REPUBLIC OF KENYA

DIAGNOSTIC STEWARDSHIP

A CLINICIAN'S HANDBOOK ON APPROPRIATE USE OF MICROBIOLOGIC DIAGNOSTIC TESTS

Foreword

Antimicrobial resistance (AMR) is an increasing global threat that calls for collaborative action among various stakeholders in the health sector.

Kenya has been at the forefront of the global fight against antimicrobial resistance. Following alarming rates of antimicrobial resistance in both human and animal health, the Global Action Plan on AMR was adopted in 2015 after the World Health Assembly, the FAO Governing Conference and the World Assembly of OIE Delegates agreed to jointly combat AMR. Member states committed to develop national actions plans on AMR consistent with the Global Action Plan.

In 2014 the National Antimicrobial Stewardship Advisory Committee was formed to coordinate the AMR agenda. Their efforts culminated in the development of the AMR Policy and National Action Plan (NAP).

The NAP is anchored on the following key strategic objectives: to improve awareness and understanding of antimicrobial resistance; to strengthen knowledge through surveillance and research; to reduce the incidence of infection; to optimize the use of antimicrobial agents; and to ensure sustainable investment in countering antimicrobial resistance.

In order to support AMR surveillance and ensure ensure the most appropriate choice of antibiotics for patients, it is essential that clinical teams work closely with laboratory teams to ensure optimal use of microbiology services.

The Clinicians handbook on diagnostic stewardship will go a long way in guiding clinical teams on appropriate sample collection for microbiology and enhance the processing, reporting and interpretation of bacteriology results, ultimately improving patient outcomes.

Dr. Rashid Aman, PhD

Chief Administrative Secretary

Ministry of Health

Preface

Diagnostic stewardship is the process of co-ordinating clinical and microbiologic work to ensure that appropriate samples are collected, transported to the lab, process and accurate results availed in a timely manner. This is a process that is essential in ensuring that patients receive the most appropriate antimicrobial for their infection and promotes good clinical outcomes.

Diagnostic stewardship supports the development of guidelines for antibiotic use by ensuring that the data generated by microbiology labs is accurate and credible. This goes a long way in supporting antimicrobial stewardship programs.

This handbook provides guidance on appropriate identification of infection, appropriate collection, handling and processing of samples to ensure that resulting culture and antibiotic susceptibility results are correctly interpreted. The handbook also gives pointers to appropriate empiric antibiotic prescribing even as laboratory results are awaited.

Dr. Patrick Amoth,

Ag. Director General for Health

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What is "diagnostic stewardship"?

Diagnostic stewardship is defined as:

"coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. It should promote appropriate, timely diagnostic testing, including specimen collection, and pathogen identification and accurate, timely reporting of results to guide patient treatment." [1]

The objectives of microbiological diagnostic stewardship are:

- To improve patient management by providing accurate microbiological data in a timely manner.
- To collate accurate and representative AMR surveillance data to inform development of antibiograms, treatment guidelines, and AMR control strategies.

Diagnostic stewardship is a central component of antibiotic stewardship programmes and is also important for infection prevention and control activities in health-care facilities. By getting correct microbiological results in good time, clinicians are able to select the most appropriate antibiotics or antibiotic combinations for their patients, as well as put in place precautions to reduce nosocomial transmission of resistant organisms.

Having well equipped labs with the capacity to isolate, identify and accurately report pathogenic bacteria in a timely manner is essential. To make the most out of labs, it is essential that clinical teams work closely with laboratory teams. Diagnostic stewardship embraces all stages of the diagnostic process in clinical microbiology and laboratory management: begins with early and proper recognition of infection and describes the procedures that guide appropriate specimen selection, collection and the completion of clinical, demographic and epidemiological data that must accompany each specimen; it includes the correct storage and transportation of specimens to the laboratory; it covers how laboratories receive, register and process specimens, including how appropriate tests are selected and performed; and it extends subsequently to how results are reported and interpreted and then used to guide patient management. How well available resources are used determines the success of each stage of diagnostic stewardship.

Implementation of diagnostic stewardship within health-care facilities requires institutional commitment and support. This guideline will complement the work of antimicrobial stewardship and infection control committees within health-care

facilities. It should be used alongside the national guidelines for Antimicrobial stewardship, Antimicrobial stewardship and Infection control and prevention. [2]

1. WHO Diagnostic stewardship. Guide to implementation in AMR surveillance sites

2. Dik JW, Poelman R, Friedrich AW, Panday PN, Lo-Ten-Foe JR, van Assen S et al. An integrated stewardship model: antimicrobial, infection prevention and diagnostic (AID). Future Microbiology 2015 Sep 1

Overall goal

Optimal patient care depends on effective communication between personnel at points of clinical care and microbiology laboratories through strengthening of the lab- clinical interface. The purpose of this clinician's handbook is to provide information for the clinicians on the microbiology laboratory services available, the types of specimens to submit to the laboratory for analysis and how to effectively use the results for optimal patient outcomes.

Target audience:

All health care workers involved in patient care, this includes clinicians, pharmacists, nurses, Infection prevention and control personnel, laboratory personnel as well as health care managers and administrators.

ROLE OF CLINICIANS

- Identifying patients with suspected infections
- Determine appropriate tests
- Completing the detailed laboratory request forms to include relevant clinical information, patient biodata and source of sample
- Obtaining appropriate number and types of clinical samples as indicated by the clinical presentation before initiation of empiric antibiotics
- Reviewing and appropriately interpreting the laboratory results and consulting with the microbiology or infectious disease teams where necessary.
- Choosing the most appropriate antibiotic regimen based on the culture and sensitivity results

Identifying patients with suspected infections

Patients with suspected bacterial infections may have the following clinical findings

- Fever
- Leucocytosis with neutrophilia and toxic granulation
- Raised inflammatory markers e.g. CRP, ESR, procalcitonin
- Specific organ dysfunction (tachypnoea, dysuria, inflamed skin, stiff neck, diarrhoea) - this helps to identify the focus of infection and determine the most appropriate samples to collect for microbiology.

A thorough history and physical examination is key in identifying the focus of infection

Examples of presentations of common bacterial infections are shown in the table below

Infection	History	Physical exam	Appropriate diagnostic investigations	Appropriate samples for microbiology	Infectious mimics
Pneumonia	Cough, rusty sputum, pleuritic chest pain, fever, difficulty breathing	Fever, tachypnoea ,other features of respiratory distress, brachial breath sounds, crackles	Chest Xray	Blood culture, Sputum for AAFBs and/or gene Xpert	Bronchitis, Pulmonary TB
Bacterial Meningitis	Acute onset Fever, headache, photophobia	Fever, stiff neck, fundoscopy to rule out papilloedema	Lumbar puncture, CT scan of the brain if there are features of raised intracranial pressure or space occupying lesion	CSF for microscopy and culture	Viral encephalitis
lower Urinary tract infection (cystitis)	Dysuria, frequency, foul smelling urine, suprapubic pain	Suprapubic tenderness	Urinalysis	Urinalysis **consider urine culture in recurrent UTI	PID

Infection	History	Physical exam	Appropriate diagnostic investigations	Appropriate samples for microbiology	Infectious mimics
Ascending urinary infection (pyelonephritis, urosepsis	Dysuria, frequency, foul smelling urine, suprapubicpain, back pain, fever, chills and rigour	Fever, renal angle tenderness	Urinalysis	Urinalysis, urine culture *avoid taking urine from an indwelling urethral catheter or urine bag	PID
Urethritis in males	Dysuria, sexual history, prior STI history			Urethral swab	UTI
Pelvic inflammatory disease (PID)	abnormal bleeding, dyspareunia, vaginal discharge, lower abdominal pain, fever, and chills	fever, abnormal cervical or vaginal mucopurulent discharge, uterine or adnexal tenderness and cervical motion tenderness	Pelvic ultrasound	High vaginal swab for microscopy and culture, consider multiplex PCR for Chlamydia and gonococcal organisms	

Infection	History	Physical exam	Appropriate diagnostic investigations	Appropriate samples for microbiology	Infectious mimics
Blood stream infection	Fever, chills, possible indwelling catheters	Thorough history and physical exam may identify source of infection	Blood culture	Blood	
Skin and skin structure infections	Pain, swelling, warmth	Breaks in the skin, warm and tender skin, pustules or blisters	Often a clinical diagnosis. Pustules and blisters may be directly aspirated	Aspirates for microscopy, culture and sensitivity, tissue culture *avoid superficial swabs of wounds	
Bone and joint infections	Bone pain, joint pain and swelling, warmth, limitation of joint movement	Limb or joint swelling and tenderness	Joint aspirate, imaging of limb/joint	Joint aspirate	

Infection	History	Physical exam	Appropriate diagnostic investigations	Appropriate samples for microbiology	Infectious mimics
Diarrhoea	Duration, fever, colour, blood in stool, local outbreaks, mucoid stool	Fever, abdominal tenderness	Stool microscopy, culture and sensitivity, Cholera Ag if there is a local outbreak	Stool	

Appropriate diagnostic samples for microscopy, culture and antibiotic susceptibility testing (AST) should be obtained from patients with suspected infections **before** antibiotics are initiated.

Contamination and colonization:

Contamination occurs if samples are not collected in a sterile manner for example, a blood culture sample can be contaminated by skin commensals such as coagulase negative staphylococci if sterile technique is not observed during collection. Similarly, urine can be contaminated by organisms commonly found in the perineum.

Colonization is the presence of bacteria at a body site without causing disease. Colonization often occurs where there is communication with the skin or environment. Examples of colonization are highlighted in the table below:

Body site or prosthetic device	Bacterial colonization
Pressure sores	Skin flora e.g. Staphylococcus species Enteric flora e.g. Escherichia coli, Pseudomonas spp
Breaks in skin e.g. wounds	Skin flora e.g Staphylococcus spp. Enteric flora e.g. Escherichia coli, Pseudomonas spp.
Urinary catheter	Enteric flora e.g. Escherichia coli, Pseudomonas spp.
Endotracheal tube OR Tracheostomy tube	Mixed enteric flora in patients given antibiotics or who have been in healthcare settings for more than 4 days e.g. Escherichia coli, Pseudomonas spp.
Central venous catheter	Skin flora e.g. Staphylococcus spp.
Urine	Bacterial growth in the absence of pus cells reflects contamination or colonization. Always request a urinalysis with urine culture

Antibiotics should not be prescribed for contamination or colonization. It is important to differentiate true infection from both contamination and colonization to avoid unnecessary antibiotic prescriptions.

The table below provides a summary of how to differentiate colonisation and contamination from infection

Infection	**Colonization/ Contamination**
Fever/ Hypothermia	Normothermia
WBC elevated or decreased with left shift WBC may be decreased in overwhelming sepsis or may be unchanged in indolent or subacute infection	Normal WBC count, no left shift
Often associated with pure growth of single organism on culture	Often associated with mixed growth on culture
Usually associated with other local or systemic signs of inflammation	Not associated with other signs of infection

For central venous catheters, same organism grown from sample collected from the catheter and from a blood sample collected from a peripheral site. For endotracheal or tracheostomy tube isolates, there needs to be evidence of new pneumonia (new infiltrates on CXR, increasing oxygen requirement)	Organisms grown only from a CVC and not from a blood sample collected from a peripheral site. Organism grown on samples collected from an indwelling catheter, drain, endotracheal tube or tracheostomy, chronic ulcer or other wound
For wounds, surrounding cellulitis or abscess, organisms grown from a tissue culture	For wounds, no features of surrounding cellulitis or underlying abscess
Coagulase negative staphylococcus spp. grown from 2 blood culture bottles taken at the same time	Coagulase negative staphylococcus spp. grown from a single blood culture bottle

Completing the laboratory request forms

Clinicians should complete the detailed standard laboratory request form containing patient data that should accompany every sample sent for bacteriology.

MICROBIOLOGY LAB REQUEST FORM								
Patient identification								
IP NO						Gender	M	F
Name (Surname, Given Name)								
Ward/ Clinic		Facility			County	Sub-county		
Date of Birth (DD/MMM/YYYY								
Age	Years		Months if < 1 year					
Specimen Information								
Blood	Body fluid (specify)	Urine	Swab (specify)	Faeces		Tissue	Urethral swab	
High vaginal swab			CSF	Sputum/tracheal aspirate				

Had the patient been hospitalized for more than 48 hours at the time of sampling?				
Yes		No		
Current **antibiotic therapy**				
Prior antibiotic therapy in the last 3 months				
Patient history				
Working diagnosis				
Date of Sample Collection (DD/MMM/YYYY)				
Name of person collecting the sample				

Collecting appropriate clinical samples for bacteriology:

1. Sterile technique should be observed. Appropriate sterile containers should be used
2. Samples should be collected at time of patient presentation/ onset of illness and before administration of any antibiotics
3. Samples should be collected only when clinically indicated. **Avoid** routine screening cultures.

Adequate specimen collection: (Refer to appendix 1)

1. **Blood** - should be taken from 2 sites e.g. from a central line and a peripheral site or 2 peripheral sites. When taking a blood culture sample from a peripheral site, clean the site with an alcohol swab and allow 30seconds to dry before puncture, do not palpate the vessel before puncture unless sterile gloves are worn. Central venous catheter tip cultures must be accompanied by blood for culture. For adults draw 10ml of blood from each site, for children under 5 years, collect 1-5ml (volume as per the blood culture bottle)

2. **Urine** - should be a clean catch midstream sample, from a freshly inserted catheter or cleaned catheter hub where urine will be collected directly from the tubing. Do not collect urine from a urine bag or an indwelling catheter. Urine catheter tips should not be sent for culture

3. **Abdominal fluid** - should be taken straight from the abdomen or from a newly placed drain. Do not collect samples from existing drains

4. **Wound swabs** are often not useful due to contamination, to collect a swab, first clean the wound with normal saline and attempt to get a swab from the base or alternatively, get a tissue specimen for culture. Do not collect a superficial sample from the surface of a wound

5. **CSF**- a sterile procedure should always be used for collection of CSF. A mask should be worn to avoid respiratory contamination

6. **Abscesses, bullae, blisters** - aspirate directly from the abscess with a sterile needle and syringe.

Interpreting bacteriology culture results:

1. The clinical context must be taken into account when interpreting culture results as this will help in differentiating true infection from colonization and contamination. Infection is the presence of one or more microorganisms with an inflammatory response. Colonization is the presence of microorganisms without significant inflammation. Contamination is when a culture contains a microorganism(s) that did not originate from the intended anatomical site.

   Sterile sites

   CSF, lungs (below the glottis), urinary tract, biliary tract, and blood are normally sterile sites. Bacteria cultured from these sites are likely to be causing infection but may represent colonisation or contamination. If the organism corresponds with the clinical scenario then this should be considered to be causing infection.

   Non-sterile sites

   Cultures from non-sterile sites are often much harder to interpret. Non-sterile specimens include sputum (as it must pass through the oropharynx and the mouth), pus swabs from skin, samples from GI tract and vagina. Specimens from these sites are expected to culture bacteria (unless growth is inhibited by laboratory techniques). Interpretation therefore depends on the organism(s) being compatible with the clinical scenario.

2. Coagulase negative *Staphylococci* in blood will only be considered relevant if grown in more than 1 bottle in an appropriate clinical scenario (site of infection).

3. True infection is almost always present if blood culture is positive for one of the following:

   - *Streptococci* (non-Viridans)
   - *Staphylococcus aureus*

- Aerobic and facultative gram-negative rods e.g. *E.coli, K.pneumoniae, Enterobacter, Pseudomonas*
- Anaerobic cocci eg *Peptococcus, Peptostreptococcus*
- Anaerobic gram-negative rods eg *Bacteroides, Prevotella, Fusobacterium*
- Yeast eg *Candida sp.*

4. Suspect contamination if only one of several cultures is positive, if detection of bacterial growth is delayed (≥5 d), or if multiple organisms are isolated from one culture
5. Tracheal aspirates should only be collected if clinically indicated, consider the organism cultured as the possible cause of infection if the Chest radiograph shows infiltrates consistent with pneumonia

NOTE : If you are unsure of how to interpret culture and sensitivitity results, consult the Infectious Disease, microbiology or antibiotic stewardship team.

GENERAL GUIDANCE ON ANTIMICROBIAL PRESCRIBING

Antimicrobial prescribing should be an individualized, rational and methodical process putting into consideration the available clinical, epidemiological, pharmacological and microbiological information and evidence. Further, antimicrobial therapy should be a dynamic process, requiring periodical re-assessments and monitoring.

The following are practical tips when prescribing antimicrobials

1. **Clinical justification and initial patient assessment:** Antimicrobial therapy should be started with clear clinical justification and evidence of infection or established prophylactic benefit. The indicators of active disease such as, clinical findings, laboratory parameters and radiological imaging must be clearly documented.

   Ideally the pathogen should be identified before antimicrobial therapy is started, however this usually takes more than 48 hours hence the need to start empiric treatment then tailoring after culture and susceptibility results are known. Whenever possible, samples for CST should be taken before starting empiric therapy so that growth is not inhibited.

2. **Documentation:** The indication and rationale of antimicrobial use should be clearly documented in the clinical notes and the antibiotic section of the treatment sheet clearly completed. Name of the antimicrobial agent, dose prescribed, route of administration and duration of treatment should be clearly documented in the clinical notes and treatment sheet. Proper documentation is an anchor to guide therapy and makes it easier to detect errors.

3. **Allergy to Penicillin** or other Antibiotics -BEFORE any drugs are prescribed or administered, the patient should be consulted about the nature of their allergy and the allergy box MUST be completed on the drug chart and details recorded in the medical/nursing notes.

4. **Start date:** The commencement date of antimicrobial therapy should be clearly documented on the clinical notes and the treatment sheet. Antimicrobial therapy should not be delayed in an emergency, but every effort should be made to obtain all necessary appropriate specimens before therapy starts. In severe infections such as sepsis and septic shock,

start fast as prompt initiation of effective antimicrobial treatment has a high impact on morbidity and mortality.

5. **Choice of antimicrobial agent**: Consider local epidemiology and patient's individual factors. Local antimicrobial treatment guidelines are a great help in choosing empiric therapy.

   Factors to consider when selecting an antimicrobial:

   - If patient has an infection that requires treating
   - Diagnosis/likely source of infection/ anatomical site of infection
   - Severity or potential severity of infection and possible consequences
   - Patient's underlying condition and vulnerability to infection
   - Identity of pathogen and its sensitivity to antimicrobials
   - Evidence of efficacy of the antimicrobial agents against the pathogen and at the site of infection
   - Bacteriocidal versus bacteriostatic agents
   - Spectrum of activity: Narrow spectrum are preferred if the organism has been identified while broad spectrum might be required in empiric therapy or mixed infection. Indiscriminate use of broad spectrum agents increases the risk of development of resistance and super infection.
   - Appropriate route of administration. Possible side effects and drug interactions
   - Pharmacokinetics: tissue penetration, clearance in renal/liver impairment

6. **Route of administration**: Oral therapy is preferred and most antimicrobials have good bioavailability. Intravenous therapy might be necessary if the infection is severe, drug has poor oral bioavailability or the patient is unconscious or unable to take oral drugs. Intravenous antimicrobial therapy should be switched to a suitable oral alternative as soon as possible, (patient can swallow and absorb), generally within 48 hours of treatment. Avoid widespread use of topical antibiotics except where indicated such as in eye or ear infections.

7. **Dose optimization**: Ensure that an appropriate dose is prescribed; if uncertain consult the clinical pharmacist/pharmacist or check in the hospital formulary if available. Dose optimization needs consideration of factors that will affect drug choice and dose such as age, pharmacokinetic and pharmacodynamics properties, renal and hepatic dysfunction, drug interactions, hypersensitivity reactions, pregnancy and lactation etc.

8. **Duration of treatment**: To prevent unnecessary use, all antibiotics must be prescribed with a course length or review date on the prescription - prescribe the shortest antibiotic course likely to be effective. For most infections 5 days of antimicrobial therapy is sufficient. Exceptions include: Meningitis, deep seated abscesses, infective endocarditis, osteomyelitis, pyelonephritis, blood stream infections secondary to MRSA and Pseudomonas.

9. **Treatment review:** The need for antimicrobial therapy should be reviewed at 48 hours and regularly thereafter. Once Culture and sensitivity results and/or PCR results are available, a clinical review and decision shall be made to either stop empiric therapy, change to narrow spectrum agent (de-escalate) or continue therapy. If investigations do not suggest an infection, antibiotics should be stopped and other appropriate management instituted.

10. **Patient education**: For all antibiotic-prescribing strategies, patients should be given advice about the usual natural history of the illness, including the average total length of the illness and advice about managing symptoms, including fever (particularly analgesics and antipyretics).

    When the no antibiotic prescribing strategy is adopted, patients should be offered reassurance that antibiotics are not needed immediately because they are unlikely to make a difference to symptoms and may have side effects, for example,

    diarrhea, vomiting and rash.

11. **Restricted antimicrobials:** Reserve/restricted antimicrobials shall be prescribed based on the Reserve Antimicrobial protocol (where a facility specific protocol is not available, prescribing should be based on the MoH

AWARE classification) and provided that the prescriptions are accompanied by culture and sensitivity results. The hospital shall develop a list of restricted or reserve antimicrobials which are restricted for use in patients with severe infections, strictly based on clinical evidence of infection or sepsis and microbiological confirmation of multi-drug resistant micro-organisms. The rationale for their use shall be clearly indicated in the clinical notes and laboratory results are a prerequisite for their use.

ROLE OF THE MICROBIOLOGY LABORATORY

Operating Hours

The Microbiology Laboratory operates five days a week from 8.00 am to 5.00 pm and over the weekend (**Each lab should define it's hours of operation and avail this information to the clinical teams)**

Important contacts

All important contacts should be indicated to ensure any queries are promptly handled. Relevant contacts include:

- Head of microbiology lab
- Clinical microbiologist
- Antimicrobial stewardship committee

Advisory Services

The laboratory shall establish arrangements for communicating with users on the following:
a) advising on choice of examinations and use of the services, including required type of sample, clinical indications and limitations of examination procedures and the frequency of requesting the examination;
b) advising on individual clinical cases;
c) professional judgments on the interpretation of the results of examinations
d) promoting the effective utilization of laboratory services;
e) consulting on scientific and logistic matters such as instances of failure of sample(s) to meet acceptance criteria.

Resolution of Complaints and Feedback

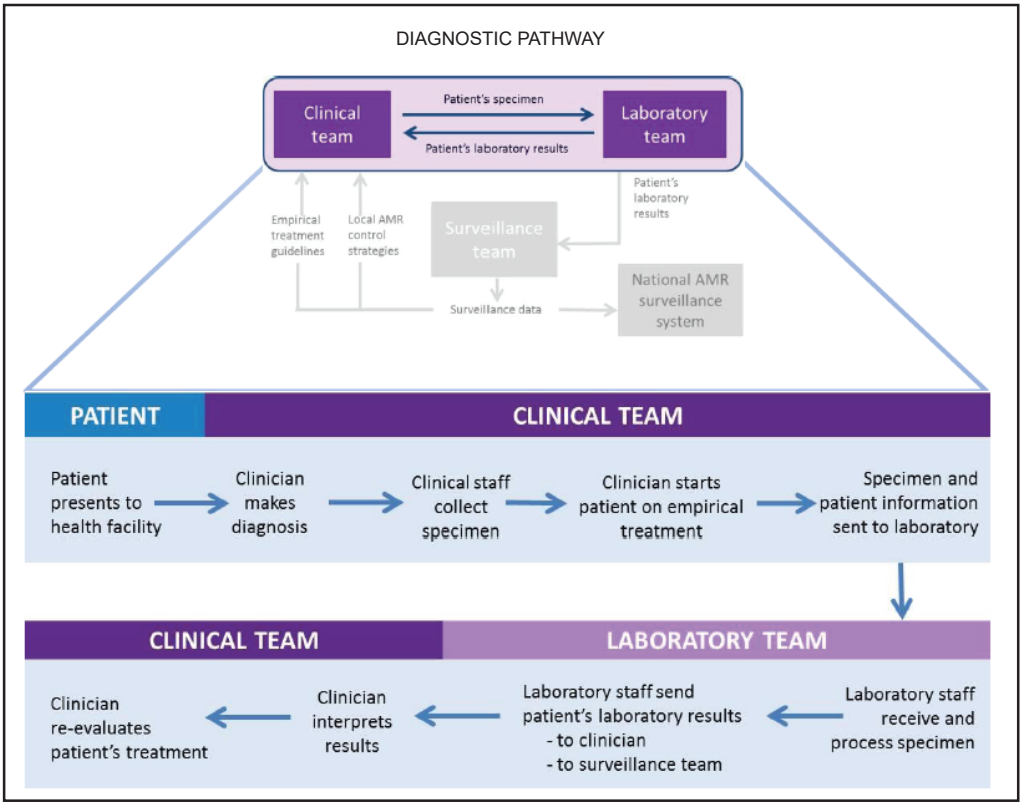
- The laboratory should have a mechanism to assess customer satisfaction and receive feedback. Each lab should assign a quality officer/manager

- The laboratory shall evaluate the data and resolve the complaints. The feedback to the customers shall be done through phone calls, email and meetings. The customer is requested to write their contacts on the sample receiving log book at the reception. The manager shall discuss the problem with the clinicians to arrive at equitable resolutions.

- The laboratory will effectively monitor complaints to prevent the reoccurrence of the same

Flow of Responsibilities

Adequate diagnostic and antimicrobial stewardship requires collaboration between the clinical and laboratory personnel. Adequate communication is key in ensuring optimal patient outcomes



From WHO. Diagnostic stewardship - A guide to implementation in AMR surveillance sites

SPECIMEN MANAGEMENT

Specimen management should be clearly stipulated in the lab microbiology procedures

Patients Samples

Patients samples will include:

- Urine
- Stool
- Rectal swabs
- Sputum
- CSF
- Blood
- Pus swabs
- Genital swabs

Specimen collection

The use of specimens for bacteriological analysis requires that specific clinical material be collected, stabilized, and transported according to exact specifications to ensure valid results. Poor specimen quality contributes to misdiagnosis and inappropriate antimicrobial therapy. Communication between the laboratory and clinicians is essential to the proper selection of laboratory tests and interpretation of their results. Laboratory personnel are responsible for monitoring and educating those collecting and transporting specimens.

There are factors that can affect the performance of the examinations in the laboratory which might lead to wrong results, these factors are addressed in each sample category in this handbook.

General specimen selection and collection guidelines include the following.

1. Verify the client identity by counterchecking with the request form

2. Explain the procedure to the clients.
3. Select the proper anatomic site from which to collect the specimen.
4. Observe careful skin preparation before procedures such as blood cultures and spinal taps to decrease the chance that organisms normally present on the skin will contaminate the specimen.
5. Avoid contamination with indigenous flora. Growth and reporting of normal flora can be mistaken by the physician as the cause of infectious process. The flora can also overgrow and obscure pathogens in cultures.
6. Collect sufficient volume of specimen to enable all test requests to be performed satisfactorily. Insufficient specimen may yield false negative results.
7. Maintain the correct temperatures during the transportation as indicated in each sample category
8. Any deviation from the documented collection procedure should be recorded, documented in the final report and communicated to the referring clinician

Specimen Labelling

All the required information must be provided on the specimen container so that the specimen can be matched with the lab request form when it is received in the laboratory.

This information includes the patient's name and identification number, type of specimen, date and time of collection, and name of the collector.

Transporting Samples to the laboratory

General Specimen transport guidelines include the following.

- Ideally, specimens for bacterial cultures should be transported to the laboratory within 30 minutes of collection. Exposure to extremes of temperature must be

avoided. If transport will require more than 2 hours, storage at 4º to 8ºC is required. *Note*: Never refrigerate spinal fluid, blood, genital, eye, or internal ear specimens.

- Do not store specimens for bacterial culture for more than 24 hours even with appropriate holding medium or refrigeration temperature.
- Bacteria that are especially sensitive to ambient conditions include *Shigella spp., Neisseria gonorrheae, Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae,* and anaerobes. Reliable detection of these organisms requires immediate processing. Delays of up to 6 h result in minimal loss of CFU when transport media are used. Longer delays, even with the use of transport media result in significant loss of bacteria.
- Transportation of clinical specimen and transportation of infectious substances from one health care facility or laboratory to another, regardless of the distance, requires strict attention to specimen packaging and labelling instructions

Specimen receiving

Once a specimen is delivered to the microbiology laboratory, personnel must ascertain that all pertinent information has been provided on the request form.

Specimen rejection

Specimen will be rejected and documented as per the criteria below

Request form

- Request form not received with specimen
- Missing collection date on specimen container or request form
- Name and signature of requesting clinician missing

- Mismatch of information details on request form with details on the specimen container
- Request form contaminated with specimen

Specimen rejection criteria

- Container used not appropriate for investigation requested.
- Specimen unlabelled or has inadequate labelling.
- Specimen container broken.
- Specimen container leaking or cracked.
- Duplicate specimen received.
- Specimen volume not sufficient for required investigation.
- Delay between collection of specimen and arrival in laboratory.
- Specimen not appropriately packaged.
- The specimen has been transported at improper temperature
- Specimen is dried-up
- If processing of the specimen would produce information of questionable value (e.g., Foley catheter tip)

Tests requested

- Requested test not performed by the laboratory
- Inappropriate specimen is provided for the requested tests.

If a sample is rejected, the reason for rejection should be documented and clinical personnel notified immediately. A record of this communication should be maintained.

Examination referred to other laboratories

In some cases, the laboratories might not be able to provide all the testing required by its clinicians and will therefore have to refer the tests to a referral laboratory. The laboratory might also refer samples when there is a short term interruption of service caused by instrument breakdown, unavailability of personnel, sudden increase in volume, or any other unscheduled or unanticipated situation. The laboratory will make all the referral arrangements and ensure that clinicians get the results within the stipulated time

Specimen processing

Specimen processing involves detection of pathogens by staining and culturing and performing immunologic assays for microbial antigen. Specimens should be processed in a timely manner. Improper handling prior to processing can result in death of bacteria or overgrowth of contaminating bacteria. Correct interpretation of culture results generally requires a rough quantitation of bacterial densities in the clinical specimen. Allowing bacteria to multiply out of proportion to their original numbers may result in erroneous interpretations.

Storage conditions

Specimens that are not processed immediately are stored in appropriate storage conditions as stipulated in appendix 1.

Interpretation of results

The test results will indicate whether an organism was isolated and its antibiotic susceptibility.

Specific sample handling

Urine sample

Use a clean, sterile, leak-proof, wide mouthed container and collect ≥ 15ml. ***Urinalysis must be performed for every urine culture requested.

Females: Hold the labia apart, pass several mls of urine and collect a midstream portion without stopping the flow of urine. The midstream portion is used for culture.

Males: Hold and retract the foreskin. pass several mls of urine and collect a midstream portion without stopping the flow of urine. The midstream portion is used for culture.

Commonly isolated bacterial pathogens in urine include:

- *E. coli*
- *Klebsiella* species
- *Proteus* species
- *Enterococcus* species
- *Staphyloccus saprophyticus*.

Interpretation of results

- The results will be interpreted as the number of Colony Forming Units/ml of the urine sample and the pathogen isolated will be recorded. I.e 10^5CFU/ml, *Escherichia coli* isolated.

Urine Culture – Turnaround time up to 72**hours**

Stool and rectal swabs

Pass specimen directly into a clean, dry, leak-proof, wide mouthed container. Put approximately 5 grams (pea sized). Rectal swabs should be collected using a clean, sterile swab. Rotate the swab for 360^{o}C in the rectum to make sure that enough sample is collected.

The rectal swab samples are collected and transported in appropriate transport medium preferably i.e. Cary Blair for stool isolates, alkaline peptone water or Ames media. If a delay in transport or processing is anticipated, the specimen should be kept refrigerated.

Stool is the most ideal sample because it yields higher recovery rate of the organisms as compared to rectal swabs. The bacterial pathogens isolated in stool include: *Salmonella Spp, Shigella Spp, Campylobacter, Vibrio Spp, Yersinia enterocolitica* Pathogenic *E.coli* (Enterotoxigenic *E.coli*, Enteroinvasive *E.coli*, Enteropathogenic *E.coli* , Enteroaggregative *E.coli* and Enterohaemorrhagic *E.coli*)and C*lostridium difficile*. The isolated organisms are subjected to antibiotic susceptibility testing as per standard guidelines.

Interpretation of results

The result shall contain the pathogen isolated i.e *Salmonella typhi* isolated. When the pathogen being tested is not isolated, the results shall be writen as No *salmonella or Shigella* isolated

The turnaround time is up to 4 days

CSF

CSF should be obtained before antimicrobial therapy commences in order to avoid loss of viability of the etiological agents. Treatment of the patient, however, should not be delayed while awaiting collection of specimens or results from the laboratory. Specimen should be obtained in all suspect cases as bacterial pathogens can still be detected even after antimicrobial therapy has begun

Specimens should be delivered to the laboratory in sterile containers and processed as soon as possible. CSF specimens for bacteriology are transported at ambient temperature. They must never be refrigerated as these pathogens do not survive well at low temperatures. Where molecular analysis is suggested the CSF should either be refrigerated if shipment will occur within a week or frozen for long term storage. For culture purposes, cerebrospinal fluid (CSF) should be processed within 1 hour of collection. Preliminary results for Gram stain (cell count and gram stain) should be reported immediately. Specimens for culture should not be refrigerated or exposed to extreme cold, excessive heat, or sunlight. They should be transported at temperatures between 20°C and 35°C.

The primary pathogens includes: Group B Beta haemolytic *streptococcus* (newborns) ,H*aemophilus influenzae* (2 months - 2 years old), *Streptococcus pneumoniae* and *Neisseria meningitides*, *E.coli* carrying the K1 antigen (newborn) and *L. monocytogenes (*newborn and occurs in epidemics)

Interpretation of results

The result shall contain the amount and the type of the pathogen isolated i.e Moderate *Streptococcus pneumoniae* isolated. When the pathogen being tested is not isolated, the results shall be No *growth obtained*

The turnaround time for CSF culture is 6 days.

Blood samples

The samples are collected aseptically into the sterile blood culture bottles. Blood can either be withdrawn using sterile disposable syringes or vacutainers needles. Two blood culture sets should be collected. The amount of blood per set required for adult is 10 ml of whole blood, 2-5ml from children and 0.5-2ml for infants (for automated systems using commercial blood culture bottles, the volume withdrawn should follow manufacturer's instructions).

The sample should be transferred to the laboratory at room temperature as soon as possible and processed immediately. The samples must never be refrigerated. Preliminary results for Gram stain should be reported immediately.

Primary pathogens include: *Salmonella*, other gram-negative rods, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae, yeasts*. The following organisms will **NOT** be considered as pathogens unless recovered from >1 blood culture set: *Bacillus sp*., Coagulase negative staphylococcus*, viridans Streptococci*, *Coryneform Gram + rods*

Interpretation of results

The result shall contain the type of the pathogen isolated i.e *Streptococcus pneumoniae* isolated. When no pathogen isolated, the results shall be No *growth* obtained

The turnaround time is 7 days

Sputum

Expectorate sputum in a sterile container. The specimen should be stored refrigerated if there is a delay in transport to the laboratory. A delay in processing of >2 hours will compromise the ability to isolate fastidious organisms such as *S.pneumoniae* and *H.influenzae*

Gram stain should be conducted prior to culture to confirm quality of sample.

Primary Pathogens: *S.pneumoniae, H.influenzae,* heavy or predominant growth of *S.aureus* and gram-negative bacilli such as *Klebsiella sp*. or *P.aeruginosa*.

Interpretation of results

The result shall contain the amount and the type of the pathogen isolated i.e Moderate *Streptococcus pneumoniae* isolated. When the pathogen being tested is not isolated, the results shall be No *growth* obtained

The turnaround time is 4 days

Swabs

Swabs include genital swabs (High vaginal swabs, urethral swabs), wound swabs, ear, eye swabs, throat and nasopharyngeal swabs

Wound swabs: Specimens are collected using a clean, sterile swab and sent preferably in transport medium. If fluid or pus are sent this is placed in a sterile container with a screw cap.

If there is a delay in transport or processing the specimen should be refrigerated.

Common organisms include *S. aureus*, beta-hemolytic streptococci groups A, B, C and G and *P. aeruginosa.*

Interpretation of results

The result shall contain the amount and the type of the pathogen isolated i.e Moderate *Pseudomonas aeruginosa* isolated. When the pathogen being tested is not isolated, the results shall be No *growth* obtained

Genital swabs (High vaginal swabs, urethral swabs) excluding swabs for GBV screen

Cervical and urethral swabs for isolation of *N.gonorrhoeae should be* placed in appropriate transport media, delivered to the laboratory as soon as possible and cultured without delay.

Vaginal swabs in transport media may be refrigerated if there is a delay in processing.

Vagino-anal swab in transport media for detection of Group B beta-hemolytic *Streptococcus* should be processed immediately.

Interpretation of results

N.gonorrhoeae and *C.trachomatis* cause cervicitis in females and urethritis in males and females: *Candida sp.* and *Trichomonas vaginalis* cause vaginitis in females. Group B streptococcus is a colonizer in females and is usually considered significant only in pregnancy.

Eye swabs

It is preferable that both eyes be swabbed, even if the infection is unilateral. Swabs should be collected prior to the instillation of topical antibiotics, and sent in transport medium

Interpretation

Potential pathogens: *S. aureus, H. influenzae, M. catarrhalis*, *N.gonorrheae*, Gp.A Strep, *S. pneumoniae*, *Moraxella* species, and *P. aeruginosa.*

Ear swabs

The ear swab is collected using a clean, sterile swab and sent in transport medium. If a delay in transport or processing is anticipated, the specimen should be kept refrigerated

Interpretation

Potential pathogens *S. aureus*, *P. aeruginosa, S. pneumoniae,* Group A streptococcus or yeast is significant.

The turnaround time is 4 days

Quality assurance

Quality assurance system should be established to ensure that all laboratory results are accurate, reliable and reproducible by ensuring that:

- All procedures, media and reagents in the laboratory are subjected to quality control.
- The laboratory participates in external proficiency testing.
- The staff are regularly subjected to competency assessment to improve on their skill for the production of quality results

Factors known to significantly affect performance of the examination or interpretation of results:

1. Sample transportation
2. Antimicrobial therapy
3. Sample collection site
4. Method of sample collection
5. Timing of sample collection

Protection of personal information

The laboratory shall ensure that confidentiality of information is maintained through secure storage of medical records. All lab personnel should sign a confidentiality form.

REFERENCES

1. WHO Diagnostic stewardship. Guide to implementation in AMR surveillance sites
2. WHO GLASS Manual for Early Implementation 2015
3. Dik JW, Poelman R, Friedrich AW, Panday PN, Lo-Ten-Foe JR, van Assen S et al. An integrated stewardship model: antimicrobial, infection prevention and diagnostic (AID). Future Microbiology 2015 Sep 1
4. The KNH Guide to Empiric Antimicrobial Therapy 2018
5. ISO 15189-2012
6. WHO SLIPTA checklist
7. National Antimicrobial Stewardship Guidelines
8. National Policy and Action Plan

Appendix 1: Sample collection

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Enteric pathogens	Faeces	Patient should collect a sample during acute stage of diarrhoea and avoid contamination with urine, transfer a portion (about a spoonful) of specimen containing mucus, pus or blood.	Put specimen into clean, disinfectant free, dry and wide-necked container, transport to laboratory within 1 hour or transfer to Carry-Blair holding medium and label the Patient ID ,type of	Clean, leak-proof, disinfectant-free, wide mouth container or transfer to Carry-Blair holding medium (2-5g)	Unpreserved: <1 h, room temperature Transport medium <24h, room temperature	<24h, cold chain Transport medium <48h, R.T or cold chain	Do not perform stool cultures which have stayed longer than 6 days.

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
			sample, date and time				

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Enteric pathogens	Rectal swab	Patient should be explained about the procedure to avoid contamination with anal skin flora.	Carefully insert the swab beyond the anal sphincter, gently rotate the swab to collect sample for about 10 seconds, faeces should be visible on the swab	Leak-proof container or container with Carry Blair medium	<24h, cold chain	<72h, cold chain	-

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Mycobacterium tuberculosis	Sputum	Patient should rinse or gaggle with water to remove excess oral flora then cough deeply to produce sputum.	Cough deeply to produce sputum not saliva. Collect into clean, dry, wide-necked, leak-proof container.	Leak-proof, clean and dry container 1-10ml	<24 hrs, R.T >24hrs in cold chain	<24 hrs, R.T >24hrs in cold chain	Don't process sputum which has much saliva.

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Septic wound	Pus swab	Specimens should be collected by a medical officer or an experienced nurse, pus is best collected at the time the abscess is incised and drained	Aspirate or pass a swab deep into the lesion to firmly take the specimen	Stuart transport media	<2h, R.T	<24h, R.T	-Samples of the base of the lesion and abscess wall are most productive -Avoid contamination of with commensal organisms from the skin

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Sexually transmitted infection	Urethral swab	The patient should not have passed urine for about 2 hours before specimen is collected. Clean round the urethral opening using swab moistened with sterile saline	Gently massage the urethra from the above downwards and collect the sample of pus on a sterile cotton swab.	Insert the swab in a container of Amies transport medium breaking off the swab stick to allow the bottle top to be replaced tightly.	<2h, R.T	<24h, R.T	-

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Sexually transmitted infection	Cervical swab	-Moisten a vaginal speculum with sterile warm water and insert it into the vagina -Clean the cervix using a swab moistened with sterile saline	Pass a sterile swab into endocervical canal and gently rotate the swab to obtain a specimen	Insert the swab in a container of Amies transport medium breaking off the swab stick to allow the bottle top to be replaced tightly.	<2h, R.T	<24h, cold chain	-

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Sexually transmitted infection	Vaginal swab	Wipe away old secretions and discharge	Collect a sample of vaginal discharge on a sterile swab by gently rotating the swab to obtain a specimen from mucosal membrane of the vaginal wall	Insert the swab in a container of Amies transport medium breaking off the swab stick to allow the bottle top to be replaced tightly	<2h, R.T	<24h, cold chain	For intrauterine devices, place entire device into a sterile container and submit at RT. Gram stain is recommended for bacterial Vaginosis.

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Sexually Transmitted Disease (STD)	Genital ulcer swab	Clean around the ulcer using a swab moistened with sterile saline	While pressing the base of the lesion's surface, firmly rub base with sterile swab to collect fluid.	Stuart transport media where possible.	<2h, R.T	<24h, cold chain	-

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Bacterial meningitis	Cerebral spinal fluid.	CSF is collected by an experienced medical officer. Collect the specimen by using strict aseptic technique. The patient should be fasting. When one tube is available microbiology should receive it first, if more than one is collected then microbiology should receive the less bloody.	The fluid is collected by lumbar puncture and drip into two dry sterile containers	Sterile container	<2h, R.T	<24h, R.T	-If CSF is purulent or markedly cloudy, make Immediately Gram staining and report as soon as possible

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Septicaemia and Bacteraemia.	Blood	Blood should be taken before antimicrobial treatment has been started	Sterile the skin with 70% alcohol and use disposable syringe to punch the vein.	Blood culture medium or Vial	<72h, R.T	<72h, room temperature (R.T)	-
Susceptibility testing	Isolates	-	Single colony from pure growth	Storage media	< 1 week, Refrigerator	<1 year, Freezer	-

Name of Test	Sample	Patient Preparation Procedures	Sample Collection Guidelines	Transport device and/or minimum vol.	Transport Time and Temp	Storage time and Temp	Comment
Urinary Tract Infection (UTI)	Morning Mid-stream Urine	Patient has to clean genital area with clean water, then void the urine to come out and collect mid-stream urine into a container	Sample collected into a dry, clean and sterile universal bottle, 5-10ml	Sterile universal bottle	<2h, R.T	<24h, cold chain	Once received in the laboratory, the sample should be stored in refrigerator

DIAGNOSTIC STEWARDSHIP

A CLINICIAN'S HANDBOOK ON APPROPRIATE USE OF MICROBIOLOGIC DIAGNOSTIC TESTS

The Fleming Fund
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