MICRO

RR-8.0

Contents

GENERAL MICROBIOLOGY

----- Active space -----

Kary B Mullis :
Invented PCR (Molecular) : Detects gene.

Eva Engvall (Along with Peter Perlman) :
Developed ELISA (Immunological) : Detects Ag/Ab.

Microscopy & Staining

00:09:58

MICROSCOPY

Detection of organism → Morphology : Staining (Killed).
→ Motility : Hanging drop (Live).

Types of Microscopes to Visualize Motility :

	Bright field microscope (Light)	Darkfield microscope	Phase contrast microscope
Features	M/c used	Darkfield (Special condenser) > Phase contrast (Phase plate)	
Characteristics	Bright organism with bright background	 Bright organism with dark background	 Dark organism with bright background
Motility Observed	All except thin organisms	Thin organisms motility visualized (Spirochetes : Corkscrew motility)	

Darkfield microscope : Contains dark field condensor.
Phase contrast microscope : Contains phase plate.

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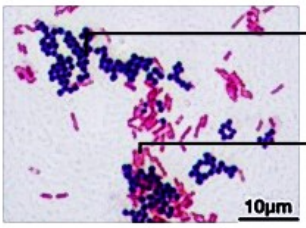
STAINING

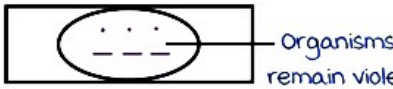
Gram Stain :



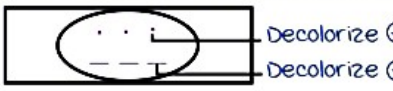
Smear preparation :

Primary staining : Crystal violet/Gentian/Methyl violet
(1 min)
↓ Wash

Add mordant : Gram's Iodine
(1 min)
↓ Wash

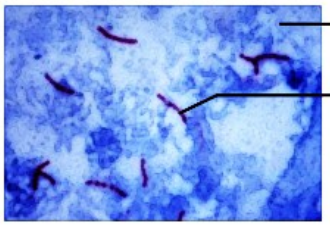


Add decolourization (Critical step) : Acetone (2-3 secs)/ Alcohol (20-30 secs)
(Few seconds)
↓ Wash



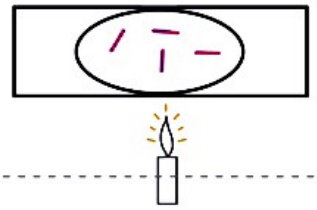
Counter staining : Saffranin/Dilute carbol fuschin
(1 min)

Acid Fast Stain/Ziehl-Neelsen Stain :



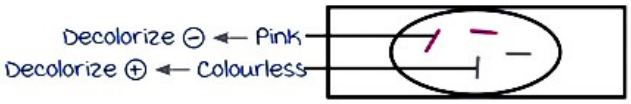
Smear Preparation :

Primary staining + Mordant : Concentrated Carbol fuchsin (Pink)
(5 min)
+ Intermittent heating



↓ Wash

Decolorization (Critical step) : H_2SO_4
(1 min)



↓ Wash

Counter staining : Methylene blue

Note :
Concentration of H_2SO_4 →
- 20% : M. Tb
- 5% : M. leprae.
- 1% : Nocardia & parasites.

Culture & Molecular Methods

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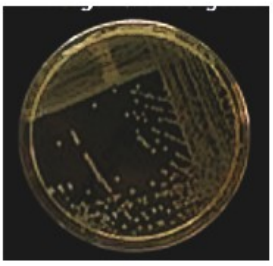
Culture media (CM) :

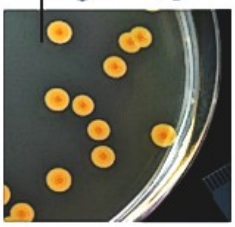
Simple/Basal CM :

- Nutrient agar (Straw-colored).
- Appearance : White-grey colonies.
- Exceptions :



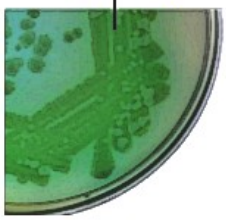
White-grey colonies

Golden yellow pigment



Staphylococcus aureus

Bluish-green pigment



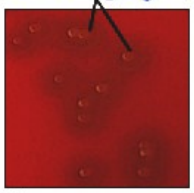
Pseudomonas aeruginosa

Enriched Media :

- Blood agar.
- Normal Appearance : No zone surrounding colonies.
- Exceptions :

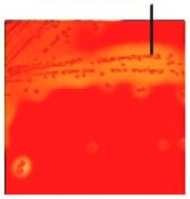
No zone

α-hemolysis (Partial) :
Greenish-grey zone



Pneumococcus

β-hemolysis (Complete) :
Clear zone



Staphylococcus

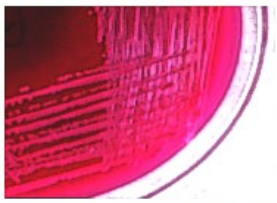
Double zone/Target hemolysis :
Complete followed by partial hemolysis

Clostridium perfringens

Differential Media :

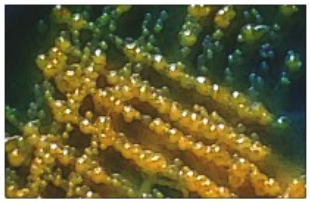
- McConkey agar :
  Only gram -ve organisms grow.
- CLED (Cystine-lactose-electrolyte deficient) agar :
  Gram ⊖, Gram ⊕ & Candida grows.

Pink colonies : Lactose fermenters (LF)

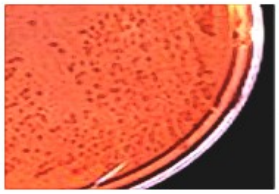


← E. coli, Klebsiella →

Yellow colonies (LF)

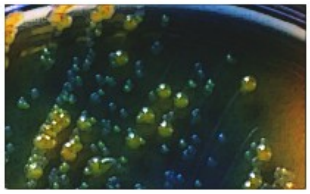


Pale colonies : Non-lactose fermenters (NLF)



← Many organisms →

Blue colonies (NLF)



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Enrichment CM :

- Use : Inhibit commensals in stool sample.
- Types :

	Selenite F broth	Tetrathionate broth	Alkaline peptone water
Colour of CM	Light brown	Bluish green	Straw colour
Pathogen	Salmonella, Shigella		Vibrio

Note :

NALC-NaOH method : Inhibits commensals in sputum sample

- N-Acetyl-L-cysteine : Liquefies sputum.
- NaOH : Inhibit commensals.

Transport media :

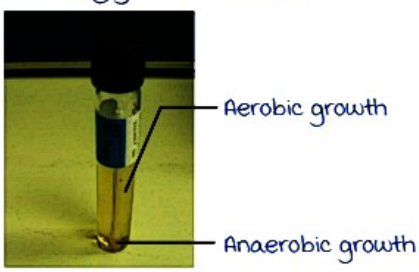
Transport of Stool Sample

- Cary Blair media (Best) : For all stool pathogens.
- Buffered glycerol saline : For all except Vibrio & Campylobacter.

Anaerobic CM :

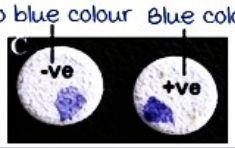
- Robertson cooked meat (RCM) : Best

- Thioglycollate broth :



Culture Identification :

Biochemical Tests :

	Catalase Test	Oxidase Test	Urease Test
Features			• Pink color : Urease +ve • No pink color : Urease -ve
Organisms	Catalase -ve : • Streptococcus • Pneumococcus • Enterococcus	Oxidase +ve : • Vibrio • Pseudomonas • Campylobacter • Helicobacter	Urease +ve : • Helicobacter • Proteus

----- Active space -----

MOLECULAR METHOD

PCR :

Steps :

Nucleic acid extraction : Enzyme method (Add Lysozyme).

↓

Nucleic acid amplification in thermocycler → Denaturation (95°C) : ds to 2 single strands.
→ Annealing (55°C) : Primer attachment.
→ Extension (72°C) : Primer extension.

↓

Nucleic acid detection : Gel electrophoresis/Fluorescent method.

Uses :

- Diagnostic test : Detects gene.
- Prognostic test : To monitor Rx response.
  - Gives organism load.
  - If PCR ⊕ : If ↑CT (Cycle Threshold) value → ↓Organism → ↑Prognosis.

Modifications :

- Conventional PCR : Detects only DNA.
- Real-time RT (Reverse Transcriptase) PCR :
  - Detects DNA & RNA.
  - Semiautomated.
- Automated Realtime RT-PCR : CBNAAT (Cartridge based) & TruNAT (Chip based).
- Multiplex Realtime RT-PCR : Detects multiple organisms.

Antimicrobial Susceptibility Testing (AST) 01:00:40

Phenotypic method :

Culture method.

Kirby Bauer disk diffusion : m/c.

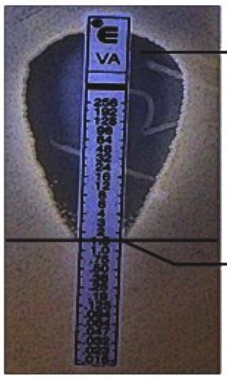
- Mueller Hinton Agar (MHA).
- Zone of inhibition → Present : Antibiotic sensitive.
  → Absent : Antibiotic resistant.
- Minimum Inhibitory Concentration (MIC) of antibiotic not obtained.

Zone of inhibition

Antibiotic disk

E-strip :

- MHA.
- MIC obtained.



Zone of inhibition : Antibiotic sensitive

MIC

Antibiotic strip

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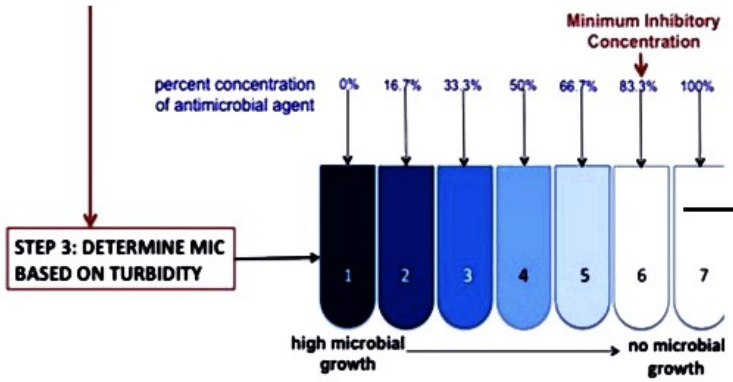
Broth dilution : Gold standard.

- Mueller Hinton Broth (MHB).
- MIC obtained.



Clear (No turbidity) : Sensitive.

Genotypic method :

Molecular method.

PCR :

Detects resistant gene → If present : Antibiotic resistant.
→ If absent : Antibiotic sensitive.

Example : CBNAAT detects → M. Tb
→ rpo-B gene (If ⊕ : Rifampicin resistance).

HOSPITAL INFECTION CONTROL

----- Active space -----

Hand Hygiene & PPE

00:00:42

Hand Hygiene :

Methods :

1. Hand wash	2. Hand rub
• Better method.	• If visibly soiled : Not acceptable.
• Soap & water used.	• Disinfectant used.

Steps :

Rub hands palm to palm.

Rub hand's back with other palm, fingers interlaced.

Rub palm to palm, fingers interlaced.

rub backs of fingers to opposing palms, hands clasped

Rub tips of fingers onto palm in a circular motion.

Rub each thumb with rotational motion.

Rub each wrist

5 moments for hand hygiene :

- Before & after touching patient.
- Before & after procedures & fluid exposure.
- After touching patient surroundings.

Personal Protective Equipment (PPE) :

Essential PPE : Gown, gloves, mask, goggles & faceshield.

Two methods :

	1. Donning	2. Doffing
Action	Putting PPE on	Removing PPE
Sequence	Gown → Mask → Goggles/Face shield → Gloves.	Gloves → Goggles/Face shield ⇄ Gown → Mask

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Biomedical Waste Management (BMW)

00:11:40

method of segregation & disposal of waste contaminated with microorganisms.

	Yellow Bag	Red Bag	White Bag	Blue Bag
Type of Waste	• Cotton, Linen • Tissues, culture media • Chemicals, medicines • Blood bags (Plastic)	• Plastic : Urine bag • Rubber	Metallic Sharps	• Metals • Glass
Disposal method	Incineration	Autoclave ↓ F/b Recycling	Na hypochlorite ↓ Shredding/Pack in puncture proof containers then bury	Na hypochlorite ↓ Recycling.

Sterilization

00:20:41

Sterilization vs Disinfection :

- Complete killing of all forms of microorganisms → Including spore form : Sterilization. / Excluding spore form : Disinfection.
- Sterilization > Disinfection.

Types of methods :

	Moist Heat	Dry Heat	H_2O_2/Plasma	Ethylene oxide (ETO)
Example	Autoclave	Hot air oven	-	-
m/c method	121°C for 15 min under 15 lbs pressure	160°C for 2 hours	-	-
Control	Bacillus Stearothermophilus	Bacillus subtilis/ Bacillus atrophaeus/ Clostridium tetani	Bacillus stearothermophilus	Bacillus subtilis/ Bacillus atrophaeus/ Clostridium tetani

materials Sterilized :

materials	method
• Surgical instruments except sharps (Eg : Linen, sutures without needle) • Plastic & rubber materials (Eg : Syringe, gloves)	Autoclave > H_2O_2 > ETO
Glass & sharps (Eg : Flasks, scalpels)	Autoclave > Hot air oven
All culture media (CM)	Autoclave
Exceptions in CM : • Serum CM (Loeffler's serum slope) • Egg CM (Lowenstein-Jensen medium)	Inspissation > Tyndallization
Oily & powdery materials (Eg : Liquid paraffin, glove dust powder)	Hot air oven