampling procedures and are meant to give additional information on ensuring sample sterility and using good microbiological practice to do so.

General comments:

- Use sterile containers. Always check sterility (unopened) and expiration date
- Use adequate personal protective equipment (PPE) as per protocol
- With the exception of blood culture bottles, if samples cannot be analysed at the laboratory immediately sample can be refrigerated for a maximum of 48 hours prior to analysis

Blood	
Preparation and Procedure	• Identify a vein via inspection and palpation. • Clean the skin with an appropriate skin antiseptic solution. • Allow the skin to dry. • Draw blood without re-palpating the vein. • Remove the cap of the blood culture bottle(s) and clean with 70% ethanol. • Inoculate blood into the bottle(s).
Storage / Transport requirement	• Transport inoculated blood cultures to the microbiology laboratory without delay. • **If delays occur, keep the blood culture at room temperature (do not refrigerate or incubate).**

Additional details	• It is recommended to inoculate both an aerobic and an anaerobic culture bottle per adult blood culture set:
	  • A volume of 20-30ml of blood per set is recommended for adults (check manufacturer's instructions).
	  • Whenever possible, more than one set from different venepuncture sites should be taken.
	• For children, a single aerobic bottle is usually considered adequate. Most paediatric bottles require 1 – 4ml blood. The appropriate volume of blood to be cultured can be determined from the child's age and weight, to a maximum of 1% of the blood volume.

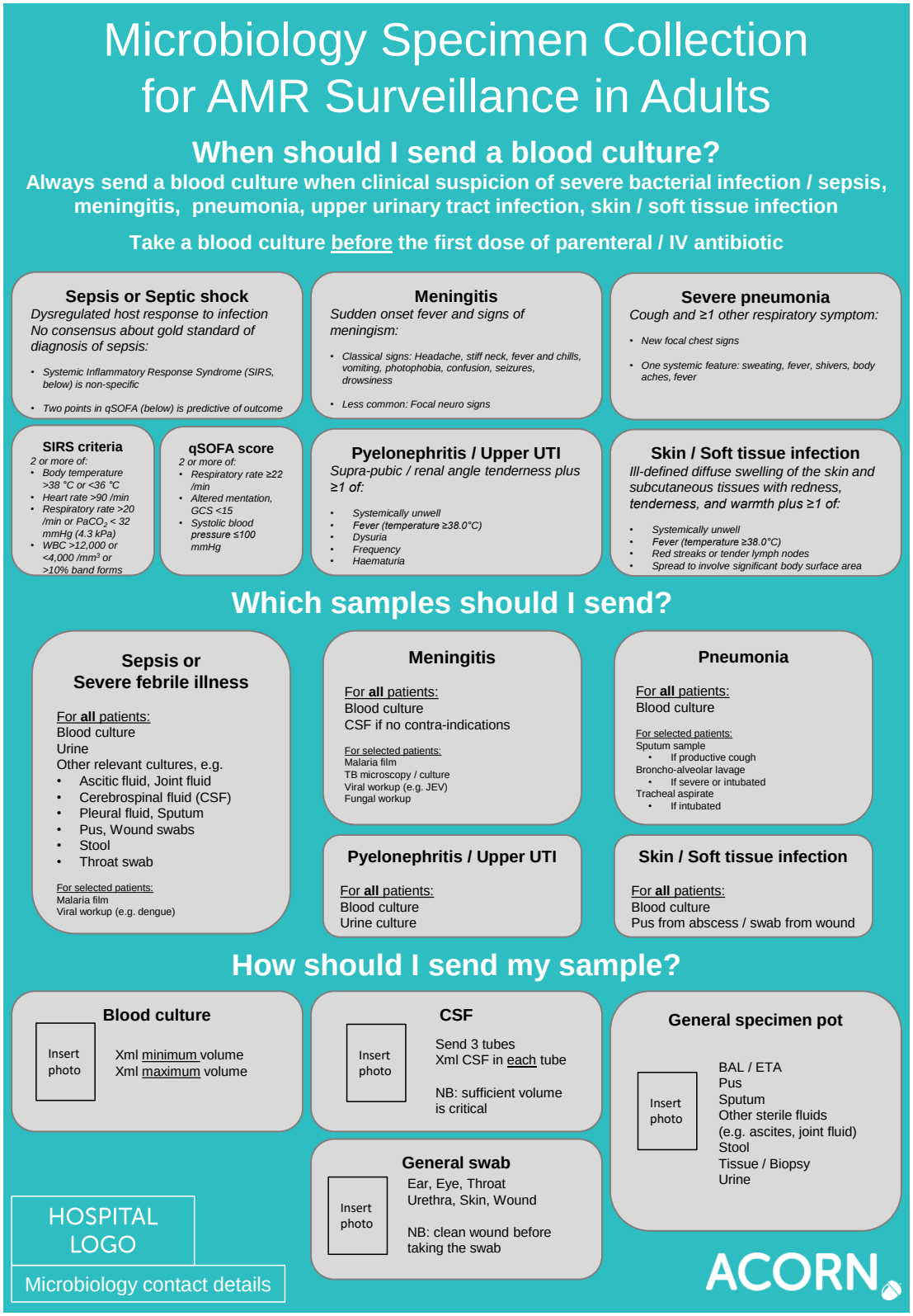
Cerebrospinal fluid (CSF)	
Supplies required	• 3 CSF containers. • Sterile lumbar puncture set.
Preparation and Procedure	• Create sterile field covering patient and bed with an opening over site of insertion • Use local anaesthetic per protocol. • Clean the skin with antiseptic solution. • Allow the skin to dry. • Do not touch the skin again while inserting the needle. • Depending on availability of manometers, measure CSF opening pressure first. • Allow CSF to drip in 3 sterile containers (haematology, biochemistry and microbiology).
Storage / Transport requirement	• Transport containers to the laboratory without delay for STAT diagnostics and incubation.
Additional details	• In children a minimum 3x1ml is recommended, for adults a total of 8-15ml is recommended. Especially for culture and TB diagnostics larger volumes (10ml or more) increase diagnostic yield.

	• A sufficient volume is crucial. It is unethical to expose a patient to the intrinsic risks of the procedure and not take sufficient sample volume for adequate diagnostics.
Sputum	
Preparation and Procedure	• Use a specific area for patients to collect sputum to minimise risk of transmission, especially if there is a risk of tuberculosis. • Care must be taken in the sample collection process to ensure that the sample is from the lower airways and not from the upper respiratory tract / saliva. • Consider using sputum induction with saline if no sputum is produced. • Alternatives in severe cases are bronchoalveolar Lavage, an invasive specialised procedure, or taking endotracheal aspirates when patients are intubated for ventilation.
Additional details	• Before Gram-staining and inoculation perform a wet stain and observe at 10x10 to ensure the sample contains white blood cells and little or no (<25) epithelial cells. NB: Some immunocompromised patient populations may not produce white blood cells.
Urine	
Preparation and Procedure	• Patients should be instructed in how to collect midstream urine ("clean-catch" urine) in a sterile container in order to minimize contamination. • In pre-continent infants obtaining a midstream or 'clean catch' specimen can be challenging. For infants aged one to 12 months, the 'Quick-wee' method can be considered to increase the voiding and success rate of a 'clean-catch' urine. This method uses gentle cutaneous suprapubic stimulation with gauze soaked in cold 0.9% saline to trigger faster voiding. • Urine collection pads or urine collection bags are often used for incontinent or non-toilet trained children, but are more susceptible to contamination due to close contact with the anogenital area. NICE guidelines suggest urine collection pads as the next best option to clean catch. When using urine collection pads, manufacturer's instructions should be followed. • Alternatively, urine can be sampled by sterile urethral or suprapubic catheterisation. Ultrasound guidance should be used to indicate the presence of urine in the bladder before a suprapubic aspirate is attempted. • Taking urine from catheters that are already in situ should follow specific protocols dependent on the type of catheter, while avoiding introduction of bacteria into the specimen and the catheter / urinary tract of the patient.
Pus	
Preparation and procedure	• Pus can be collected from a variety of different sites. When collecting pus from an unopened vesicle, abscess or empyema use a sterile syringe and empty into a sterile container. Larger volumes have higher yields. • Pus from already opened wounds or drained abscesses can be collected as wound swabs or from the drain system in situ following manufacturer's instructions.
Wound / Skin swabs	
Preparation and Procedure	• Remove wound dressing and rinse wound with sterile saline (do not use disinfectant). • Swab the wound by gently rotating a sterile swab between your fingers. Swab the wound from margin to margin in a zigzag fashion. Use enough pressure to express fluid from within the wound tissue.

6 Appendix B – Specimen request form

Microbiology Laboratory Request Form				
Patient details				
Identification Number		**Gender**	☐ Female ☐ Other	☐ Male ☐ Unknown
Family name		**Given name**		
Date of birth (dd-mmm-yyyy)	|___|___| – |___|___|___| – |___|___|___|___|			
Age (if DOB unknown)	|___|___| Years |___|___| Months (if <1 year) |___|___| Days (if <1 month)			
Ward / Clinic				
Clinical details				
Suspected diagnosis				
Other details				
Has the patient been hospitalised for more than two calendar days at the time of sampling?	☐ Yes ☐ No			
Specimen details				
Specimen type	☐ Blood ☐ CSF ☐ Sputum	☐ Urine ☐ Pus (specify below) ☐ Other (specify below)		
Specimen details (e.g. location)				
Date of specimen collection (dd-mmm-yyyy)	|___|___| – |___|___|___| – |___|___|___|___|			
Specimen collected by				
[Enter laboratory location and contact details here]				

7 Appendix C – Specimen collection poster (adult patients)



8 Appendix D – Specimen collection poster (paediatric patients)

Microbiology Specimen Collection For AMR Surveillance in Children

When should I send a blood culture?

Always send a blood culture when clinical suspicion of severe bacterial infection / sepsis
Take a blood culture before the first dose of parenteral / IV antibiotic

Sepsis
Dysregulated host response to infection
Features to alert suspicion ("red flags"):
- ***Abnormal core temperature***
  < 36.0°C / > 38.5°C (tympanic) OR
  < 35.5°C / > 38.0°C (axillary)
- ***Inappropriate tachycardia***
  <1y: ≥ 160 /min 1-2y: ≥ 150 /min
  3-4y: ≥ 140 /min 5y and above: ≥ 130 /min
- ***Altered mental state***
  GCS < 15 OR sleepy, irritable, lethargic, floppy
- ***↓peripheral perfusion or ↑capillary refill time***
  Cold feet or hands OR CRT ≥3 sec

Neonatal sepsis
Patient < 28 days old plus ≥ 1 of:
RR > 60 /min *T > 37.5°C or T < 35.5°C*
Respiratory distress *Reduced movement*
Convulsion *Poor feeding*

Severe febrile illness
Fever (T ≥ 38.0°C) or hypothermia (T < 36.0 °C) plus any danger sign:
- *Unable to drink or breastfeed*
- *Vomiting everything*
- *Convulsions*
- *Lethargic or unconscious*
- *Severe malnutrition*

Meningitis
Sudden onset fever (T ≥ 38.0°C) plus ≥ 1 of:
- *Neck stiffness*
- *Altered consciousness*
- *Other meningeal sign (e.g. Kernig / Brudzinski sign)*

Severe pneumonia
Cough or dyspnoea plus ≥ 1 of:
- *Central cyanosis*
- *O_2 saturation < 90% (in air)*
- *Severe respiratory distress*
- *Danger sign*

Pyelonephritis / Upper UTI
UTI often present non-specifically in young children. Consider in children with:
- *Fever (T ≥ 38°C) for ≥ 24 hours without clear cause*
- *Vomiting or poor feeding*
- *Irritability, lethargy, failure to thrive, abdominal pain, jaundice (neonates)*
- *Specific symptoms, e.g. increased frequency, pain on passing urine, renal angle tenderness*

Skin / Soft tissue infection
Ill-defined diffuse swelling of the skin and subcutaneous tissues with redness, tenderness, and warmth plus ≥1 of:
- *Systemically unwell*
- *Fever (T ≥ 38.0°C)*
- *Red streaks or tender lymph nodes*
- *Spread to involve significant body surface area*

Which other samples should I send?

Sepsis or Severe febrile illness
For **all** patients:
Blood culture
Urine
Other relevant cultures, e.g.
- Ascitic fluid, Joint fluid
- Cerebrospinal fluid (CSF)
- Pleural fluid, Sputum
- Pus, Wound swabs
- Stool
- Throat swab

For selected patients:
Malaria film
Viral workup (e.g. dengue)

Meningitis
For **all** patients:
Blood culture
CSF if no contra-indications

For selected patients:
Malaria film
TB microscopy / culture
Viral workup (e.g. JEV)
Fungal workup

Pneumonia
For **all** patients:
Blood culture

For selected patients:
Sputum sample
- If productive cough (older children only)

Broncho-alveolar lavage
- If severe or intubated

Tracheal aspirate
- If intubated

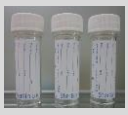
Pyelonephritis / Upper UTI
For **all** patients:
Blood culture
Urine culture

Skin / Soft tissue infection
For **all** patients:
Blood culture
Pus from abscess / swab from wound

How should I send my sample?

Blood culture

1ml minimum volume
4ml maximum volume

CSF

Send 3 tubes
1ml CSF in each tube

General swab

Ear, Eye, Throat
Genital, Skin, Wound

Remember to **clean** a wound before taking the swab

General specimen pot

BAL / ETT aspirate
Pus
Sputum
Sterile fluids
(e.g. ascites, joint fluid)
Stool
Tissue / Biopsy
Urine

HOSPITAL LOGO

Microbiology contact details