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Review article

Preparing SOP for Microbiology Laboratory: A Short Guideline

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Introduction

In recent years, enormous changes in the organization of health services have taken place throughout the world. The rapidity and extent of these changes in the healthcare delivery system have been nothing short of remarkable. Despite these dramatic progress in the treatment and prevention, infectious diseases remain a major cause of death and debility and are responsible for worsening the living condition of million of people around the world.[1]

Infections frequently challenge the physician's diagnostic skill and must be considered in the differential diagnoses of syndromes affecting every organ system. For that reason, diagnostic microbiology has obviously become an integral and inseparable component of modern medicine and public health. Microbiology laboratories play a decisive role in the diagnosis, treatment, prognosis and monitoring of communicable diseases. Therefore, reliable, reproducible and rapid laboratory services, organized in a cost-effective manner, is very much essential for providing quality health services. Accurate detection of microbes and quality assurance in laboratory services, aimed at improving reliability, efficiency and facilitating inter-laboratory comparability in testing, is the backbone of quality healthcare delivery. The use of standard operating procedure (SOP) in clinical microbiology laboratory is one of the most crucial factors in achieving quality health services.[2]

Standard operating procedure (SOP)

Microbiology investigations are important in the diagnosis, treatment, and surveillance of infectious diseases and policies regarding the selection and use of antimicrobial drugs. It is, therefore, essential that test reports are relevant, reliable, and timely and interpreted correctly.[3] The primary goal of the microbiology laboratory is to diagnose the potentially pathogenic microorganisms and to provide high quality service at the lowest cost for the customers. Achieving this goal requires a detailed analysis of the laboratory processes which include: (i) reduction of reagent and laboratory cost, (ii) improvement of productivity, (iii) improving and specifying of 'turnaround times' (TATs) for each test, (iv) improvement of the quality of specimens submitted, and (v) improvement of the clinical relevance of test results.[1]

A written set of instructions must be available for every test, process or procedure to set the minimum acceptable standard. These instructions are now termed as standard operating procedures or SOPs.[4] Every laboratory must have SOP also referred to as the 'laboratory procedure manual, hand book or local laboratory bench manual'.

The difficulty in preparing the SOP for microbiology laboratory is that written methods of how to do tests are not enough. Microbiologists also require documentation relating to : (a) what to look for, (b) when and what to report, (c) what sensitivities to test, (d) which, if any, to report, (e) the level of identification required, and (f) the need for confirmatory test in-house or referral to a reference laboratory, etc.

It is obviously very difficult to cover every eventuality, but comprehensive criteria for processing, secondary testing and reporting of all routine specimens must be covered. The manual should lay down the policy of the laboratory for the kinds and sequence of examinations to be made on each of the different kinds of specimen, the criteria for determining the content of specimen, and the standardized wording of reports.[4]

Need of SOP s in laboratory work

Standard operating procedures in a microbiology laboratory are needed for following reasons: [3]

- To improve and maintain the quality of laboratory service to patients and identify problems associated with poor work performance;
- To provide laboratory staff with written instructions on how to perform test consistently to an "acceptable standard" in the laboratory;
- To provide written standardized techniques for use in the training of laboratory personnel;
- To facilitate the preparation of a list of essential reagents, chemicals and equipments;
- To promote safe laboratory practice.

Important features of SOP

An individual SOPs must be:[5]

- Applicable and achievable in the laboratory in which they will be used;
- Clearly written and easy to understand and follow; and
- Kept up-to-date using appropriate technologies.

Preparation of SOP

The procedure manual, a reference for standardization and organization of all tests and functions, is the most important document in the microbiology laboratory. It is written for new inexperienced personnel but also serves as a reference for experienced laboratory personnel.[6] For that reason, SOPs must be written and implemented by qualified experienced laboratory officers, and followed exactly by all members of staff. Each SOP must be given a title and identification number, and be dated and signed by an authorized person.[3]

Types of SOP

Standard operating procedures can be described under two broad headlines:

- One of them is 'standard procedure for general laboratory practices' which include: (a) request form, (b) collection and transportation of clinical specimens, (c) specimen acceptability and criteria for rejection, (d) procedure for processing specimens, (e) systematic descriptions of the tests performed, (f) antimicrobial susceptibility testing, (g) safety in laboratory and (h) quality assurance.

- The other one is 'standard procedures for specific diseases' like (i) cholera, (ii) enteric fever, (iii) diphtheria, (iv) meningitis, (v) dysentery, (vi) sexually transmitted diseases, (vii) UTI, (viii) tuberculosis, (ix) malaria, (x) parasitological examination of stool, (xi) mycological techniques, (xii) water bacteriology, etc.

There must be a guideline regarding collection and transportation of clinical materials (like bacterial food poisoning, viral diseases such as AIDS, viral hepatitis, poliomyelitis, dengue etc) to referral laboratories.

Stages of SOP

Total activities performed by laboratory personnel in order to provide accurate diagnosis from a clinical specimen can be divided into three stages. These are :

- Pre-Analytical stage- primarily deals with the general concepts for specimen collection and handling
- Analytical stage- deals with testing the specimens, and
- Post-Analytical stage- comprises of reporting and interpreting test results.

Pre-analytical stage :

In terms of effectiveness of the laboratory, nothing is more important than proper filling of request form, appropriate selection, collection, and handling of a specimen for microbiological diagnosis.

The pre-analytical stage of SOP deals with the appropriate specimen management, is the key to accurate laboratory diagnosis that directly affects patient care and patient outcome. It influences therapeutic decisions, affects hospital infection control, patient's length of stay, and overall hospital costs. It also plays a major role in laboratory costs, and clearly influences laboratory efficiency. So, it is the responsibility of the individual laboratory to provide complete and accurate specimen management information in a form that can be easily incorporated into the laboratory bench manuals. This will provide a clear and precise guidance to healthcare workers, who have primary responsibility for the collection of specimens. For that reason, it is essential for every laboratory to develop a rational, sound and relevant specimen management policy and enforce it as strictly as possible.[7]

Request form :

Accurate information must be obtained on the request form to ensure unequivocal identification of the patient and the requesting physicians. Each specimen must be accompanied by a request form which includes:

1. Patient name, sex, date of birth, address and telephone number, and record number for outpatient and hospital inpatient;
2. Printed name, practice address and telephone number of the requesting physician;
3. Space should be provided for a description of the specimen (e.g., clotted blood, stool, rectal swab etc), the date of request, date and time of collection;
4. The form should be so designed that the submitting clinician gives all of the information needed by the laboratory staff to determine the natures and range of test to be done;
5. Clinical note summarizing the patient's illness, the date of onset of illness and the provisional diagnosis;
6. The request form should specifically seeks details of the type of examination requested, with notes on the patients occupation, history of recent foreign travel, relevant immunization and any antibacterial therapy or prophylaxis.

Carelessness in submitting specimens with inadequate information would markedly reduce value of the report in many cases.[8]

Collection of clinical specimens:

The diagnosis of an infectious disease begins with the collection and transportation of a clinical specimen for examination in the laboratory.

It is a critical consideration because any result, the laboratory generates, is limited by the quality of the specimen and its selection on arrival in the laboratory.[9] In order to obtain the appropriate diagnosis, the specimen must be-

- the right one,
- collected at the right time,
- transported in the right way, and
- submitted to the right laboratory.

Guidelines for collection and transportation of the specimens should be made available to clinicians in a lucidly written format. The guidelines must emphasize two important aspects:[10]

(i) Collection of the specimen before the administration of antimicrobial agent;
(ii) Prevention of contamination of the specimen with externally present organism or normal flora of the body.

World Health Organization had formulated general rules for collection and transportation of specimens which is modified and summarized as below:

- Strict aseptic techniques must be applied throughout the procedure;
- Hands of the phlebotomist should be washed before and after the collection;
- Specimen should be collected from the site representative of the infectious process (e.g., sputum is the specimen for pneumonia not the saliva and cervical not vaginal swab for *Neisseria gonorrhoeae* isolation). Even careful collection methods will produce a little clinical value if it is not obtained from a site where the infection is active;
- Specimen should be obtained at the appropriate phase of disease (e.g., acute phase of illness, and before antibiotics are administered);
- Specimen should be placed in an appropriate sterile container;
- Clean and uncontaminated outside of the container should be ensured;
- Specimen should be collected in adequate volume, insufficient material may yield false negative result;
- Specimen container should be leak-proof and closed tightly so that its contents do not leak during transportation;
- Specimen should be labeled with the patient's name, identifying number and date. Enough information must be provided on the specimen label so that the specimen can be matched up with the requisition when it is received in the laboratory;
- Specimen containing dangerous pathogens should be labeled HIGH RISK and if possible a warning symbol (i.e., red dot, star or triangle) should be attached.[11]

Note: As because of any specimen may contain infectious pathogens, it is important for laboratory personnel to handle all specimens with adequate safety precautions and to wash their hands after handling specimens.

Transport of microbiological specimen:

Ideally specimen should be transported to the laboratory within 30 minutes of collection. Many microorganisms are susceptible to environmental conditions, such as the presence of oxygen (anaerobic bacteria), changes in temperature (Neisseriae, *H. influenzae*) or change in pH (Shigellae). When a delay in delivery is unavoidable, appropriate measures like a chemical preservative or transport media or refrigeration at 4-10^{o} C must be used.[7]

Transport media- maintain the viability of microorganisms present in a specimen without supporting the growth of commensal organisms in a state of suspended animation, so that no organisms die out.[9] These media will help to prevent organisms from dying due to enzyme action, change of pH or lack of essential nutrients. Common transport media used in microbiology laboratory are:[13]

- Cary-Blair transport media- for faeces that may contain Salmonella, Shigella, Campylobacter or Vibrio species;
- Amie's transport medium- is a modification of Stuart's transport medium. It is effective in ensuring the survival of pathogens in specimen collected on swabs especially fastidious organisms such as *Neisseria gonorrhoeae* or *Bordetella pertussis;*
- Venkataraman-Ramarkishnam medium (V-R fluid) or alkaline peptone water preserve Vibrio for more then six weeks and can be kept at room temperature.

Specimen preservation:

Chemical preservatives, such as boric acid may be added to urine, are designed to maintain the appropriate colony counts. Polyvinyl alcohol (PVA) and buffered formalin can be used for preservation of ova and maintaining the integrity of trophozoites and cysts.

Note: preservatives that contain formaldehyde solution such as merthiolate iodine formaldehyde (MIF) and formal saline must not be used when culture is required because formaldehyde kills living organisms.[12]

Refrigeration at 4-10^{o}C can help to preserve cells and reduce the multiplication of commensal in unpreserved specimen.[14]

Note: However, specimens (such as spinal fluid, genital, sputum, blood, eye, or internal ear specimen) for the isolation of *Haemophilus influenzae, Streptococcus pneumoniae* or *Neisseria* species must never be refrigerated because cold kills these pathogens.[3,12]

Rejection criteria for improper samples:

Criteria should be developed by a laboratory on the basis of which the processing may not be done by the laboratory. The following are some examples of unacceptable specimens:[2,9,15]

- Missing or inadequate identification;
- Insufficient quantity;
- The specimen has been transported at the improper temperature in improper medium (e.g., specimen for anaerobic bacteria submitted in aerobic transports);
- Specimen collected in an inappropriate container;
- Contamination suspected;
- Unknown time delay;
- Leaking container or open mouthed container;
- Specimen is dried up;
- Saliva instead of sputum; and
- Inappropriate request, e.g., Folley's catheter tip, oral swab, etc.

It is an important rule to talk to the requesting physician before discarding unacceptable specimens. In some cases, such as mislabeling of a specimen or requisition, the person who collected the specimen or requisition can come to the laboratory and correct the problem. Correction of a mislabeled specimen or requisition should not be done over the telephone.[7]

Reception of specimen:

For safety, the reception of specimen should be done in a room separate form the working laboratory and the reporting office. The reception staff must be trained in the appropriate safety precautions and must know the procedure to be followed when leaking or contaminated container are received.[5,6]

Checking of specimen and request form:

When the specimen reaches the laboratory, these should be checked to ensure that correct specimen has been sent and the specimen is the same as that on the request form. Also included should be the comment that the specimen require immediate attention e.g., CSF, urine, swabs not in transport media or faecal specimen containing blood and mucus etc.[3]

Collection procedure of different specimens:

The clinical state of the patient will not be reflected in the result of laboratory investigation (despite the correct performance) unless the specimen is collected under optimal conditions required for the analysis. Few examples of the important specimens and their proper collection and transportation methods are described below in order to ensure quality.[15,17]

Blood:

- Whole blood is required for bacteriological examination;
- Skin antisepsis is extremely important and performed by using tincture of iodine (1-2%), povidone iodine (10%), and chlorohexidine (0-5%) in 70% alcohol;[13]
- Blood should be collected during the acute and early stages of disease and during paroxysms of fever (since the number of bacteria is higher during this period);
- In the absence of antibiotic administration, 99% culture positivity can be seen with three- samples blood culture;
- Small children usually have higher number of bacteria in their blood as compared to adults and hence less quantity of blood is required from them; and
- If immediate transportation to the laboratory is not possible, specimen should be inoculated into liquid media and kept in the incubator. If not available, should be kept at room temperature but never be refrigerated.[15]

Urine:

- Best collected soon after patient wakes up in the morning;
- Mid-stream urine is collected after giving proper instructions to the patient, such as: (a) genitalia should be cleaned properly, (b) mid-stream, clean catch urine collected in a sterile container;
- In case of catheterized patients, urine should be collected from an area over the collecting tubes after cleaning and the sample is collected by puncturing with a sterile needle and syringe;
- Urine should be transported immediately to the laboratory. If delay is more than half an hour, sample should be refrigerated at 4^{o}C.

Pus, Discharge and Swabs:

- The site of collection (i.e., wound) should be cleaned with normal saline and antiseptics, and should not be applied before collection;
- In case of discharge, 1-2 ml sample in a sterile vial is preferable;
- Two swabs in sterile containers should be sent - one for microscopic examination and another for culture. A third swab dipped in suitable transport media is preferable in special situation like, urethral or HVS swab;
- Vaginal swab should be high vaginal swab and should not touch the sides of the vaginal wall;
- Two throat swabs should be taken in the morning before mouth washing. Swabs should be collected under direct visualization without touching the tongue or buccal mucosa;
- Conjunctival swab should be collected in the morning before washing of the face and eyes.

CSF and other sterile body fluids:

- Should be colleted under aseptic condition (preferably by attending physician) and transported immediately to the laboratory;
- They should not be refrigerated, if delay in transport is expected, than should be kept at room temperature.[12]

Analytic stage

The microbiology laboratory should have a carefully considered and clearly designed policy for accurate detection of etiological agent. They should formulate a sequence of laboratory tests, like gross examination of specimen, microscopy, culture procedures, biochemical tests, serological test and antibiotic sensitivity tests. This sequence will be used in the examination of most of the specimens and microbial isolation.

Gross examination of specimen:

All processing should begin with a macroscopic or physical examination of specimen. Areas with blood or mucus should be located and sampled for direct microscopy and culture.

Direct microscopic examination:

All appropriate specimens should have a direct microscopic examination. The direct examination serves several purposes:

- First, the quality of the specimen can be assessed, for example, sputum can be rejected that represent saliva by quantitation of WBCS and squamous epithelial cells (should be 10: 1);
- Infection can be assumed by observing plenty of pus cells

in the specimen.

- The work up of the specimen can be guided by comparing what grows in culture to what was seen on smear;
- A situation in which three different morpho-types (cellular types) are seen in gram stain but only two types seen in culture and will alert the microbiologists to the fact that the third organism may be an anaerobic bacterium.[9]

Isolation of microorganism in culture media:

The role of suitable quality of culture media for cultivation of microorganism can not be over emphasized. Selection of media to inoculate any given specimen is usually based on organisms, most likely to be involved in the disease process.

The following points should be incorporated in the microbiological SOPs covering the analytical stage: [3]

- Detailed procedure for examining different specimens;
- Staining technique and quality assessment of stains;
- Aseptic technique and safe handling of infectious materials;
- Preparation and quality assessment of culture media and preservation of stock strains;
- Inoculation of liquid and solid media;
- Reading and interpretation of culture;
- Techniques used to identify pathogens;
- Antimicrobial sensitivity testing and quality control of procedures and antibiotic discs;
- Cleaning and quality control of equipment used in microbiology laboratory;
- Immunologic techniques and quality control of antigen-antibody reagents;
- Safe working practices;
- Safe disposal of specimen and cultures;
- Cleaning of glasswares, plasticwares, etc and
- Sterilization procedures and their control.

Control of stains and reagents:

- All stains and reagents must be clearly labeled, dated and stored correctly;
- These should not be used by their expiry dates or when they show signs of deterioration, such as, abnormal turbidity and decoloration;
- At regular intervals and whenever a new stain is prepared control smear should be stained;
- Control smear for gram stain can be prepared from mixed culture of reference strain of Staphylococci and *Escherichia coli;*
- Control smear for Ziehl-Neelsen stain should include smears with few to moderate number of AFB;
- It should be noted that when a smear is too thick the decolarization being often incomplete which can result in gram negative organisms being reported as gram positive.

Control of equipment:

- All equipment used for tests, should be checked and calibrated at regular intervals;
- For each item of equipment, there should be clear operating and cleaning instructions, and service sheets;
- Specimen containers should be inspected regularly, especially the caps of bottles and tubes for missing or warn liners.

Post analytical stage

The result of microbiological examination usually becomes available in stages on successive days. So, the SOP needs to include: (a) reporting and verifying test results, (b) interpreting test reports correctly, and (c) taking appropriate action when a result has serious implications for a patient or public health.[3]

Wording of reports:

The aim of the clinical microbiologist is to provide clinicians and health officers with reports that are understandable, instructive, relevant and reliable.

The laboratory should, therefore, have a carefully constructed policy for the wording of reports and all staff should adhere to the policy.[18]

Reporting policy:

The laboratory policy for reports should specify not only the wording of interpretative comments, but also the circumstances in which the different comments are to be made. It should, for instance,

- Lay down the circumstances in which the finding of coagulase-negative Staphylococci in a blood culture to be reported with the comment 'probably a contamination from the skin', and without giving its antibiotic sensitivity report.
- In different circumstances as, in a compromised patient when the finding is to be reported as 'possibly of clinical

significance', and the antibiotic sensitivity given.

A policy is also required for reporting the finding of AFB in different specimens. Thus their finding in sputum might be reported: 'many AFB resembling tubercle bacilli seen in film, culture for Mycobacterium is in progress (or is advised). However, there finding in urine might be reported more cautiously: 'a few AFB seen in film which may be commensal Smegma bacilli, culture for Mycobacterium is in progress (or is advised).

Particular care must be given to the policy for the wording of negative reports. These should be phrased in such a way as to indicate which pathogens were sought and not found. The uninformed recipient of a report on a throat swab stating 'no pathogen on culture', might well imagined that a search had been made for every kind of respiratory tract pathogen including viruses, Mycoplasmas and Chlamydiae, when the specimen had been cultured only for pyogenic bacteria. If a throat swab from acute sore throat has been examined the report might properly read 'mixed upper respiratory organisms present. No *Streptococcus pyogenes* on culture. Not cultured for viruses, mycoplasmas and other pathogens. Similarly, if faeces from acute diarrhea has been examined the report should read 'no Salmonella, Shigella, Vibrio or Campylobacter found'.[12]

Conclusions on reporting:

The terminology and format in reporting should be standardized and agreed between laboratory personnel and clinicians.

- Any preliminary report should be followed by a full written report;
- All the completed reports should be scrutinized for credibility by senior staff before signature and issued by the consultant;
- Copies of the reports should be filed in the laboratory for later reference and for response to enquiries;
- Report distribution and delivery systems must be efficient and urgent reports should be informed over telephone;
- Confidentiality of reports should be ensured; and
- Microbiologist should be prepared to give advice on the type of investigation that might be helpful in the diagnosis and be prepared to advice on antibiotic treatment.

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