Short operational manual for clinical microbiology lab

SPECIMEN

Specimen isolated from non-sterile sites:

- Sputum,
- Stool,
- Urine,
- Genital tract secretion etc.

Specimen isolated from normally sterile sites:

- Blood,
- Cerebrospinal fluid (CSF),
- Pleural fluid,
- Abdominal fluid,
- Joint fluid etc.

CULTURE MEDIA AND REAGENT

Culture media:

- ✓ Columbia blood agar plate
- ✓ Chocolate agar plate（non-selective）
- ✓ Chocolate agar plate（selective）
- ✓ EMB agar plate
- ✓ MacConkey agar plate
- ✓ SS agar plate
- ✓ HE agar plate
- ✓ TCBS agar plate
- ✓ MH agar plate
- ✓ Gonococcal (GC) agar plate
- ✓ Sabouraud's Dextrose Agar Plate etc.

Reagent

- ✓ Gram stain
- ✓ Ziehl-Neelsen Stain
- ✓ India ink (ink stain)
- ✓ 3% H2O2 (Catalase reagent)
- ✓ Oxidase test strip
- ✓ Sterile mineral oil

- ✓ Cedar oil
- ✓ Dimethylbenzene etc.
- ✓ Sterile saline (0.9% NaCl)

INSTRUMENT AND CONSUMABLES

Instrument and consumables:

- ✓ Bio-safety cabinet: for routine operation such as inoculation etc.
- ✓ CO2 incubator: incubation of streptococcus, fastidious bacteria etc.
- ✓ Air incubator: incubation of regular bacteria such as staphylococcus, enterobacteriaceae etc.
- ✓ Microscope: bacteria morphological examination.
- ✓ Centrifuge: pretreatment of some specimen such as CSF etc.
- ✓ Medicine refrigerator: reagent preservation.
- ✓ Refrigerator(-70℃): bacteria strain preservation.
- ✓ Inoculation needle: puncture inoculation.
- ✓ Inoculation loop: inoculation
- ✓ Quantitative inoculation loop: quantitative inculation.
- ✓ Glass slide: basic operation such as Gram staining, catalase test etc.
- ✓ SS-spreader: spread the plate (or use sterile cotton swab instead)
- ✓ Electronic sterilizer/alcohol lamp: sterilize the inoculation loop etc.
- ✓ Pipettes and pipette tips
- ✓ Pressure steam sterilizer (autoclave) etc

SPECIMEN PLANTING PROCEDURES

Streaking for isolation:

Before identification of bacteria or fungi may be achieved, pure colony must be isolated. That is only one type of colony containing one type of bacteria must be transplanted to the identification system in use. A mixed colony or culture will not only produce confusing results but also inaccurate results as well. Cultures must be planted by streaking for isolation.

Plate streaking:

1. A small portion of specimen (sputum, feces etc.) is placed on the agar plate with an inoculating loop or sterile cotton swab, streaked back and forth on approximately 1/4 of the plate (this is the Area Ⅰ in Fig1).
2. The inoculation loop is burned to remove remaining specimen on loop. The second 1/4 is streaked after the loop is cooled. To streak the second 1/4 streak out from Area Ⅰ, using close streaks until approximately 1/4 of plate is covered (Area Ⅱ).
3. Burn the loop, turn the plate and begin streaking process as above, again covering 1/4 of the plate (AreaⅢ), repeat the procedures until AreaⅣ is completed.

   CAUTION: when streaking AreaⅣ, streak from areaⅢ but do not re-touch Area Ⅰ.

   This streak by area method will "spread out" the colonies into thinner areas (AreaⅢ/Ⅳ) so that these are true isolated colonies that are pure colonies for identification and susceptibility testing(ID/AST)(Fig2).

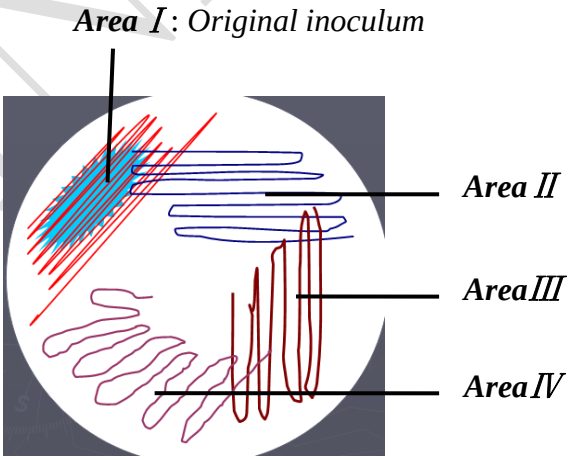


Fig1: Diagram of streak plate

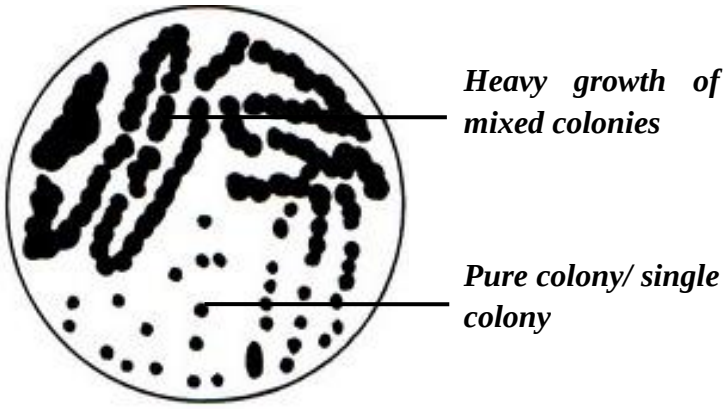


Fig2: Bacteria growth on plate after streaking

Quantitative Streaking

To determine urinary tract infection, the actual colony count of a urine specimen is necessary (colony-forming unit or CFU). For a colony count a calibrated loop (1 µl or 10 µl usually) is used.

1. Quantitative streaking is usually used in urine specimen.
2. A calibrated loop of 1 or 10 ml is dipped vertically into a well-mixed specimen. One loopful is streaked down the center of a plate (original streak).
3. Without flaming, cross-streaks at a 90 degree angle are made perpendicular to the original streak. Make the streaks densely cover the whole plate. Fig3.

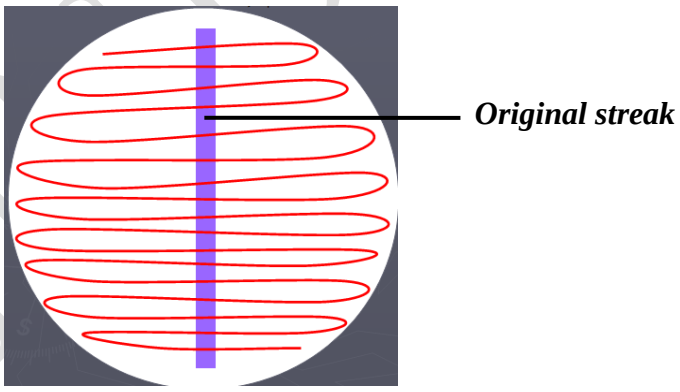


Fig3: quantitative streaking

Reference:

Patrick R. etc., Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

Procedures/Guidelines for the Microbiology Laboratory, College of Physicians & Surgeons of Saskatchewan, 2010.

MICROBIOLOGY SPECIMEN PROCESSING

Blood culture procedure

The detection of microorganisms in a patient's blood has great diagnostic and prognostic importance. Positive culture result can not only confirm the disease was caused by infectious pathogens, what is more important is via subsequent ID/AST which can optimize the antibiotics therapeutics.

1. Specimen volume and number of cultures:

- ✓ The volume of blood drawn for culture is the most important variable in detecting bacteremia or fungemia. For adult patients, the positive rate of blood culture increases as the volume of blood that is cultured from 2 to 30 mL.
- ✓ Blood should be diluted in broth media at a blood-to-broth ratio of 1:5 to 1:10.
- ✓ The optimal number of blood cultures to be drawn multiple, a single blood culture set can't distinguish a "false-positive" blood culture which was due to skin contaminants.

Inoculate the blood into blood culture bottle beside sickbed.

2. Transport blood culture bottles into microbiology lab directly, and conduct the incubation and culture as the manufacture's instructions of automated blood culture system OR following the manual blood culture instructions.
   (Mindray Automatd Blood Culture System will be available in Q3 2013.)
3. Detection and work-up for cultures

   Positive cultures

   Examined by preparation of a gram smear (results of gram smears must be called to the ward/physician stat).
   Subculture on agar plate (Blood agar plate, Chocolate agar plate or MacConkey agar plate, Anaerobic plate if necessary).
   Identify isolates and conduct susceptibility testing.

   Negative cultures

   The standard incubation period for routine blood cultures performed by automated systems is five days; manual blood culture bottle should be incubated for seven days.

Reference:

M47-A, Principles and Procedures for blood Cultures; Approved Guideline. Clinical and Laboratory Standards Institute.

Patrick R. etc., Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

Procedures/Guidelines for the Microbiology Laboratory, College of Physicians & Surgeons of Saskatchewan, 2010.

Cerebrospinal fluid and other sterile body fluid

Just like blood, there should be no bacteria or fungi exist in a health person's CSF and some other sterile body fluid such as joint, pleural, or abdominal fluid. So any isolates should be reported. The operation procedures for these kind of specimen are as following:

1. Specimen collection
   CSF and other sterile body fluids should be collected in a sterile tube, if the specimen is enough, inoculate blood culture bottle.
2. Record the appearance of specimen, i.e., clear, bloody, cloudy etc.
3. Centrifuge the specimen (2500-3000 rpm, 15min), discard the supernatant and vortex the sediment. [if the specimen volume is low (eg: £1 ml), do not to centrifuge but vortex the specimen]
4. Conduct gram staining (report the staining result to clinical doctor by phone) and inoculate agar plate by streaking. Usually Columbia blood agar plate + Chocolate agar plate (or GC agar plate) + MacConkey agar plate
5. Identify isolates and conduct susceptibility testing

Reference:

Patrick R. etc., Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

Procedures/Guidelines for the Microbiology Laboratory, College of Physicians & Surgeons of Saskatchewan, 2010.

Performance Standards for Antimicrobial Susceptibility Testing; M100-S22 Vol. 32. No.3.CLSI.

Clear middle stream urine

Urinary tract infections (UTI) are caused by a range of common bacteria such as *E.coli*, *Klebsiella spp.*, sometimes maybe *Neisseria gonorrhoeae*. Diagnosis of UTI is accomplished by semi-quantitative culture of urine (fig3. quantitative streaking).

1. Specimen collection

   The optimal urine specimen is the morning urine. Instruct the patient to collect a mid-stream specimen of urine into a sterile specimen container.
2. Record the appearance of specimen, i.e., clear, slight turbidity, turbid etc.
3. Mix specimen well by vortex, and conduct the quantitative streaking with a calibrated loop on blood agar plate and MacConkey agar plate. Chocolate agar plate is necessary if the patient was suspected to be infected by *Neisseria gonorrhoeae*. Incubate the plate in an aerobic incubator overnight at 35-37℃. For *Neisseria gonorrhoeae* it should incubate in 5%-10% CO_2 incubator and if there is no bacteria growth overnight we should incubate for another 24 hours.

   To examine *Mycobacterium tuberculosis*, we have to first centrifuge the specimen then make a smear to conduct acid fast staining.
4. Inspect the plate and count colonies. When gram negative bacilli colonies count > 10^5 CFU/ml or gram positive cocci colonies count > 10^4 CFU/ml was considered to be diagnostic significance. If there are more than 3 kind of dominant organisms it was considered to be contamination, the specimen should be collected again.
5. Identification and susceptibility testing should be conducted for potential pathogens.

Reference:

Patrick R. etc., Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

Procedures/Guidelines for the Microbiology Laboratory, College of Physicians & Surgeons of Saskatchewan, 2010.

National Guide to Clinical Laboratory Procedures (Third Edition). Medical Administration Department of Ministry of Public Health , P. R. C

Sputum

Sputum or lower respiratory tract specimen such as bronchial lavage was collected to detect the causative agent of pneumonia. For bacterial pneumonia the most commonly pathogen is *S. pneumoniae, H. influenzae, M.catarrhalis, S. aureus, K. pneumonia etc.*

1. Specimen collection

   Patient should rinse mouth with water, cough from deep in lung, do not spit. Collect the sputum into a sterile container (early morning specimens generally more suitable).
2. Make Gram stain to screen if acceptable for culture. White blood cell≥25/LPF and squamous epithelial cell≤10/LPF is the qualified specimen. If the specimen is unqualified, ask clinician to collect specimen again.
3. Inoculate blood agar plate, chocolate agar plate and MacConkey agar plate, incubate 24-48hs under 5%-10% CO_2 incubator at 35-37℃.
4. Check the plate 24hs later, identification and susceptibility testing are performed if necessary. If plate shows only normal flora incubate for another 24hs and re-check in the following day.

Reference:

Patrick R. etc., Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

Procedures/Guidelines for the Microbiology Laboratory, College of Physicians & Surgeons of Saskatchewan, 2010.

Stool

Stool specimen is usually collected when a patient who develop diarrhea, the pathogens can be *Salmonella spp., Shigella spp., Toxigenic E. coli, Yersinia enterocolitica, Vibrio Cholera, Vibrio parahemolyticus, Campylobacter, Clostridium difficile etc. S. aureus* and *Candida albicans* can be found for patients who after broad-spectrum antibiotic therapy. *C. difficile* is the most common pathogen for adult patients who develop diarrhea⩾3 days after hospitalization.

1. Specimen collection

   Stool should be transported to laboratory within 30 minutes after collection, and processed in 2 hours. If there is a delay in transport and/or processing, the specimen should be better to collect with Cary-Blair medium.
2. Record the appearance of specimen: soft stool, formed stool, mucous stool, watery stool, bloody purulent stool etc.
3. Inoculate Columbia blood agar plate +SS agar plate+ EMB agar plate (or MacConkey agar plate). If the patient was suspected to be infected by *Vibrio*, hanging drop examination should be conducted, by the same time inoculate TCBS culture media (thiosulfate citrate bile salts sucrose agar culture media)
4. Identification and susceptibility testing should be conducted for potential pathogens.

Reference:

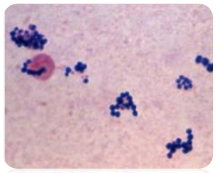
Patrick R. etc., Manual of Clinical Microbiology, 9th ed.: American Society for Microbiology, 2007.

Procedures/Guidelines for the Microbiology Laboratory, College of Physicians & Surgeons of Saskatchewan, 2010.

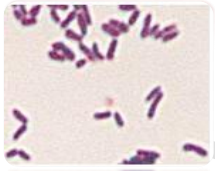
National Guide to Clinical Laboratory Procedures (Third Edition). Medical Administration Department of Ministry of Public Health , P. R. C

Identification and susceptibility testing with microorganism analysis systems

1. Gram staining

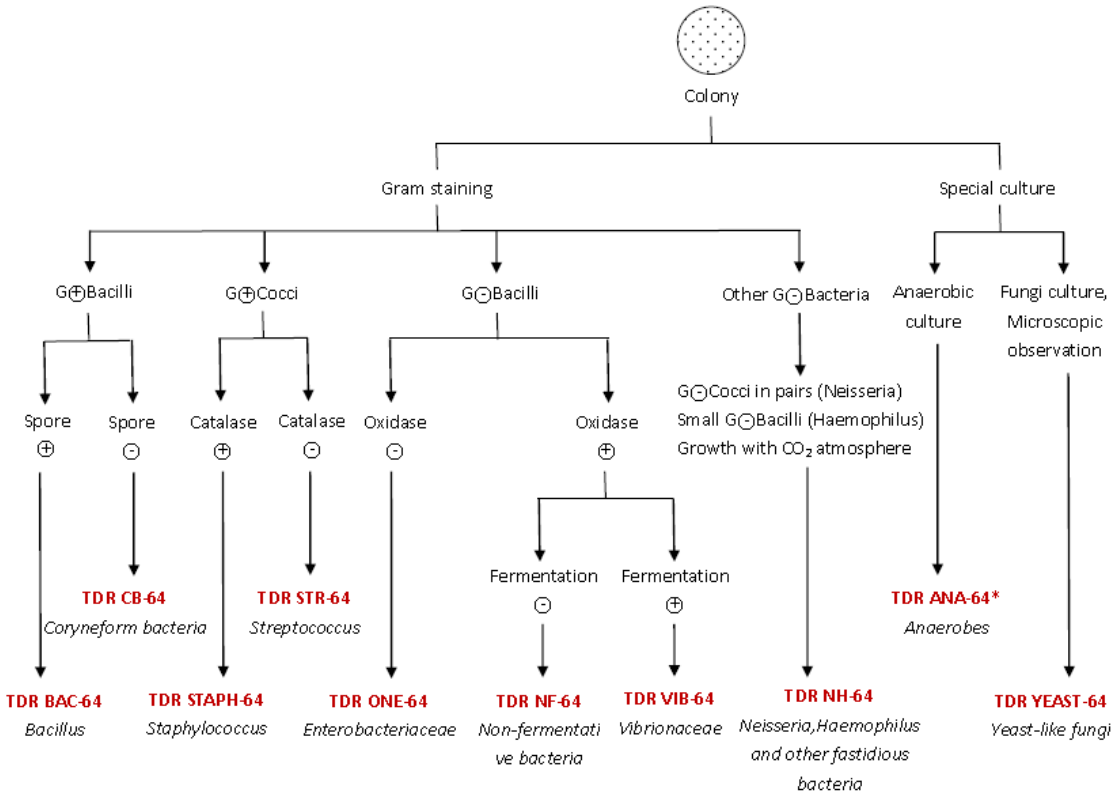


Gram positive

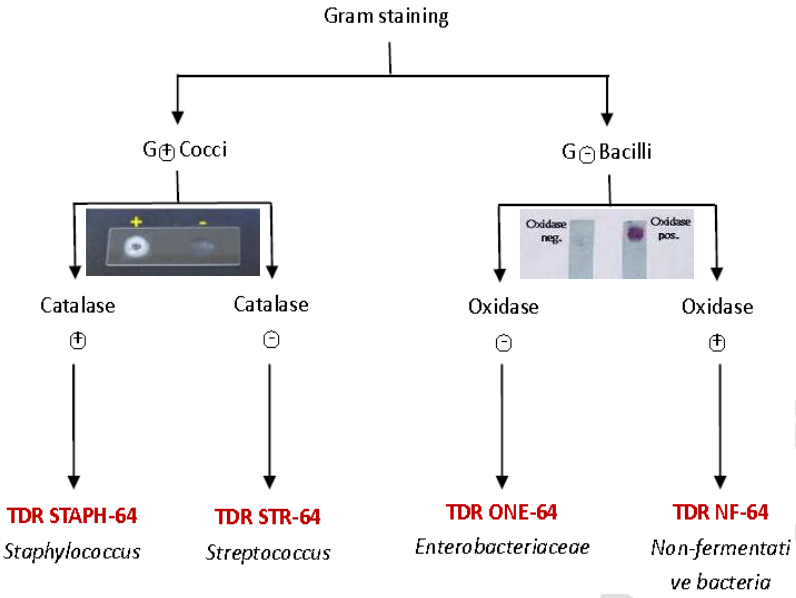


Gram negative

2. Preliminary classification (orientation test) to choose a test card



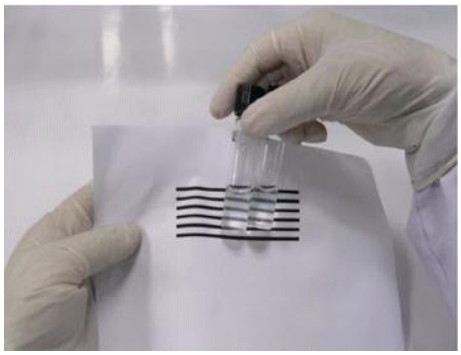
Ps: Orientation test for frequently-used test card



Ps: for Mindray TDR-300B test card the relevant culture medium are included in each test card kit make the whole operation more convenient.

3. Bacteria suspension medium preparation

- The concentration of bacteria suspension is determined by turbidimeter (Mindray turbidimeter TDR-Z200 will be available in June. 2014) or compared with standard turbidity tube (McFarland tube) with naked eye.

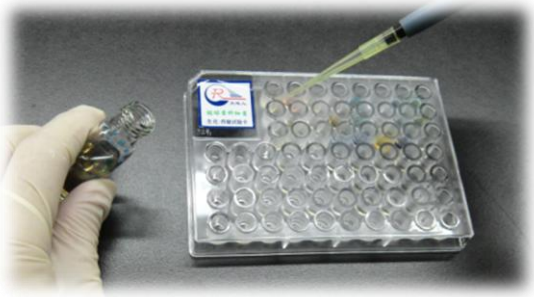
- 

IMPORTANT NOTICE: Pure culture is the precondition for the correct identification and susceptibility testing. So make sure to pick 2-3 similar pure colonies to prepare the bacteria suspension. If it is a bacteria lawn on the agar plate please purify it with streaking method, and make the identification and susceptibility testing one day later.

4. Test card inoculation

After finishing the preparation of ID medium suspension and AST medium suspension, inoculate them to the test card wells by using pipette or Automated Dosing System(TDR-J100, will be available in June.2014)

Automated dosing system



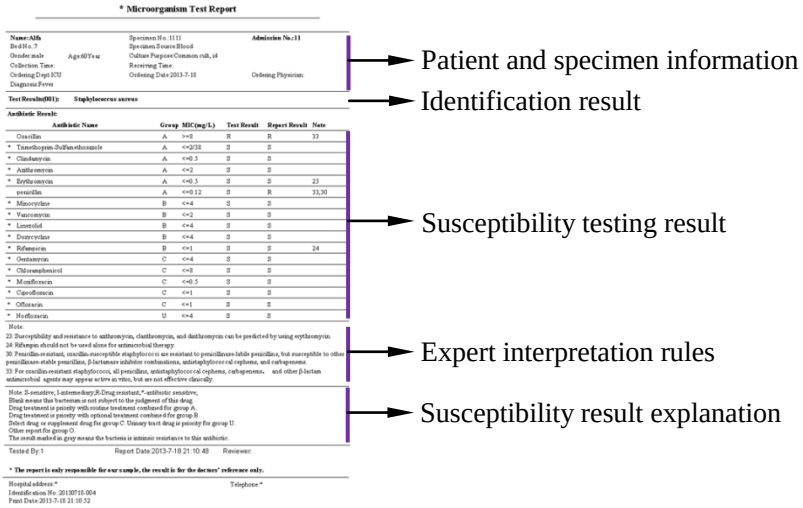
Comment: Some reaction should be sealed with sterile mineral oil after inoculation.

5. Test card incubation

Normally 35℃ 18-24h, with some exceptions such as streptococcus and fastidious bacteria should incubation under 5%-10% CO_2 atmosphere, yeast-like fungi should incubation under 30℃ for 24-48hs.

6. Test card reading and result analysis

7. Reporting



Operation procedures of microorganism analysis system and different test card please contact Mindray sales for operation manual or instructions.