

MANUAL METHODS IN MICROBIOLOGY – ISSUES AND TROUBLESHOOTING



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Microbiology
lab setup is
unique.

Observes strict
hygiene and
discipline.

Follows all
standards of
bio-safety
precautions.

DEPARTMENT OF **MICROBIOLOGY**

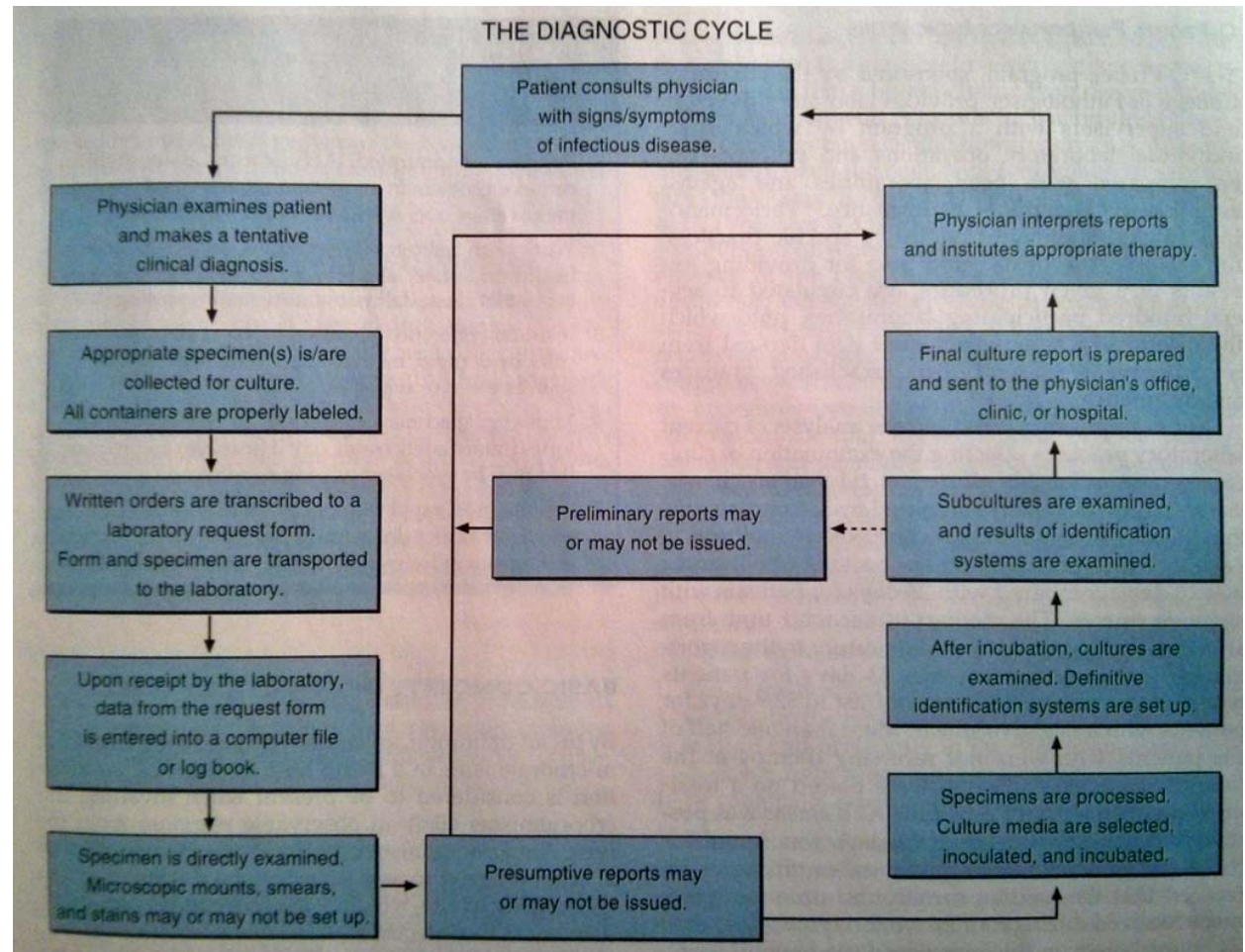


WORKFLOW

Pre-analytic test process

Analytic test process

Post-analytic test process



Pre-analytic test process



- **Specimens Management**

- ✓ Requisition Form
- ✓ Specimens identification/gross inspection
- ✓ Proper collection & Transport
- ✓ Sample Accession
- ✓ Rejection Criteria
- ✓ Storage
- ✓ Specimens Handling
- ✓ Impact- Accurate Lab Diagnosis which directly affect Patients care & Therapeutic decision.

Pre-analytic test process



• Media Preparation & Sterilization

- ✓ Media preparation should be under standard Aseptic conditions
- ✓ Important parameters to check
- ✓ Avoid Wastage while Preparation
- ✓ Rehydrate/ Powder media in use
- ✓ pH check
- ✓ Proper Sterilization
- ✓ Slopes/ Slant Preparation
- ✓ Blood Agar preparation **temperature** is Critical
- ✓ Sheep blood is Mandatory for Accreditation
- ✓ Cold Storage

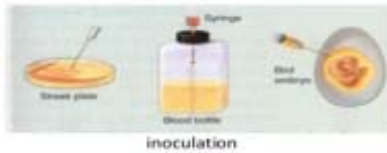
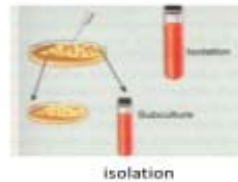


Analytic test process

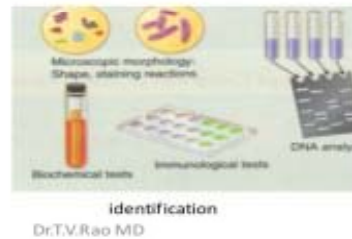
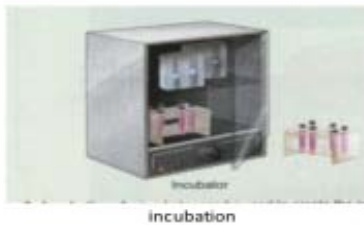


• Bacteriology

- Specimen Processing is very crucial step
- Staining



5 "I" s



Analytic test process



Microbe Identification

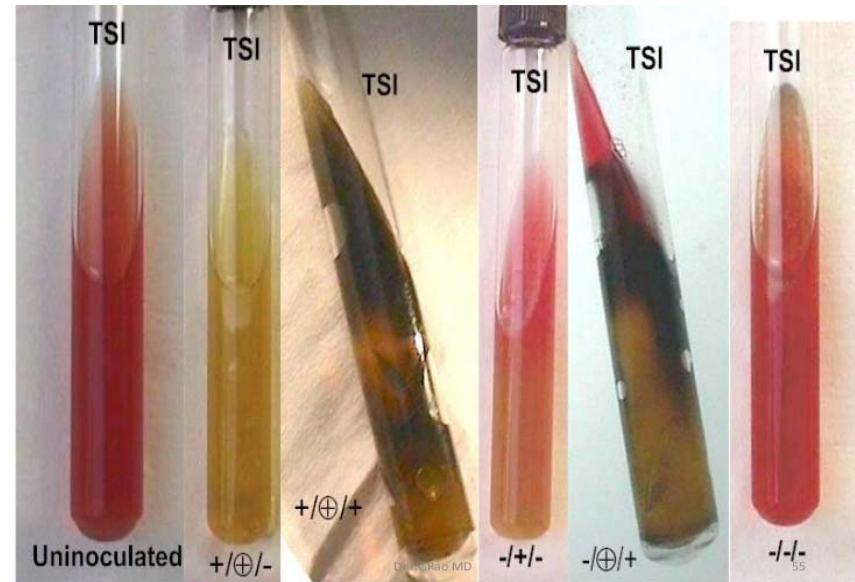
- Identification measures include:
 - ♣ Microscopy (staining)
 - ♣ growth on enrichment, selective, differential or characteristic media
 - ♣ specimen biochemical test (rapid test methods)
 - ♣ immunological techniques
 - ♣ molecular (genotypic) methods.
- After the microbe is identified for clinical samples it is used in **susceptibility tests** to find which method of control is most effective.



Analytic test process



Isolate Identification



Analytic test process



• Antibiotics Sensitivity Testing

- Important Task
- Goal-measure the growth response of isolated organism to a particular Drug or Drugs
- Performed under standardized condition to get reproducible results
- Different methods-
 - Agar disc diffusion method
 - MIC estimation
 - E-tests



Analytic test process



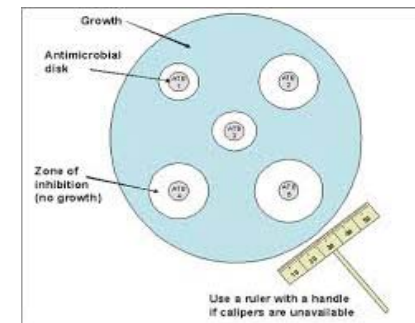
- **Agar disc diffusion method**

- **Kirby-Bauer method**

- Each & every step in this process is important and crucial in quality assurance-

- Critical point-

- Culture media
- Size of inoculums
- Disks storage
- Incubation condition
- Control with reference strains
- Accurate measurement of dimension of inhibition zone



Analytic test process



• Mycobacteriology

- Manual testing, risky & time consuming
- Risk of transmission by inhalation
- All work should be in a biosafety cabinet
- L J media preparation itself very laborious
- Liquid media preferred for better results
 - ZNCF Staining- possibility of carrying over
 - ADA level testing
 - Identification
 - Antibiotics susceptibility test



Analytic test process



- **Mycology**

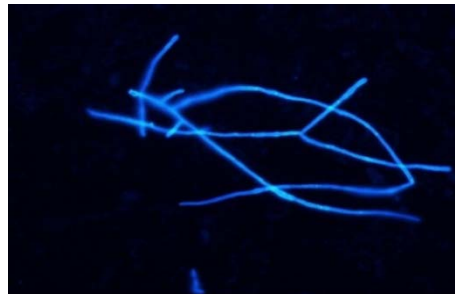
- All test are done manually-

- KOH Mount
- CFW Staining
- Serum galactomannan
- Germ tube test
- Crypto latex
- Yeast identification-sugar assimilation, on chrome agar *Candida albicans*-green, *Candida tropicalis*- dark blue, *Candida cruzi*-dry colony, violet
- Antifungal susceptibility Testing



Yeast—calcofluor white
<http://www.med.sc.edu/85/mycology/mycology-3.htm>

35



Mould—calcofluor white

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36



Analytic test process



- **Serology**

- Serological tests are of importance in epidemiological studies
- Screening tests
- ELISA test-Avoid cross contamination in manual washing
- Rapid card tests less sensitive

ELISA plate



Analytic test process



- **MOLECULAR MICROBIOLOGY**

- Molecular Methods –are now part of routine diagnostic Microbiology
- Simple detection of viral infection to become an integral component of the management of blood borne virus & other viral infection
- **Limitations**
 - Cannot replace conventional methods since many common tests performed in lab are rapid & economic
 - False positive & false Negative Results
 - False positive results can be due to lab contamination
 - So needs physical separation of areas for extraction & amplification
 - Inhibitors checks should be added to avoid negative results or actual inhibitor of PCR test
 - Skilled & dedicated personnel is required.



Post-analytic test process



- **Preparation of final culture report**
 - **Inform clinician/physician**
 - **Clinician/physician interprets the reports at the institute's appropriate therapy.**
-
- **Preservation of isolates and laboratory results**

Thank
you

