Microbiology Department

STANDARD OPERATING PROCEDURES

(SOPs)

April 2016

بسم الله الرحمن الرحيم

قال تعالى: (و قل اعملوا فسيرى الله عملكم ورسوله و المؤمنون) صدق الله العظيم

الأخوة والأخوات الزملاء الأكارم...

السلام عليكم ورحمة الله وبركاته..... أما بعد

انه لمن دواعي سروري أن أضع بين أيديكم (دليل طرق الفحوصات المخبرية الموحد) (Standard Operating Procedure SOPs) والذي يتضمن توثيقاً وتفصيلاً لطرق عمل الفحوصات التي تقدمها مختبرات وبنوك دم المستشفيات، وذلك من أجل تنظيم العمل وضمان جودته في جميع جوانب خدمات المختبرات وبنوك الدم.

تأتي الحاجة إلى هذا العمل تأكيداً وترسيخاً لمبدأ العمل بروح الفريق الواحد وتوثيق وتوحيد طرق العمل المبني على أسس علمية والذي تنتهجه وزارة الصحة والإدارة العامة للمستشفيات في كافة مناحي العمل وتطلعاً للرقي بمهنة التحاليل الطبية خاصةً، و بجودة الخدمات الطبية المقدمة في الوزارة بشكل عام.

كما يعتبر هذا الدليل الأول والموحد لجميع مختبرات وبنوك دم المستشفيات و الذي سيكون له بمشيئة الله الأثر الايجابي على جودة الخدمات المخبرية ومجمل الأداء الفني من أجل الارتقاء بالخدمات المقدمة للمواطن.

في النهاية لا يسعني إلا أن أتقدم بخالص الشكر و التقدير لفريق عمل دائرة مختبرات وبنوك دم المستشفيات وجميع من ساهم في انجاز هذا العمل سواءً في الإعداد أو المراجعة أو الطباعة والإخراج.

د. عبد اللطيف الحاج

مدير عام المستشفيات

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Contents

Quality Assurance In Microbiology (*Equipment* ,Safety Measures)

SOPs\HGA \.....H\ M / 01

Version: …1……. Date effective: …… Copy number: ……	Head of department:…….. Quality Officer: …… Director of :……

Introduction

Microbiology can be defined as a branch of biology which is studying of microorganisms that cant seen by naked eye (bacteria-viruses-fungi-parasite).
Quality assurance programmers are an efficient way of maintaining the standards of performance of diagnostic laboratories, and of upgrading those standards where necessary. In microbiology, quality goes beyond technical perfection to take into account the speed, cost, and usefulness or clinical relevance of the test.

Purpose & Definition:

To be of good quality, a diagnostic test must be clinically relevant, i.e. it must help in the prevention or treatment of disease.

Care of equipment:

It is particularly important to take good care of laboratory equipment. Good quality tests cannot be performed if the equipment used is either of poor quality or poorly maintained.

Maintenance Of Equipment :

Good quality equipment is absolutely essential to generate quality results .

Suggested maintenance of commonly used equipment :

Autoclave :

1. Adjust Clean and change water monthly .
2. Adjust water level before each run .
3. Record time , temperature and pressure for each run .
4. Inspect gasket in the lid weekly .
5. Technical inspection every six months .

Incubator:

1. Clean inside walls once in a month .

2. Record temperature at the start of each working day .
3. Technical maintenance every six months .

Microscope :

1. Wipe lenses with lens paper at the end of each day's work .
2. Protect the microscope from dust , vibrations , and moisture .
3. Place a shallow plate containing dry blue silica gel in a box to absorb moisture
4. Check alignment of the condenser once a month .
5. Technical inspection once in a year .

Balance :

1. Keep the balance clean and dry .
2. Always use a container or weighing paper , do not put material directly on the pan Prevent the balance from drafts of air .

Refrigerator:

1. Place at least 10 inches away from the wall .
2. Clean and defrost at least every two months .
3. Records temperature daily .
4. Technical service at least once a year .

Centrifuge *:*

1. Wipe inner walls with antiseptic solution weekly .
2. Check brushes and bearings every six months .

Laboratory Safety Measures:

Every microbiology laboratory must have a strictly enforced safety program to protect the technical personnel working in the area , to ensure the validity of the testing procedures by preventing contamination , and to prevent the spread to infectious microbial agents .

The following regulations are recommended for the safety program of general microbiology laboratories:

1. Benches should be washed with a suitable disinfectant (e.g. chlorine compound) before work is begun.
3. When work is completed , all benches should be disinfected again with a suitable disinfectant .
4. Infectious organisms should be performed in safety cabinet.

5. Infectious Materials should be placed in biohazard containers and sent to incineration.
6. The laboratory is restricted access to authorized personnel only .
7. No smoking , drinking , or eating is permitted in the area .
8. No mouth pipetting is allowed in the lab .
9. All syringes should be placed in a puncture –proof container .
13. safety cabinet should be checked yearly for efficiency .
14. All biohazard materials should label clearly .
15. The use of gloves is recommended for handling infectious materials .
16. Hands should be washed before and after the work.
17. All spills and accidents should be reported to supervisor.

Blood Agar Preparation and Sterilization

SOPs \ HGA \.....H\ M \ 02

Version: …1……. Date effective: …….. Copy number: ……..	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media, blood agar used to differentiating hemolytic organisms.

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Equipment and Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic)
- Glass conical flask (and cotton Plugged).
- Dehydrated Media.
- Defibrinated Blood (sterile).
- Beakers and Magnetic stirrer .
- Distilled water.
- Analytical balance .
- Water bath.
- Autoclave.
- Cotton.

Abbreviation:

(BA): Blood Agar

(R.T): Room Temperature.

(Ibs): Pounds Per Square.

Procedures:

1-Suspend 40 gm in 1000 ml distilled water

2-Heat to boiling to dissolve the medium completely

3-Sterile by autoclaving at 15 Ibs Pressure 121º C for 15 min

4-Cool to 50ºC & aseptically add 5 % v/v Sterile blood

5- Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 C^{o}.

Quality control of blood agar media:

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- Culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Chocolate Agar Preparation and Sterilization

SOPs \HGA \.....H \ M \ 03

Version: …1……. Date effective: …….. Copy number: ……..	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media, chocolate agar is used specially for fastidious organisms **as Heamophilus Influenza.**

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Equipment and Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).

-Glass conical flask (and cotton Plugged).

- Dehydrated Media.

- Defibrinated Blood (sterile).

- Beakers and Magnetic stirrer .

- Distilled water.

- Analytical balance .

- Water bath.

- Autoclave.

- Cotton

Abbreviation:

(CA): Chocolate Agar

Procedures:-

The same procedures of blood agar media except , we heat the blood agar to 80ºC until change the color .

Quality control of chocolate agar media:

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

MacConky Agar Preparation and Sterilization

SOPs \ HGA \.....H\ M \ 04

Version: 1.....	Head of department:.....
Date effective:	Quality Officer: .....
Copy number:	Director of :.....

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for gram negative bacteria).

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).

-Glass conical flask (and cotton Plugged).

- Dehydrated Media.

- Distilled water.

- Analytical balance .

- Water bath.

- Autoclave.

- Cotton.

Abbreviation:

(Mac): MacConky Agar

Procedures:-

1-Suspend 51.5 gm in 1000 ml distilled water

2-Heat to boiling to dissolve the medium completely

3-Sterile by autoclaving at 15 Ibs Pressure 121º C for 15 min

4-Cool to 50ºC then Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 c◦.

Quality control of MacConky agar media:

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- Culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Muller Hinton Agar Preparation and Sterilization

SOPs \HGA\.....H\ M\ 05

Version: …1……. Date effective: …….. Copy number: ……..	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for sensitivity).

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).

-Glass conical flask (and cotton Plugged).

- Dehydrated Media.

- Distilled water.

- Analytical balance .

- Water bath.

- Autoclave.

- Cotton.

Abbreviation:

(MH): Muller Hinton Agar

Procedures:-

1-Suspend 38 gm in 1000 ml distilled water

2-heat to boiling to dissolve the medium completely

3-sterile by autoclaving at 15 Ibs Pressure 121º C for 15 min

4-Cool to 50ºC then Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 c◦.

Quality control of Muller Hinton Agar (MH) agar media:

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria. \

Note :refer the quality control sheet in the index.

Sabouraud Dextrose Agar Preparation and Sterilization

SOPs \HGA\.....H\ M\ 06

Version: …1……. Date effective: …….. Copy number: ……..	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for fungi).

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).
-Glass tubes (screw capped and cotton Plugged).
- Dehydrated Media.
- Flasks , Beakers and Magnetic stirrer .
- Distilled water.
- Analytical balance .
- Water bath.
- Autoclave.
- Cotton.

Abbreviation:

(Sab): SabouraudDextrose Agar

Procedures:-

1-Suspend 65 gm in 1000 ml distilled water

2-heat to boiling to dissolve the medium completely

3-sterile by autoclaving at 15 Ibs Pressure 121º C for 15 min

4-Cool to 50ºC then Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 c◦.

Quality control of SabouraudDextrose Agar

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Shigella Salmonella Aagr Preparation and Sterilization

SOPs \HGA\.....H\ M\ 07

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for stool).

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).

- Dehydrated Media.

- Flasks , Beakers and Magnetic stirrer .

- Distilled water.

- Analytical balance .

- Water bath.

- Cotton.

Abbreviation:

(SSA): Salmonella ShigellaAagr.

Procedures:-

1-Suspend 63 gm in 1000 ml distilled water

2-heat to boiling to dissolve the medium completely

Note:

don't autoclave or over heat , because overheating may destroy the selectivity of media

3-Cool to 50ºC then Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 c◦.

Quality control of Salmonella ShigellaAagr (SSA)

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- Culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Hekton Enteric Aagr Preparation and Sterilization

SOPs \HGA\.....H\ M\ 08

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for stool).

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction:-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).

- Dehydrated Media.

- Flasks , Beakers and Magnetic stirrer .

- Distilled water.

- Analytical balance .

- Water bath.

- Cotton.

Abbreviation:

(HE): Hekton Enteric Aagr

Procedures:

1-Suspend 76.67 gm in 1000 ml distilled water

2-heat to boiling to dissolve the medium completely

Note:

don't autoclave or over heat , because overheating may destroy the selectivity of media

3-Cool to 50ºC then Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 c◦.

Quality control of Hekton Agar:

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Xylose lysine deoxycholate Preparation and Sterilization

SOPs \HGA\.....H\ M\ 09

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for stool).

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

- Sterile Petri dishes (plastic).

- Dehydrated Media.

- Flasks , Beakers and Magnetic stirrer .

- Distilled water.

- Analytical balance .

- Water bath.

- Cotton.

Abbreviation:

(XLD): Xylose lysine deoxycholate agar.

Procedures:

1-Suspend 56.68 gm in 1000 ml distilled water

2-heat with frequent agitation until the media boils.

Note:

don't autoclave or over heat , because overheating may destroy the selectivity of media

3-Cool to 50ºC then Pour the media in sterile Petri dishes and wait to cool at R.T. then we can stored in a refrigerator at 4-12 c◦.

Quality control of Xylose lysine deoxycholate

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- Culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Fluid Selenite Cystine Medium Preparation and Sterilization

SOPs \HGA\.....H\ M\ 10

Version: …1……. Date effective: …….. Copy number: ……..	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

A culture media is special medium to grow different kinds of microorganisms and distinguish the growth characteristics of microorganisms in various differential, selective and enriched media.(for stool).

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Restriction :-

A culture medium is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms. The effective culture medium must contain all the nutrients required for the growth of the microorganism.

Items Required for preparation of Media :(Materials):

-Glass tubes (screw capped and cotton Plugged).

- Dehydrated Media.

- Flasks , Beakers and Magnetic stirrer .

- Distilled water.

- Analytical balance .

- Water bath.

- Cotton.

Abbreviation:

(SF): SeleniteFluidCystine Medium.

Procedures:

1-Suspend 76.67 gm in 1000 ml distilled water

2-heat to boiling to dissolve the medium completely

3-distribute in sterile test tubes

Note:

don't autoclave or over heat , because overheating may destroy the selectivity of media

Quality control of Fluid Selenite Cystine Medium

Negative control:

- To control prepared media,5% of prepared media selected randomly in an incubator 24hour, if there is any growth of any organism in the media we must be discard the media and look for the problem to solve it and prepare another one .

Positive control:

- Culture two different known of microorganisms on random media to testing growth and inhibition growth of bacteria.

Note :refer the quality control sheet in the index.

Catalase Test

SOPs \HGA \.....H \ M \ 11

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

The enzyme catalase catalyzes produce by small amount bacteria that liberated of water and oxygen and water from hydrogen peroxidase, metabolic end product toxic to bacteria.

1. Differentiate staphylococci (catalase positive) from streptococcus are catalase negative
2. Differentiate Listeria monocytogenes (catalase positive) from beta hemolytic streptococci.
3. Differentiate bacillus Spp. (catalase positive) from Clostridium Spp. (mostly catalase positive).
4. All Neisseria Spp. are catalase positive.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect colony growth on agar.

Equipment & Items required:

Loop or sterile wooden, glass slide, 30% (H_2O_2) hydrogen peroxide.

Abbreviations:

H_2O_2 : hydrogen peroxide.

Spp. :species

Procedures:

1. Use loop or sterile wooden to transfer small amount pure colony growth to clean, dry glass slide.
2. Place drop of 30% H_2O_2 onto colony on slide.
3. Observe for the evaluation of bubble of gas, indicating positive test.

Quality control procedures:

Positive control: colony of staphylococcus spp.

Negative control:colony of streptococcus spp.

Note :refer the quality control sheet in the index.

Limitations/ Interfering substance:

1. The test should be performed only on isolates grow on non-blood-containing media because red blood cells contain some catalase.
2. Some bacteria produce peroxidase that catalase breakdown of H_2O_2; the catalase test may thus appear to be weakly positive (few bubbles slowly elaborated) this reaction should not be confused with truly positive catalase test.

Expected result:

Positive : air bubble

Negative: no air bubble

Interpretation of the results:

The staphylococcus are catalase positive and produce copious bubbles; streptococci are catalase negative and do not yield any bubbles of H_2O_2.

Bacitracin test

SOPs \ HGA\.....H\ M\ 12

Version: …… Date effective: …… Copy number: ……	Head of department:…… Quality Officer: …… Director of :……

Purpose & Definition:

The test used to determine the effect of a small amount of bacitracin (0.04 U) on organism. Streptococcus pyogenes is inhibited by small amount bacitracin in the disk; other beta- hemolytic streptococci usually are not.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect β-hemolytic streptococci colony growth on blood media.

Equipment & Items required:

Blood agar, sterile swab, bacitracin disk, sterile loop, forceps, incubator.

Abbreviations:

(U): unit

Procedures:

1. Using inoculating loop or sterile swab, steak 2-3 of pure colony culture on blood agar plate.
2. Using heated forceps, place bacitracin on the inoculate.
3. Incubate the plate for 18-24 hr. at 37 C^{o}.
4. Look for zone of inhibition around disk.

Quality control procedures:

Positive: Streptococcuspyogenes.

Negative:other beta-hemolytic streptococci.

Note :refer the quality control sheet in the index.

Expected results:

1. **Positive**: Any zone of inhibition around the disk (Positive: Streptococcus pyogenes)
2. **Negative:** No zone of inhibition (Negative: other beta-hemolytic streptococci *)*.

Interpretation of the results:

- The test usually used to differentiate streptococcus pyogenes (pathogen) from normal flora which growth on throat.
- Some S. aureus may be susceptible, so only coagulase negative strain should be tested by bacitracin.

Optochin susceptibility test

SOPs \ HGA\.....H\ M\ 13

Version: …1……. Date effective: …….. Copy number: ……	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

Hydrocupreine hydrochloride (optochin) at the concentration 5.0 µg inhibits the

growth of S. pneumoniae, but not of other streptococci. S. pneumoniae, therefore, be differentiated from other alpha-hemolytic streptococci by the formation

of a zone of inhibition around a disk impregnated with this compound.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect α-hemolytic streptococci colony growth on blood media.

Equipment & Items required:

Blood agar media, swab , Optochin disk, incubator, 5%-10% CO_2.

Abbreviations:

(Op) :optochin

Procedures:

1. Using inoculating loop or sterile swab, steak 2-3 of pure colony culture on blood agar plate.
2. Using heated forceps, place optochin on the inoculate.
3. Incubate the plate overnight at 37 C^o in 5%-10% CO_2.
4. Look for zone of inhibition around disk.

Quality control procedures:

Positive: Streptococcus pneumonia

Negative: Viridans Streptococcus .

Note :refer the quality control sheet in the index.

Expected results:

1. **Positive:**zone ≥ 14 mm surrounding a 6 mm disk or ≥ 16 mm surrounding 10 mm disk (according to disc used).
2. **Negative:**No zone of inhibition or < 14 (6 mm disk) or < 16 (10 mm disk)(according to disc used).

Interpretation of the results:

The pneumococcus should be yield a positive test result, and the viridans streptococcal species should grow right up to and under optochin disk.

Limitations/ Interfering substance:

1. Culture do not grow as well in ambient air and large zones of inhibition occur result false positive.
2. Positive test for *S. pneumoniae*should be tested for bile solubility.

Oxidase test(Kovac's Method)

SOPs \ HGA\.....H\ M\ 14

Version: …1……. Date effective: …….. Copy number: ……	Head of department:…….. Quality Officer: …….. Director of :……

Purpose & Definition:

The oxidase test is based on the production of the enzyme indophenol oxidase by organisms containing cytochrome C. Indophenol oxidase, in the presence of atmospheric oxygen, oxidizes a redox dye (N-tetramethyl-p-phenylenediaminedihydrochloride) to form a dark-purple indophenol compound. Bacterial genera characterized as oxidase positive include *Neisseria* and *Pseudomonas*. Genera of the Enterobacteriaceae family are characterized as oxidase negative.

This test is used for differentiating *Micrococcus* from *Staphylococcus* . Micrococci should yield a positive result. Staphylococci should yield a negative result, with the exception of *Staphylococcus sciuri.*

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect colony growth on agar media

Equipment & Items required:

Loop or sterile wooden applicator, moisten filter paper with N-tetramethyl-p-phenlenediamine (commercial as filter paper or disk).

Abbreviations:

(**OX) :** oxidase.

(E): Escherichia

Procedures:

1. Use platinum wire or wooden applicator to remove small portion of bacteria colony (preferably not more than 24 hr old) from agar and rub the sample on filter paper or commercial disk.
2. Observed development of a violet or purple color (positive reaction). No color change indicates a negative result within 10 second (time critical).

Quality control procedures:

Positive: Neisseria gonorrhea

Negative: E coli .

Note : refer the quality control sheet in the index.

Expected results:

Positive:dark purple color

Negative:colorless

Interpretation of the results:

Oxidase positive organisms give blue color within 5-10 seconds, and in oxidase negative organisms, color does not change.

Limitations/ Interfering substance:

1. Wire loops containing iron may give a false-positive reaction and reactions from weak oxidase-positive organisms may be inaccurate. Colonies growing on selective media or differential media containing glucose cannot be used for oxidase. determination because fermentation inhibits indophenol oxidase activity resulting in false negative results.
2. The reagents used in the oxidase test have been shown to auto oxidize, so it is very important to use fresh reagents, no older than 1 week , Steel found that the auto oxidation can be slowed by the addition of 1% ascorbic acid.
3. Both bacteria and yeast grown on media containing high concentrations of glucose show inhibited oxidase activity, so it is recommended to test colonies grown on media without excess sugar, such as nutrient agar. Tryptic soy agar is also an excellent media.
4. Older cultures are less metabolically active and are thus unreliable for this test in a clinical setting. In the classroom, if by necessity older cultures must be used, expect longer reaction times.
5. All reaction times listed are based upon freshly made reagents without stabilizing agents. If you use commercially prepared reagents, these often contain stabilizing agents and thus you should follow the manufacturer's instructions.

Coagulase tube test

SOPs \ HGA\.....H\ M\ 15

Version: …1……. Date effective: …….. Copy number: ……	Head of department:…….. Quality Officer: …….. Director of :……

Purpose & Definition:

Coagulase is a thermostable enzyme found primarily in *S. aureus*and is used to differentiate *S. aureus*from other commonly isolated staphylococci. Two forms of coagulase exist: one is bound to the cell wall (clumping factor) and react with fibrinogen, and one is liberated by the cell as "free coagulase.

Slide coagulase test detects the bound coagulase, which acts directly on the fibrinogen in plasma and causes clumping of bacteria. whereas tube method detect free coagulase when *S. aureus*suspension with plasma cause formation clot.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect colony growth on agar media

Equipment & Items required:

Loop or sterile wooden applicator, tube, 0.5 ml plasma, incubator.

Abbreviations:

***(S. aureus*):** staphylococcus aureus

***(S. epidermidis*):**staphylococcusepidermidis

(EDTA): ethylenediaminetetraacetic acid.

Procedures:

1. Prepare a heavy suspension of the Staphylococcus colonies in 0.5 mL of water.
2. Place the suspension into a tube containing rabbit plasma and incubate at 35-37◦C for 4 hr.
3. Examine for the presence of a clot. If negative for a clot, re-incubate the tube and reexamine at 24 h. Any degree of clot formation at 4 h or 24 h is considered a positive reaction. No clot formation at 24 h is considered negative coagulase reaction.

Quality control procedures:

Positive:*S. aureus*

Negative: *S. epidermidis*.

Note :refer the quality control sheet in the index.

Expected results:

Positive: aggregation (visible clot)

Negative:no agglutination (remain smooth milky)

Limitations/ Interfering substance:

Rabbit coagulase plasma with (EDTA) or citrate is preferable to human plasma which may contain substances inhibitory to the reaction.

Interpretation of the results:

Coagulase test is used to screen *S. aureus which almost coagulase positive.* Although other species of staphylococci *may be coagulase positive (e.gS.intermedius, S.hyicus)*, they are not important agent of human disease. Not all *S. aureus* strain produce clumping factor.

Slide Staphylococcus aureus Kit

SOPs \ HGA\.....H\ M\ 16

Version: …1……. Date effective: …….. Copy number: ……..	Head of department:…….. Quality Officer: …….. Director of :……..

Purpose & Definition:

Staph kit is rapid latex agglutination test for the detection of staphylococci which produce clumping factor and/or protein A from those species of staphylococci which do not.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Specimen should be fresh 18-24 hrs. cultures, 2-3 Suspect staphylococcus colony growth on blood or chocolate media.

Equipment & Items required:

Staph kite reagent, loop, slid, shaker, positive and negative control.

Abbreviations:

(Staph) : staphylococcus.

Procedures:

1. Emulsify 1-2 staphylococcus colony with one drop of sterile normal saline
2. Then added one drop of reagent on slide.
3. Mix reagent and culture using sterile loop
4. Gently and evenly, rock and rotate the test slide for 1 minute and examining for agglutination.

Quality control procedures:

Positive control.

Negative control.

Expected results:

Positive:agglutination pattern of latex in clear solution.

Negative:no agglutination

Limitations/ Interfering substance:

Some species of staphylococcus other than S.aureus, notably S. intermedius and S. hyicus may give positive results in conventional coagulase test and may also agglutinate latex reagent

DNase test

SOPs \ HGA\.....H\ M\ 17

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

The test used to determine ability of organism to hydrolyze DNA. S. aureus can be identified by produced heat-stable deoxyribonuclease enzyme which hydrolyze DNA and addition 1 N HCL after 24 hrs.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect staphylococcus colony growth on blood or chocolate media.

Equipment & Items required:

DNase agar media, swab , incubator.

Abbreviations:

(DNA): deoxyribonucleic

(DNase):deoxyribonuclease enzyme

Procedures:

1. Inoculate the DNase agar with organism to be tested and streak for isolation.
2. Incubate aerobically at 37 C^{o} for 13 to 24 hr.
3. Add 1NHCL to the DNA agar.
4. Wait 5 minutes and read the clear zone around S. aureus.

Quality control procedures:

Positive control: *S. aureus*

Negative control: *S. epidermidis*.

Note : refer the quality control sheet in the index.

Expected results:

Positive: when DNA is hydrolyzed, The acid(1NHCL) precipitates unhydrolyzed DNA. *S. aureus*colonies are therefore

surrounded by clear areas due to DNA hydrolysis.

Negative:no change(clear areas) in the media.

Novobiocin test

SOPs \ HGA\.....H\ M\ 18

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

The Novobiocin test is used to differentiate coagulase negative staphylococci.

S. saprophyticus is only to E. Coli as the most frequently causative organism of urinary tract infections in women

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

The test should be performed on isolated colonies, catalase negative gram positive cocci from blood agar.

Abbreviations:

(Nov): Novobiocin

Procedures:

1. Inoculate the Muller Hinton or blood agar with organism to obtain confluent growth.
2. Aseptically apply one µg Novobiocin disk onto the inoculated agar surface.
3. Incubate aerobically at 37 C^{o} for 18 to 24 hr.
4. Measure the diameter of zone inhibition around the Novobiocin disk and record susceptible or resistant.

Quality control procedures:

Positive control (resistant):staphylococcus saprophyticus.

Negative control: staphylococcus epidermidis.

Expected results:

Staphylococcus saprophyticus- groth> 12 or uniform growth up to the edge of disk.

Staphylococcus epidermidis-zone of inhibit >16 mm or large

Limitations/ Interfering substance:

The Novabiocin disk is not helpful and can give misleading results if performed on isolates other than those from urinary specimens, occasional human isolates that are not S.Saprophyticus as S.Cohnii, S. Xylosis.

Bile Esculin Agar Slant

SOPs \ HGA\.....H\ M\ 19

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Group D streptococci (including *Enterococcus* spp.) and a few other bacteria, such as *Listeria* spp., can grow in the presence of 40% bile and also hydrolyze esculin to esculetin. Esculetin reacts with ferric ions, supplied by ferric citrate in the agar medium, to form a diffusible black complex. Most strains of viridans streptococci that are capable of hydrolyzing esculin will not grow in the presence of 40% bile.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect streptococci colony growth on blood or chocolate media.

Equipment & Items required:

Bile esculin agar media, swab , incubator.

Procedures:

1. Streak the surface of the bile esculin agar slant with several colonies of the organism to be tested.
2. Incubate at 35◦C in non-CO2 for 24 to 48 h.

Quality control procedures:

Positive: *enterococcusfaecalis*
Negative: *Streptococcusmitis*.

Expected results:

Positive: blackening of agar slant

Negative: No blackening medium.

Streptococcus – Check Kit

SOPs \ HGA\.....H\ M\ 20

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

This kit for the identification of streptococcal of Lancefield groups A,B , C , D ,F and G by agglutination of specific antibody coated latex particles in the presence of enzymatically extracted antigen, streptococci carry group specific carbohydrate antigens in their cell walls , after extraction by a specially developed enzyme preparation these antigens will agglutinate latex particles coated with the corresponding antibody ,the latex remains un smooth suspension in the absence of group specific antigen.

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Note colonies characteristics, hemolysis and cell morphology before start the test, ensure that organism are gram positive and catalase-negative isolate 2-3 Suspect streptococcus colony growth on blood or chocolate media.

Equipment & Items required:

Strep-kit reagent, loop, slide, shaker, positive and negative control.

Abbreviations:

strep : streptococcus sp.

Procedures:

1. Using a sterile loop pick 2-6 colonies of streptococci
2. Emulsify the colonies in 0.4 ml of extraction enzyme

3. Incubate the mixture in water bath 37Co for 10 minutes. Shaking tubes after 5 minutes incubation.
4. Dispense 1 drop of each latex reagent into appropriate labeled circle on the test slide.
5. Add one drop of the extract to each drop of latex reagent and mix the contents with a separate mixing stick.
6. Rock the slide for not longer than 1 minute and observe for agglutination. Record the result.

Quality control procedures:

Positive control.

Negative control.

Expected results:

Positive:agglutination of RBC

Negative: no agglutination

Interpretation of the results:

1. Positive: Strong agglutination of specimen with one latex reagent, normally within a few seconds of mixing constitutes a positive result and within the accepted limitations of the test procedure, indicates the presence of that specific Streptococci group, either A, B, C, D, F, or G.

2. Negative: No visible agglutination of latex particles in a milky liquid constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of Streptococci groups A, B, C, D, F and G.

3. Equivocal: If agglutination occurs in all groups then either the enzyme has been over-inoculated in which case repeat the test using a lighter inoculum. Or a mixed culture was tasted. In which case check for purity and retest.

Limitation

1. False positive reaction have been known to occur with organisms from unrelated genera e.g. Escherichia, klebsiella or pseudomonas.
2. Group D antigen is common to organism of group Q, R,S.
3. False negative or false positive results may occur due to
   a. Contamination of test materials.
   b. Improper storage of test material or omission reagents.
   c. Deviation from recommended techniques.

API 20 E test

SOPs \ HGA\.....H\ M\ 21

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

API 20 E test is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gram-negative rods which uses 21 miniaturized biochemical tests and database. The complete list of those organism that possible to identify with this system is given in the identification Table at end of package insert. .

Responsibilities

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

The organism to be identified must be isolated on culture medium adapted to culture Enterobacteriaceae and other non-fastidious, Gram-negative, according to standard microbiological techniques.

Equipment & Items required:

API 20 strips, incubation box, API E sheet, API E NaCl 0.85% medium ,TDA reagent, JAMES reagent, VP1+VP2 reagent, NIT1+NIT2 , mineral oil, sterile needle and syringe

Abbreviations:

(API E 20): Analytical Profile Index for Enterobacteriacea (20 Test)

(CIT): Citrate test

(VP): VogusProscuer test

(GEL): Gelatine liquefaction test

(TDA): TryptophaneDeaminase

(NIT): Nitrite

(NaCl): Sodium Chloride.

(D.W): Distiled Water.

(LDC): Lysine decarboxylase.

(GEL): Gelatinase.

(ADH): Arginine dihydrolase.

(ODC): Ornithine decarboxylase.

(H2S): Sodium thiosulfate.

(URE): Urease.

(IND): Indole

Procedures:

1. Oxidase test should be perform according to manufacturer's instructions for organism.
2. Prepare incubation box and distribute 5 ml of D.W without additive or chemicals which release gas.
3. Remove strip from its packaging
4. Place the strip in incubation box.
5. Using sterile loop to isolate single well-isolate colony from plate (culture 18 24 hrs.) and emulsify in 5 ml NaCl 0.85% medium to obtain homogeneous bacterial suspension
6. Distribute the bacterial suspension into tube of the strip
    a. For the test CIT,VP and GEL tests fill both tube and cupules
    b. For the other tests fill only the tube not cupules
    c. For the test ADH, LDC, ODC, H2S and URE create anaerobic condition by overlaying with mineral oil.
7. Close the incubation box
8. Incubate at 36Co ±2 for 18-24 hr.

Quality control procedures:

The media, strips, and reagent are systematically quality controlled at various stages of their manufacture.

Escherichia Coli	Enterobacter cloacae
Proteus mirabilis	Klebsiella pneumonia

Interpretation and reading

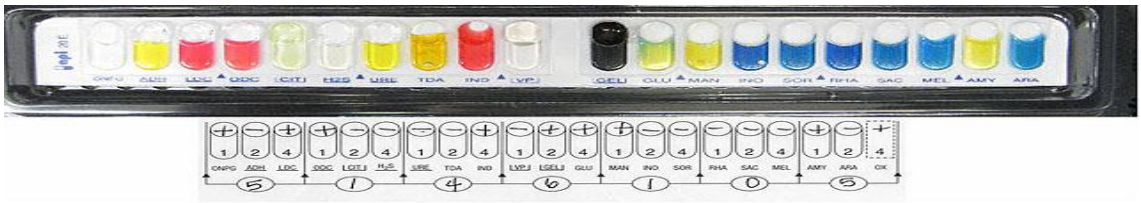
1. After incubation period, read the strip by referring to the reading Table.
2. TDA test: add 1 drop TDA reagent ,IND test add one drop IND reagent, VP test add one drop each of VP1 and VP2 reagent wait at least 10 minute.
3. The indole production test must be performed last since this reaction releases gaseous products which interfere with interpretation of other tests on strip. The plastic incubation lid should not be replaced after addition of reagent.

Determination of numerical profile:

Read the API strip according to the interpretation table, and record the result on the report sheet. On the report sheet, the test are separated into groups of three and number 1 , 2 or 4 is allocated for each test. By adding the numbers corresponding to the positive reaction within each group, a7- digit profile number is obtained for 20 tests of the API 20E strip.

The 7- digit profile is then compared with the numerical profile in the API 20 E analytical profile index book to obtain the organism identification.

On the result sheet, the tests are separated into groups of 3 and value 1,2,4 is indicate for each. As picture



Limitation

1. The API 20 E system is intended uniquely for the identification of *Enterobacteriaceae*and those nonfastidious, Gram-negative rods included in the database. It cannot be used to identify any other microorganisms or to exclude their presence.
2. Discrepancies with respect to conventional methods may be observed. They are due to the different principles of the reactions used in the API technique. In addition, substrate variations exist that also account for percentage differences.
3. On rare occasions, the glucose reactions for organisms such as *Klebsiella*or*Proteus* may revert from positive to negative, in which instance a bluish-green color is seen. This reaction will be recorded as a negative reaction. Such occurrences are reflected in the percentages indicated in the Identification Table.
4. If *Salmonella* or *Shigella*are identified, serological identification must be performed to confirm the bacterial identification.

5. Only pure cultures of a single organism should be used.

Gram stain

SOPs \ HGA\.....H\ M\ 22

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Used to distinguish between Gram-negative (pink or red) and Gram-positive cells (purple or blue).
allows determination of cell morphology, size and arrangement.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

The Gram stain is useful in the diagnosis of bacterial and some fungal infections by demonstrating the causative agent in smears prepared from clinical material or positive blood cultures, skin smear (for Neisseria spp.) and CSF turbid. Smears prepared from growing cultures demonstrate microscopic morphology that is helpful in organism identification.

Equipment & Items required:

Staining reagents: Crystal Violet, 95% ethanol/ acetone ,Lugol's Iodine ,Safranin

Loop , Slide & coverslip, Gloves, Microscope ,flame. Staining rack,Pen or maker for labeling,Immersion oil

Abbreviations:

(G.S): gram stain

Preparation of smear

From colonies:

1. Place a drop of sterile distilled water or saline onto a glass slide.
2. Using a 1 µLoop, remove a colony and emulsify in the droplet.
3. Place on slide warmer to dry
4. Fix the dried film by passing it briefly through the Bunsen flame two or three times without exposing the dried film directly to the flame.

Stain procedure

1. Flood slide with crystal violet.
2. Let stand one minute.
3. Rinse with tap water and drain off excess water.
3. Flood slide with iodine and let stand for one minute.
4. Rinse with tap water and drain off excess water.
5. Decolorize with 95% ethyl alcohol/acetone until most of the crystal violet is removed in thin areas (length of decolorizing time depends on thickness of smear).
6. Rinse with tap water and drain off excess water.
7. Counterstain with Safranin for 60 seconds.
8. Rinse with tap water and drain off excess water.
9. Place on slide warmer until dry or blot gently on paper towel.

Examination of gram stains

1. Place a drop of immersion oil on the slide.
2. Examine using oil immersion (100x) objective.
3. Focus using coarse and fine adjustment knobs until objects are in focus.

Results:

- Violet organisms – Gram Positive
- Red organisms – Gram Negative
- Yeasts stain Gram Positive
- Describe organisms by their Gram reaction (Gram Positive - blue, Gram negative – red) and their microscopic morphology and arrangement (e.g. cocci in pairs, chains, clusters; bacilli, small, large, filamentous, yeasts).

Sample Report: e.g. "Gram positive cocci in chains"

Quality control

Frequency: Once per week

Controls: Prepared slides with Gram positive and Gram negative organisms.

Gram Positive: *Staphylococcus aureus*

Gram Negative: *E. coli*

Note :refer the quality control sheet in the index

Acceptable Results:

Gram Positive: blue cocci

Gram negative: red bacilli

Corrective Actions:

1- Re-stain new control slide.

2- If still unacceptable, have a different technologist re-stain the slide.

Limitations/ Interfering substance:

1. The length of time of the decolorizing step (ethanol/acetate) is critical. Thin smears require less time than thick. Too much decolorizing will render everything on the slide red; not enough, blue.
2. Gram positive organisms, especially bacilli, from cultures that are not fresh (>48 hrs.) may not retain the crystal violet and stain red.
3. Some species of bacteria are described as "Gram variable" i.e. may stain blue or red or show both colors (e.g. *Gardnerellavaginalis*).
4. Direct smear can be taken for turbid CSF sample and skin smear.
5. To remove immersion oil from a slide without damaging the smear, lay a piece of lens tissue on the slide, add a drop or two of xylene and draw the lens tissue across the slide. Repeat if necessary.
6. Acetone is a more rapid decolorizes than alcohol and must be used with some care. Excessive decolorization turns Gram positive appear as Gram negative.
7. Some bacteria which are poorly stained by Safranin, such as Hemophilus spp., Legionella spp. , and some anaerobic bacteria, are readily stained by basic fuchsine, but not Safranin.

Acid fast stain

SOPs \ HGA\.....H\ M\ 23

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

For diagnosis & monitoring tuberculosis.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Sputum, lymph node biopsy, urine sediment, CSF, gastric lavage, pulmonary aspiration, semen , tissue biopsy.

Equipment & Items required:

- **Reagent** : Carbolfuchsin , Acid-alcohol, Methylene blue
- Slide & coverslip , Gloves , Microscope , flame
- Centrifuge , Staining rack , Pen or maker for labeling , Immersion oil

Abbreviations:

- **(AFS):** acid fast stain.

Procedure

1. Make a thin smear on slide .
2. Air dry slide 10 minutes at 60 °C, heat-fix slide by passing the slide 3 times on Bunsen burner.
3. Flood slide with CarbolFuchsin
4. Hold a flame beneath the slide until steam appears but do not allow it to boil (if hot procedure are used).
5. Allow hot slide to sit for 3 to 5 minutes, rinse with tap water
6. Flood slide with 3% hydrochloric acid in isopropyl alcohol

7. Allow to sit 1 minute, rinse with tap water
8. Flood slide with Methylene Blue
9. Allow to sit 1 minute, rinse with tap water
10. Blot dry
11. View under oil immersion lens.

Examination of AFS.

1- Place a drop of immersion oil on the slide.

2- Examine using oil immersion (100x) objective.

3- Focus using coarse and fine adjustment knobs until objects are in focus.

Results:

1. Acid-fast cells will appear pink
2. Non Acid-fast cells will appear purple

Quality control:

- Positive and negative specimens of acid fast bacilli.

Note : refer the quality control sheet in the index.

Reporting:

Finding	Recording
No AFB found in at least 100 fields	**Negative**
1–9 AFB per 100 fields	**Positive (exact NO./100)**
10–99 AFB per 100 fields	**Positive(+)**
1–10 AFB per field (count at least 50 fields)	**Positive(++)**
More than 10 AFB per field (count at least 20 fields)	**Positive(+++)**

Limitations/ Interfering substance:

Among the possible reasons for *false-negative results* are:

- poor quality of specimen, not taking proper portion of specimen for smear preparation
- excessive decolourization;
- too little time staining with carbolfuchsin;
- over-staining with methylene blue, overheating during fixing.

KOH test

SOPs \ HGA\.....H\ M\ 24

Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Potassium hydroxide (KOH) is a quick, inexpensive fungal test to dermatophytes..

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Clinical Material: Skin, hair& nail scrapings; urine, sputum and bronchial washings; cerebrospinal fluid, pleural fluid and blood; tissue biopsies from various visceral organs and indwelling catheter tips.

Equipment & Items required:

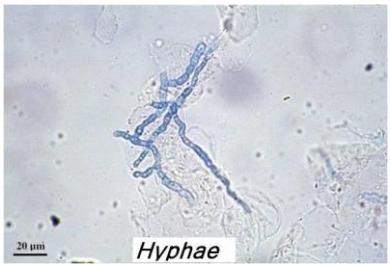
1. Microscope
2. Slides
3. Coverslips (22 x 22 mm)
4. 20-30% KOH
5. Cotton-tipped swabs
6. Bright field microscope
7. Scrape skin scales
8. Forceps**.**

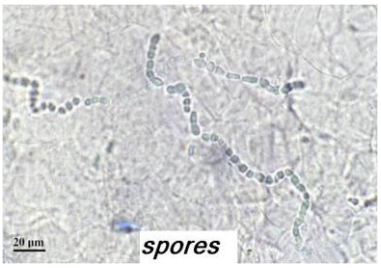
Abbreviations:

(KOH): Potassium hydroxide

Procedure

1. Collection: Skin, nail, or hair samples are collected from the infected area on the patient. For skin samples, a scalpel or edge of a glass slide is used to gently scrape skin scales from the infected area. For hair samples, a forceps is used to remove **hair shafts and follicles** from the infected site. If the test is being sent to a laboratory, the scrapings are placed in a sterile covered container.
2. The scrapings are placed directly onto a microscope slide and are covered with 30% potassium hydroxide.
3. The slide is left to stand until clear, (scale hair 30 min, skin 20min and nail 2-3 min).
4. To enhance clearing dimethyl sulfoxide can be added to the slide. To make the fungi easier to see lactophenol cotton blue stain can be added.
5. The slide is gently heated to speed up the action of the KOH.
6. Adding calcofluor-white stain to the slide will cause the fungi to become fluorescent, making them easier to identify under a fluorescent microscope.





7. Place the slide under a microscope to read.
8. Examine the prepared slide, microscopically, under 10x objective of microscope (low power objective).
9. Confirm the presence of Yeast cells and Pseudohyphae under 40x objective of microscope (high dry objective).

Result:

Hyphae, spore and mycelium.

Meningitides Antisera

SOPs \ HGA\.....H\ M\ 25

Version: 1.....	Head of department:.....
Date effective:	Quality Officer: .....
Copy number:	Director of :.....

Purpose & Definition:

Neisseria meningitides also known as meningococcus is found in the oropharynx and nasopharynx of humans. Because Survive poorly in environment, humans are primary reservoir in asymptomatic persons the carrier state lasts for variable time periods usually weeks. The microorganism is transmitted from person to person by direct contact with respiratory secretion or airborne droplets.

Typical human organism specimen for isolating the organisms are CSF, blood, skin lesions and nasopharyngeal swabs.

N. meningitides is divided into serologic groups based on presence of either capsular or outer membrane protein antigens. Among the currently recognized groups are A, B, C, D, 29E, H, I, K, X, Y, Z, Z', and W135. Group A,B, C, Y and W135 are most frequently implicated in systemic disease.

N. meningitides are gram negative cocci usually occurring in pair called diplococcic. There are strict aerobes and produce enzyme cytochrome oxidase. The growth of N. meningitides is enhance by CO_2-enriched atmosphere.

Serologic confirmation involves reaction in which microorganism antigen reacts with its corresponding antibody. This test produces macroscopic clumping called agglutination.

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

2-3 Suspect colony growth on agar.

Equipment & Items required:

Loop or sterile wooden, agglutination glass slide, sterile purified water, sterile 85%

Saline, meningitides antisera

Antiserum	Antigenic group detected	Antiserum	Antigenic group detected
Poly	A,B,C, D	D	D
Poly 2	X, Y, Z	X	X
W135	W135	Y	Y
A	A	Z	Z
B	B	Z'	Z'
C	C		

Reagent preparation

To rehydrate N. Meningitides antisera, add 1 ml sterile water and rotate gently until dissolved

Abbreviations:

(N. meningitides): Neisseria meningitides.

Procedures:

1. From test blood or chocolate agar , transfer 2-3 colony to drop of saline on clean slide then add drop of antisera and emulsify organism.
2. Rotate the slide for one minute then observed agglutination
3. Test the organism first with N. meningitides antisera Poly, Poly2, group Z' and W135 depending upon reaction, continue testing according either A,B,C listed below.

If positive reaction with this antisera	Retest with antisera
Ploy	A,B,C and D
Poly 2	X, Y and Z

4. Repeated test 1-3 for known positive and negative culture .

Quality control procedures:

At time use, test both positive and negative control cultures to check performance of antisera.

Limitations/ Interfering substance:

3. Correct interpretation of serologic reaction depend upon culture purity and morphological characteristics and biochemical reactions that are consistent with identification of microorganism as N. meningitides.
4. Serologic method can not identify N. meningitides only but other bacteria causing meningitis can cross-react with meningococcal antisera.
5. Excessive heat from external source may be prevent smooth suspension of microorganism or causes evaporation or precipitation of test , false positive reaction occur.
6. Neisseria meningitides antisera reagent have been tested using undiluted culture take from agar, not used microorganism diluted with saline
7. Rehydrated reagent are cloudy or precipitate during period of use should be discarded.

Expected values:

Read and record result as following

Positive :

(4+) 100% agglutination (background clear to hazy)

(3+) 75% agglutination (background slightly cloudy)

(2+) 50% agglutination (background moderately cloudy)

(1+) 100% agglutination (background cloudy)

Negative :- No agglutination

Cerebrospinal fluid (CSF)

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Purpose & Definition:

CSF is a sterile fluid and does not contain any commensals, however, care should be taken not to contaminate the specimen with skin normal flora during collection.

Diagnosis of the bacteria or fungal meningitis by microscopic examination and culture with identification and susceptibility test of the isolated organism.

Responsibilities:

- Microbiology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Types of specimen

CSF

Specimen collection

Only physicians

*Quantity of specimen

3 ml from tube # 2 or #3 of CSF is sufficient for culture(in sterile screw cap tube).

*Time relapse before processing the sample

CSF is an emergency specimen and should be processed immediately

Specimen reception:

All sample must labeled and record and sent as soon as possible to microbiology department.

Criteria of specimen rejection

Inappropriate specimen transport device; mislabeled specimen; unlabeled

specimen; specimen received after prolonged delay (usually more than 72

hours).

Equipment & Items required:

Media

Blood Agar (2 plates)

Chocolate Agar,

MacConkey Agar

Sab. Agar

Thioglycolate

Abbreviations:

(Sab):sabouraud's agar.

(CSF): Cerebrospinal fluid

Procedures:

1-CSF should be culture as soon as possible if delay is un voidable the sample should be kept in incubator at 35-37 C (never refrigerator) .

2-Tube for microbiology should be centrifuged 20 min at 2000 rpm and the sediment is used for inoculating the plates (Blood agar, Chocolate and Macconkey,) and gram staining ,then put 1ml ofThioglycolatebroth to the tube and putting it into incubator 37 C.

3- supernatant cab be used for serological testing .

4-incubate the inoculated plate 24hr at 35-37c in 5-10%CO2 Incubator if no grow incubate the plates for farther 24 hr .

5- After 24hr , make subculture from Thioglycolatebroth in(Blood agar, Choclate and Macconkey,) .

Limitations/ Interfering substance:

Interfering factors:

Patient on antibiotic therapy.

Improper sample collection.

Expected results:

Pathogen and commensals

Infection of C.S.F
CSF is a sterile fluid and does not contain any commensals, however, care should be taken not to contaminate the specimen with skin normal flora during collection.
Common bacterial pathogen
Haemophilusinfluenzae
Neisseria meningitis
Streptococcus pneumoniae
Group A & B *streptococci*
Gram negative bacilli
Brucella(rare)
Salmonella (rare)

Result reporting

Results of the microscopy and all positive cultures of CSF are reported immediately to the treating physician. Negative bacterial results are sent out 72 hours after the CSF is received.

Body Fluid Culture, Sterile

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Culture, Body Fluid, Sterile, Routine; Sterile Body Fluid Culture, peritoneal, pericardial, plural, ascitic, synovial, etc.

Isolate and identify pathogenic organisms from normally sterile body fluids and perform sensitivity test

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements

Types of specimen

Aseptically aspirated body fluid (e.g., , synovial, peritoneal fluid).

Specimen collection

Contamination with normal flora from skin, rectum, vaginal tract, or other body surfaces should be avoided. Indicate the specific source and pertinent clinical history on the request form.

Who will collect the specimen

Physician

Quantity of specimen:

1-5 mL is adequate.

Time relapse before processing the sample

Body fluids should be treated as CSF specimens and should processed

Immediately

Specimen reception:

All sample must labeled and record and sent as soon as possible to microbiology department.

Criteria of specimen rejection

Inappropriate specimen transport device; mislabeled specimen; unlabeled

specimen; specimen received after prolonged delay (usually more than two

hour).

Equipment & Items required

Media

1. Blood Agar (2 plates)

2. Chocolate Agar,

3. MacConkey Agar

4. Thioglycollate broth

Procedures:

1. Streak two blood agar plates, one chocolate, MacConkey and Thioglycollate broth.
2. Centrifuge clearspecimen and inoculate plates.
3. Do staining from sediments. While turbid is directly streaking.

Limitations/ Interfering substance:

Patient on antibiotic therapy.

Improper sample collection

Expected results:

Pathogen and commensals

common pathogenic of pericarditis andmyocarditis	**Pleural fluid**
Mycoplasma pneumoniae	*Staphylococcus aureus*
Staphylococcus aureus	*Streptococcus pneumoniae*
Streptococcus pneumoniae	*Haemophilusinfluenzae*
Enterobacteriacae	*Pseudomonas spp*
Bones and joint	**Peritoneal fluid**
Staphylococcus aureus	*Streptococcus pneumoniae*
Streptococcus pyogenes	Group A streptococci
Enterobacteriacae	Enterobacteriacae
*Neisseria gonorrheae*Enterobacteriacae	Staphylococci *Neisseria gonorrheae*

Result reporting:

Report Gram stain finding as an initial report

Report the isolated pathogen and its sensitivity pattern as a final report.

Blood Culture

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

To detect the causative organisms & treatment of septicemia.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Collection of blood sample :-

- **Timing of blood collection :-**

Blood should be collected before antimicrobial treatment has been started and at the time the patients' temperature is beginning to rise. It is recommended that at least two specimens should be obtained, Separated by interval of approximately 30 – 60 min .

Specimen requirements:

Aerobic and Anaerobic blood bottles , Alcohol 70% , Iodine for disinfection of skin, Syringe with needle.

Quantity of blood :-

1. 10 ml per vein puncture for adults .
2. 2 – 5 ml for children .
3. 1 – 2 ml for infants and neonates .
4. Note: The specimen must be 10-20% from amount of media.

Skin disinfection :-

1. First disinfect the vein puncture site with 70% ethanol .
2. Then use tincture of iodine to disinfect the site using progressively larger concentric circles . Iodine should remain in contact with skin for 1 min .
3. Remove the iodine with 70% alcohol .

4. If the site must again be palpated the finger must be disinfected or sterile gloves worn .

Specimen reception:

1. Sample should be transport to the lab. Immediately with best request patient details, diagnosis and antibiotic administration.
2. If the request patient details is not completed we reject it .
3. All blood-culture bottles should be carefully examined for clarity. Any medium showing turbidity should not be used.

Equipment & Items required:

Aerobic and Anaerobic blood bottles , Alcohol 70% , Iodine for disinfection of skin, Syringe with needle, Blood agar, MacConkey agar and chocolate agar, CO2 Incubator,37 Incubator.

Procedures:

1. Aerobic bottle should be vented by briefly inserting sterile cotton plugged needle into the robber septum. And leave the anaerobic bottle unvented.
2. Both bottles were then incubated aerobically at 35 – 37 C for 12 – 24 hr.

***24 hr subculture**

Subculture the aerobic blood bottle aseptically by :

1. Mix blood culture bottle gently .
2. Disinfect the top of each bottle on flame or with 70% alcohol .
3. Using a sterile needle and syringe withdraw 1 ml of the broth culture .
4. Inoculate one drop onchocolate, MacConkey ,sabouraud Agar and put it in Thioglycolate .
5. Incubate the inoculated plates for 24 hrs. at 37 C in CO2 incubator.
6. If fungal infection is suspected , sabouraud agar should be inoculate and incubate at R.T for 7 day.

Anaerobic blood bottle is inoculated on :

1. Two Blood agar plates
2. MacConkey agar
3. Chocolate agar
4. Incubate for 24 hr at 37 C in CO2 incubator .
5. One Blood agar plate incubated anaerobically at 37 C for 48 hrs.
6. Disinfect the top of each bottle and re-incubate them without agitation for 7 days in 37 C.
7. At the end of week subculture the bottles on blood , MacConkey and Chocolate agar.

Quality control procedures:

If we preparedmedia for aerobic blood culture we must work negative control by putting the bottle in incubator 37 to 24h and then culturing it as blood culture.

Limitations/ Interfering substance:

Administration of antibiotic may interfering the result , so it must be stopped for 72h before drawing blood culture .

Contamination of skin with bacteria present as normal flora through drawing the sample may cause false positive result with coagulase negative staph.

Expected results:

Negative Culture .

Positive Culture with antibiotic sensitivity.

Pathogens

Blood is a sterile body fluid and normally no commensalscontains.

Common pathogens

Pathogenic Bacteria	Pathogenic Bacteria	Pathogenic Fungi
Streptococcus spp.	*Corynebacteriumjeikeium*	*Candida albicans*
Bacteroidesfragilis	*Neisseria meningitides*	*Cryptococcus neoformans*
other anaerobic bacteria	*Haemophilus influenza*	*Other candida spp.*
Staphylococcus aureus	*Nonfermenter gram negative bacilli*	*Coccidoidesimmitis*
Coagulase negative staphylococci	*Salmonella typhi*	*Histoplasmacapsulatum*
Listeria monocytogenes	*Pseudomonas aeruginosa*	
Enteric gram negative bacilli		

Reporting result:

- Antibiotic sensitivity test is put up for the organism isolated and the result are reported .

Throat Swab Culture

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

To isolate and identify any pathogenic organisms.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

- Ask the patient to open his mouth and hold the tongue with sterile tongue depressor, without touching the buckle cavity inset the swab into the throat, ensuring good contact with inflamed area. Withdraw the swab immediately without touch the inside of the mouth. Place the swab into its original tube, label and send to the laboratory.

Equipment & Items required:

Blood agar, Maconkey agar, chocolate agar.

Plastic loop.

CO2 incubator.

Bunsen burner.

Procedure :

1. Streak one blood agar plates, Maconkey agar, chocolate agar by using sterile loop.
2. Inoculate are usually spread over the surface of agar plates in a standard pattern to achieve isolated colonies.
3. Gram stain to check the presence or absence of microorganism .

4. Incubate the inoculated plates 24 hrs. at 35 – 37 C in 5-10% CO2 incubator , if no growth , incubate the plates for farther 24 hrs.
5. The routine organism are identified by their morphology , cultural character and biochemical tests.
6. Susceptibility test should only be performed on well isolated colonies of similar appearance were considered as a significant pathogens on blood or chocolate media.

Limitations/ Interfering substance:

No attempt should be made to report routinelyother bacteria in a Gram stained smear from a throat swab because the throat contains a wide variety of commensals that cannot be distinguished morphologically from pathogens.

Expected results:

Possible pathogens

Gram Positive Bacteria	**Game Negative Bacteria**
Streptococcus pyogenes	Vincent's organisms
Corynebacteriumspp.	_____
Diphtheria spp.	_____
Corynebacteriumulcerans	_____

Reporting result:

Look for beta-haemolytic streptococci, which is sensitive to bacitracin.

Identify S. pyogenes byLancefield group .

Antibiotic sensitivity test is put up for the organism isolated and the result are reported .

Sputum Culture

SOPs \ HGA\.....H\ M\ 30

Version: 1.....	Head of department:.....
Date effective:	Quality Officer: .....
Copy number:	Director of :.....

Purpose & Definition:

An etiological diagnosis of lower respiratory tract infection by culturing , identification and susceptibility test of isolated organism.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Blood agar, MaCconkey agar, chocolate agar.

Plastic loop.

CO2 incubator.

Bunsen burner.

Type of specimen:

Sputum, trans tracheal aspirates, trans laryngeal aspiration, bronchoalveolar lavage.

Specimen collection:

1. Deep cough and collect sputum in a wide mouth sterile container. All expectorated sputum is contaminated to some degree with secretion of the oropharyngeal cavity, which contains a wide variety of commensal bacteria, some of which are potential pathogens of the lower respiratory tract (S. pneumonia, H. influenza). Since the sputum reflect the infection in the bronchi and the lung. Contamination oropharyngeal secretion should be kept to a minimum.

2. Early morning sputum is preferred because they contain pooled overnight secretion in which, pathogenic bacteria are more likely to be concentrated.
3. The specimen should be collected in a sterile wide mouth container with tightly fitted screw cap lid.
4. The patient will collect the specimen and the quantity about 3 ml.
5. Time relapse before processing the sample ; 30 min.

Specimen reception:

Don't receive sample contaminated with saliva.

Patient preparation:

Patient is asked to wash oral cavity by gargling with water 3-4 times.

Abbreviations:

(Bl. Agar): Blood agar.
(Mac agar): MaCconkey agar.
(API): Analytical profile index.

Procedures:

1. Sputum should be cultured as soon as possible, if delay is un avoidable the sample should be kept at 4 C for not more than 2 hours.

2. Incubate the inoculated plates (Bl. Agar, Mac agar, chocolate agar) in (5-10%) CO2 incubator.
3. The routine organism are identified by their cultured morphology, gram stain, oxidase test, API ; serology.

Limitations/ Interfering substance:

- Don't receive contaminatedsample.

Expected results:The common pathogens

Pathogenic Microbes	**Pathogenic Microbes**
Streptococcus pneumoniae	*Mycobacterium spp.*
Haemophilusinfluenzae	*Fusobacterium spp.*
Staphylococcus aureus	*Bordetellaspp*
Klebsiellapneumoniaeandother Enterobacteriaceae	*Chlamydia pneumoniae*
Moraxella catarrhalis	*Legionella spp.*

Pus(wound, Abscesses, Burns and sinuses) culture

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Isolate and identify aerobic and anaerobic pathogenic organisms pus

specimen.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Types of specimen

- Swabs from the infected area or aspiration from deep wounds.
- Swab inanaerobic transport media for the isolation of anaerobes.

Specimen collection

1. Pus from abscess is best to collected at the time, the abscess is incised anddrained.
2. Using sterile technique, aspirate or collect from drainage up to 5 ml of pus.
3. If pus is not being discharged usesterile swab to collect the sample from the infected site ,extend the swabdeeply into the depth of the lesion.
4. Immerse the swab in transport medium, label it and send to the laboratory as soon as possible

Time relapse before processing the sample 30 min.

Specimen reception:

All sample must labeled and record and sent as soon as possible to microbiology department.

Equipment & Items required:

Media:

Blood agar, MaCconkey agar, chocolate agar,Thioglycolate broth.

Procedures:

1. Streak one blood, chocolate, andMacConkey agar by sterile loop and inoculatethioglycolate broth tube.
2. Gram stain to check the presence or absence of bacteria.

Limitations/ Interfering substance:

Contamination of the specimen with normal flora is one of the major obstacles in obtaining good results. Care should be taken to avoid contaminating the specimen with normal flora. This could e accomplished by swabbing superficial infected wounds with 70% alcohol.

Interfering factors:

Patient on antibiotic therapy.

Improper sample collection.

Expected results:Pathogen and commensals

Pus Infection	
Pathogenic bacteria	**Commensals bacteria**
Pseudomonas aeruginosa	Alpha haemolytic streptococci
Proteus spp	*Corynebacteriumsp*
E. coli	Coagulase negative Staph
Klebsiella spp.	*Propionobacteriumspp.*
Morganella.	*Bacillus spp.*

Streptococcus pyogenes	
Staphylococcus aureus	
Enterococcus spp.	
Clostridium perfringens	
Mycobacterium tuberculosis	

Result reporting:

Report Gram stain finding as an initial report.

Report the isolated pathogen/s and its sensitivity pattern as a final report.

Genital Culture

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Isolate and identify potentially aerobic pathogenic organisms including *Gardnerellavaginalis*and group B *Streptococcus*; establish the diagnosis of gonorrhea, medical/legal cases.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements

Specimen collection

1. Swab of vagina, cervix, discharge, aspirated endocervical, endometrial,prostatic fluid, or urethral discharge.
2. Swab should also be sent in transport device.
3. Swab should be collected by gynecological physician.

Specimen reception:

all sample must labeled and record and sent as soon as possible to microbiology department.

Criteria of specimen rejection

Inappropriate specimen transport device; mislabeled specimen; unlabeled specimen; specimen received after prolonged delay .

Equipment & Items required:

Media:

Blood agar, MaCconkey agar, chocolate agar,Sabauroud Agar.

Procedures:

1. Streak one blood agar plates, chocolate, MacConkey and Sab agar plate.
2. Do wet mount to examine Clue cells and Trichomonas.
3. Do gram stain tocheck the predominant organisms.

Limitations/ Interfering substance:

Interfering factors:

Patient on antibiotic therapy.

Improper sample collection.

Expected results:

Pathogen and commensals

Pathogenic bacteria	Commensals bacteria
Neisseria gonorrheae	Coagulase negative*Staphylococci*
Group B *Streptococci*	*Corynebacterium spp.*
Gardnerellavaginalis	*E.coli*and other coliform
Enterococcus spp.	Many species of anaerobic
Certain anaerobes including: *Actinomycesspp.* *Haemophilusducreyi* *Treponemapallidum*	
Mycoplasma spp.	
Enterobacteriaceae	

Result reporting:

Report wet mount Gram stain finding as an initial report.

Report the isolated pathogen and its sensitivity pattern as a final report.

Urine culture

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

An etiological diagnosis of bacterial urinary tract infection by quantitative cultivation of the urine with identification and susceptibility test of the isolated bacteria(s).

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Urine (Midstream urine), suprapubic aspiration, catheterized urine.

Specimen collection

1- Give the patient sterile container

2- Collect the first morning Midstream urine into sterilecontainer and pass excess into toilet.

3- Clean –catch urine specimen from infant and children : Give the child water or other liquid to drink, Clean the external genitalia

4- Sample must transported to the laboratory immediately . If not possible urine should be refrigerated .

Criteria of specimen rejection

1. Un-refrigerated specimen older than 2 hours may be subject to overgrowth and may not yield valid results.
2. unlabeled specimen; mislabeled specimen.
3. Patient on antibiotic therapy.

Equipment & Items required:

1. Disposable 1 µl sterile loop
2. Blood , and macConkey agar.

Procedure

1. Mix the sample to re-suspend microorganism present.
2. Dip 1 µl calibrated loop in vertical position in the urine and remove the loop and use the collected fluid to inoculate blood agar plate that will be used for urine plate count.
3. Take another loop to streak macConkey agar.
4. Incubate the inoculated plates for 24 hrs. at 35 – 37 C .
5.

Interpretation of urine results :

1. Count the number of pathogens using correction factor to obtain the number of colony forming unit Per ml (1000 if 1 µ loop is used , 100 if 10µ loop is used).
2. No bacterial growth reported negative culture
3. No of organism > 10.000 CFU/ml record name of bacteria and susceptibility test.
4. If the sample catheter or suprapubic aspiration and count of bacteria > $3X10^3$CFU/ml recorded name of bacteria and susceptibility test.

Pathogens and commensals

Urine specimen	
Common pathogens	**commensal flora**
Neisseria gonorrhoeae	the urine is sterile except for the urethral mucosa which support the growth of microflora as:
E. coli and other Enterobacteriaceae	
Enterococcus spp	Diphtheroid bacilli
Staphylococcus aureus	*Lactobacillus* spp
Staph saprophyticus	Coagulase negative *Staphylococci*
Corynebacterium jeikeium	α Haemolytic *Streptococci*
Acinetobacter spp	Bacillus spp
Pseudomonas spp	Non pathogenic *Neisseria spp.*
Gardnerella vaginalis	Anaerobic cocci
β-haemolytic streptococci	Commensal *Mycobacterium*
Salmonella spp (early stage of infection)	Commensal *Mycoplasma spp.*

Reporting result:

Antibiotic sensitivity test is put up for the organism isolated and the result are reported .

Stool Culture

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Version: 1.....	Head of department:.....
Date effective:	Quality Officer: .....
Copy number:	Director of :.....

Purpose & Definition:

A standardized protocol for the culture and isolation of Salmonella and Shigella species from human feces.

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

1-Types of specimen

Stool or rectal swab or stool (fresh random) in fecal transport system.

2-Collection of stool sample

1. Fecal specimens should be collected in the early stages of the disease, before antibiotic treatment is started.
2. Stool specimens should be collected in a clean, dry, disinfectant free and suitable wide container. The specimen should contain at least 5 g of faeces.
3. A single stool specimen cannot be used to rule out bacteria as a cause of diarrhea. More than two specimens should only be submitted from patients for whom there is a high degree of suspicion.
4. Transfer a portion of the specimen , which contains mucus, pus or blood.
5. Label the specimen, on the container not lid, and send it with a request form to reach the laboratory within one hour.
6. Stool passed into the toilet bowel must not be used for culture.

Equipment & Items required:

- Salmonella Shigella agar (SSA) or Hekton Enteric agar(HE).
- Xylose Lysine Deoxycholate (XLD)
- Gram negative broth or Slenite –F broth.
- API 20 E kit.
- Salmonella shigella antiserum(polyvalent & monovalent)
- Inoculating loop
- Applicator swab
- Incubator 37 C^{o} (non-Co_2).

Abbreviations:

- **(SSA):** salmonella shigella agar
- **(XLD):** Xylose lysine deoxycholate agar
- **(HE):**Hekton enteric.
- **(API 20 E):** Analytical profile index 20 Enterobacteriaceae

Culturing Procedure

1. Using a swab inoculate a stool swab on SS or XLD and HE agar and place the same swab in a selenite broth tube, Incubate at 37o C for 18-24 hrs.

2. Inspect SS agar for Salmonella or shigella suspected colonies(colorless or yellowish colonies (non-lactose fermenting) or colonies with black color (H2S producing) are found confirm by biochemical tests and perform the serotyping.

3. If no Salmonella suspected colonies on SSA agar were observed, subculturethe inoculated selenite broth on SSA or XLD and HE agar.Incubate at 37o C for 18-24 hrs.

4. Inspect SSA agar again for suspected Salmonella colonies. If suspected Salmonella colonies are found confirm by biochemical testsand perform the serotyping.

5. If suspected Salmonella colonies are not observed give the result as noSalmonella and shigella growth.

Limitations/ Interfering substance:

1. Typical morphology on selective agar is not a definitive identification. All suspect isolates must be confirmed biochemically.
2. Fecal-shedding of *Salmonella* by chronic carriers may be intermittent. Three cultures, performed on three stool samples, collected 24 hours apart, may increase the likelihood of recovering *Salmonella* from a suspected carrier.
3. Formed stool, specimen contaminated with urine, residual soap, or disinfectants. Specimens received in grossly leaking transport containers; diapers; dry specimens; specimens submitted in fixative or additives.
4. Stool samples should be examined and cultured as soon as possible after collection. As the stool specimen cools, the drop in pH will inhibit the growth of most *Shigella*spp. and some *Salmonella* spp.
5. Patient on antibiotic therapy.
6. Improper sample collection.
7. *Yersinia* spp.*Vibrio, E. coli O157:H7, and Campylobacter spp.* will not be isolated **unless specifically requested**; These organisms are fastidious and have very specific requirements for growth.

Reporting results

- If no suspect colonies are present, the sample may be reported as:

"No *Salmonella spp.* or *Shigella spp*. Isolated".

- Report the isolated pathogen and its sensitivity pattern as a final report.
- Antibiotic sensitivity test is put up for the organism isolated and the result are reported.

Antibiotic Susceptibility Test In Clinical Laboratory

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Version: 1..... Date effective: ..... Copy number:	Head of department:..... Quality Officer: ..... Director of :.....

Purpose & Definition:

Antibiotic susceptibility testing is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection. Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing) is a test which uses for its Process

Responsibilities:

- Microbiologydepartment personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Preparation:

1. The media used in Kirby-Bauer testing must be Mueller-Hinton agar (or blood agar if streptococcus is suspected , chocolate agar if haemophilus is suspected) at only 4 mm deep, poured into either 100 mm or 150 mmPetri dishes.
2. The pH level of the agar must be between 7.2 and 7.4.
3. Inoculation is made with a broth culture diluted to match a 0.5 McFarland turbidity standard, which is roughly equivalent to 150 million cells per mL.

Procedure:

1. Using an aseptic technique, place a sterileswab into the brothculture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube.
2. Using the swab, streak the Mueller-Hinton agar plate to form a bacterial lawn. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction. Repeat this rotation 3 times.
3. Allow the plate to dry for approximately 5 minutes.

4. Use an Antibiotic Disc Dispenser to dispense disks containing specific antibiotics onto the plate.
5. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar.
6. Plates should be incubated overnight at an incubation temperature of 37°C

Antibiotics can be used for children:

Gram Positive Antibiotics	Gram Negative Antibiotics
Penicillin G	Ampicillin
Ampicillin	Amoxicillin
Amoxicillin	Cephalexin
Gentamicin	Cefuroxime
Cefuroxime	Gentamicin
Ceftriaxone	Cefotaxime
Trimethoprim	Ceftriaxone
Ciprofloxacin	Nalidixic acid
Vancomycin	Trimethoprim
Cephazolin	Amikacin
	Cephazolin

Antibiotics can be used for urine culture

Gram Negative Bacteria (out Patient)	**Gram Positive Bacteria (out Patient)**
Ampicillin	Penicillin
Doxycycline	Ampicillin
Amoxicillin	Amoxicillin
Trimethoprim sulfamethoxazole	Cloxacillin
Cephalexin	Cephalexin
Nalidixic acid (in urine culture only)	Gentamicin
Gentamicin	Cefuroxime
Cefuroxime	Tetracycline
Tetracycline	Doxycycline
Nitrofurantion	Trimethoprim sulfamethoxazole
	Ciprofloxacin
	Rifampin
	Erythromycin
Gram Negative Bacteria (In Patient)	**Gram Positive Bacteria (In Patient)**
Ceftriaxone	Ceftriaxone
Cefazoline	Cefazoline
Amikacin	Vancomycine
Piperacillin	Pencillin G used for *streptococci*
Aztreonam	Rifampin used for *staphylococci*
Ceftazidime	Cefotaxime
Cefotaxime	meropenem
meropenem	

Antibiotics can be used for pus , blood , sputum , C.S.F ,and fluids cultures

Gram Negative Bacteria (Out Patient)	Gram Positive Bacteria (Out Patient)
Ampicillin	penicillin G
Amoxicillin	Ampicillin
Cephalexin	Amoxicillin
Chloramphenicol	Cephalexin
Cefuroxime	Cefuroxime
Gentamicin	Gentamicin
Tetracycline	Tetracycline
Doxycycline	Doxycycline
Trimethoprim	Trimethoprim
Ciprofloxacin	Chloramphenicol
	Clindamycin
	Rifampin
	Cloxacillin
	Erythromycin
Gram Negative(In patient)	**Gram positive (In patient)**
Ceftriaxone	Ceftriaxone
Ceftriaxone	Cefotaxime
Amikacin	Vancomycin
Aztreonam	Meropenem
Ceftazidime	
Cefotaxime	
Meropenem	

Antibiotics can be used for pregnant women

Gram PositiveAntibiotics	Gram NegativeAntibiotics
penicillin G	Ampicillin
Ampicillin	Amoxicillin
Amoxicillin	Cephalexin
Cloxacillin	Cefazoline
Cephalexin	Cefuroxime
Cefazoline	Ceftazidime
Cefuroxime	Cefotaxime
Cefotaxime	Ceftriaxone
Ceftriaxone	
Erythromycin	

References:

1. Standard Operating Procedures for 2010 Guidelines for Laboratories (W.H.O)
2. The Manual of Clinical Microbiology, ninth edition.

INDEX

Media Preparationn quality control

Blood Agar

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth (+ve control)	Hemolysis		Notes	Tech. Signature
					Yes	No		

Media Preparationquality control

MacConky Agar

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth +ve control selectivity		Notes	Tech. Signature
				(Gram +ve bacteria)	(Gram-ve bacteria)		

Media Preparationquality control

Selenite-F Broth

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth +ve control selectivity		Notes	Tech. Signature
				(Gram +ve bacteria)	(Gram-ve bacteria)		

Media Preparationquality control

Salmonella Shigella Agar

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth +ve control selectivity		Notes	Tech. Signature
				(Gram +ve bacteria)	(Gram-ve bacteria)		

Media Preparationquality control

Hekton Enteric Agar

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth +ve control selectivity		Notes	Tech. Signature
				(Gram +ve bacteria)	(Gram-ve bacteria)		

Media Preparationquality control

Chocolate Agar

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth +ve control selectivity		Notes	Tech. Signature
				(Gram +ve bacteria)	(Gram-ve bacteria)		

Media Preparationquality control

Muller Hinton Agar

Media Company..... **Lot No.....**

Equipment ..... **Serial No .....**

Date of Preparation	PH	Result of biological indicator	Sterility (-ve control)	Growth +ve control selectivity		Notes	Tech. Signature
				(Gram +ve bacteria)	(Gram-ve bacteria)		

Biochemical Testsquality control

Coagulase Test

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

Oxidase Test

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

Catalase Test

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

DNAse Test

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

API Test

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

Optochin

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

Bacitracin

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

Gram stainquality control

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign

Biochemical Testsquality control

Acid fast stainquality control

Company..... **Lot No.....**

Date	Control +ve	Control -ve	Notes	Tech Sign