Microbiology

World of Revision

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Contents

Microbiology

GENERAL MICROBIOLOGY : PART 1

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Pioneers in Microbiology

00:07:32

	Contribution	Significance
Antonie van Leeuwenhoek	Simple microscope	Father of microscopy
Louis Pasteur	• Liquid culture media • Vaccines (CAR) : Cholera, Anthrax, Rabies	Father of microbiology
Robert Koch	• Solid culture media • Discovered (CAT) : Cholera, Anthrax, TB	Father of bacteriology
Kary B Mullis	Molecular methods (PCR)	-
Eva Engvall (Along with Peter Perlman)	Immunological methods (ELISA)	-

Morphology & Motility

00:16:45

Microscopy

Morphology detection :

- Primarily : Capsule, cell wall, fimbriae.
- Methods : Wet mount, staining.

Motility detection :

- Primarily : Corkscrew motility.
- Method : Hanging drop.

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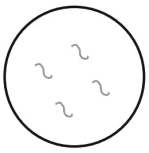
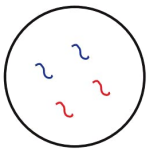
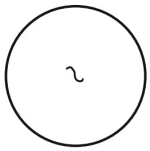
Types of microscopes :

	Bright field/light microscope (m/c)		Dark field microscope	Phase contrast microscope
	Wet mount/ Hanging drop	Staining		
Morphology	Visualised	Visualised better	Visualised : No staining	
Motility	Appreciated	Not appreciated	Corkscrew motility : Spirochetes	
Appearance	 Bright organisms with bright background	 Colourful organisms with bright background	Thin organisms  Bright organisms with dark background	 Dark organisms with bright background
Images			Dark field condensor 	Phase plate 

Capsule Detection :

	Microscopic method		Immunological method	
	Negative staining (Screening test)	McFadyean's staining	Latex agglutination test (Best/rapid test)	Quellung's reaction
MOA	Capsule staining		Detects capsular Ag	
Indicator	India ink/nigrosin	Polychrome methylene blue	Clumps	Capsular swelling
Capsule type detected	All types	Only polypeptide (B. anthracis)	All types	Only polysaccharide (Pneumococcus, Meningococcus, Hib)

Cell Wall Detection :

All bacteria except mycoplasma (mollicutes) have cell wall.

Identification :

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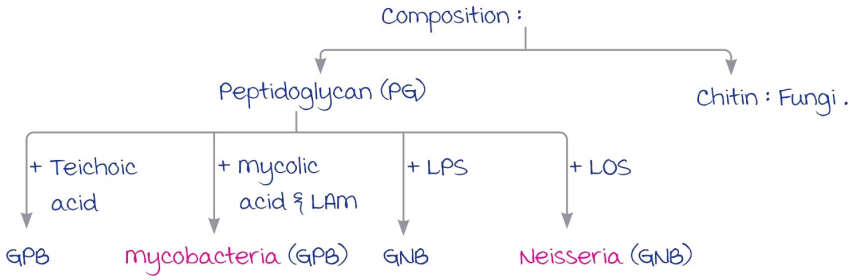


GPB : Gram positive bacteria
GNB : Gram negative bacteria
LAM : Lipoarabinomannan
LPS : Lipopolysaccharide
LOS : Lipooligosaccharide

Fimbriae/Pili Detection :

- Organ of attachment.
- UTI bacteria (m/c : E.coli).

Identification : Heme agglutination.

Culture Media (CM) 00:34:25

Types :

Simple CM : Growth of all organisms.

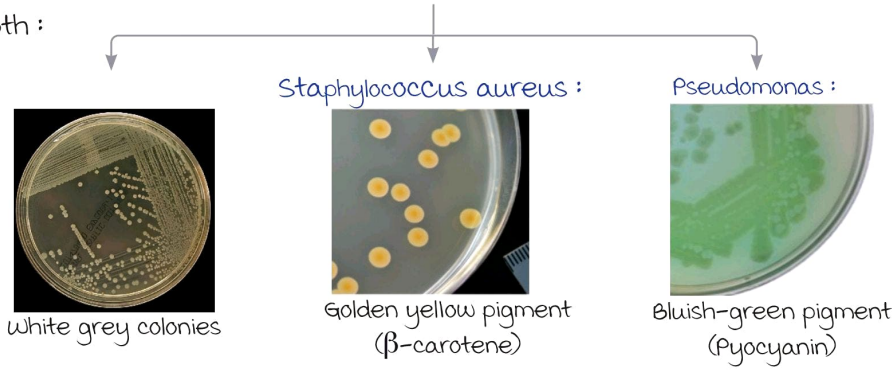
Special CM :

- Selective CM + Special substances.
- Types :

	MOA
Enriched media	⊕ Growth of pathogens
Enrichment media	⊖ Commensals in stool samples
Differential media	Differentiates organisms
Transport media	Keep organism alive during transport
Selective media	⊕ Growth of specific organisms
Anaerobic media	⊕ Growth of anaerobic organisms

Simple/Basal CM :

Nutrient agar/broth :



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Enriched Media :

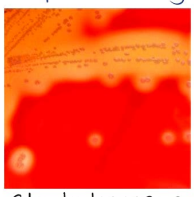
Blood agar :

Observation :

- No zone
- Greenish-grey zone (α/Partial hemolysis) :

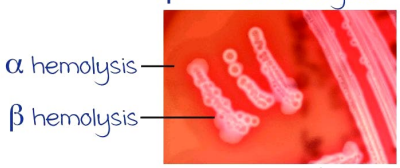
Pneumococcus

- Clear zone (β/Complete hemolysis) :



Staphylococcus & Streptococcus

- Double zone (β → α hemolysis) :



Clostridium perfringens

Enrichment Media :

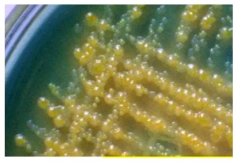
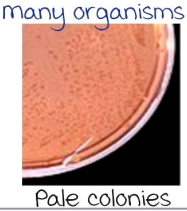
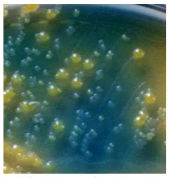
	Selenite F broth	Tetrathionate broth	Alkaline peptone water
Colour	Light brown	Bluish green.	Straw coloured
Pathogens grown	All stool pathogens (Except : Vibrio)		Vibrio

Note :

Sputum sample : NALC – NaOH method (NALC : N-Acetyl-L-cysteine).

- NALC → Liquifies sputum
- NaOH → ⊖ commensals

Differential Media :

		MacConkey agar	CLED agar
Composition		Lactose sugar	
		Bile salts (⊖ GP)	Cysteine-lactose-electrolyte deficient
Organisms		GN	GN/GN, GP, Candida
Observation	Lactose fermenters : • E. coli • Klebsiella	 Pink colonies	 Yellow colonies
	Non-lactose fermenters	Many organisms  Pale colonies	 Blue colonies

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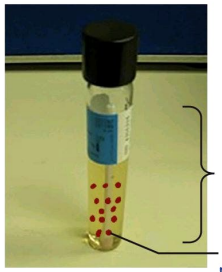
Transport Media (Stool) :

- Cary Blair Media : All pathogens
- Buffered Glycerol Saline : All pathogens except Vibrio & Campylobacter

Anaerobic Media :

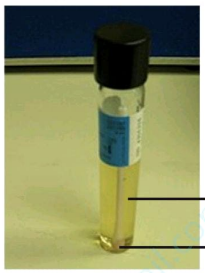
- Robertson cooked meat (RCM) :



Only anaerobic growth

Meat particles

- Thioglycollate broth :

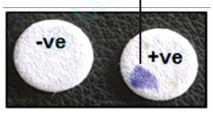
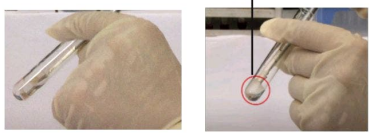


Aerobic growth

Anaerobic growth

Biochemical Tests

00:52:24

	Observations	Organisms
Catalase test	Bubbles  +ve −ve	Catalase −ve : • Streptococcus • Pneumococcus • Enterococcus
Oxidase test	Blue colour  	Oxidase +ve : • Vibrio • Pseudomonas • Campylobacter • Helicobacter
Urease test	Pink colour : +ve	Urease +ve : • Helicobacter • Proteus • Nocardia
Coagulase test	Coagulum  −ve +ve	Coagulase +ve : Staphylococcus aureus
String test	String formation : +ve 	String test +ve : Vibrio cholerae

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Molecular Methods

00:56:40

Polymerase Chain Reaction (PCR) :

Detects gene.

Steps :

NA extraction : Lysozyme

↓

NA amplification : Thermocycler [Denaturation (95°C) → Annealing (55°C) → Extension (72°C)] → 40 cycles

↓

NA detection : Gel electrophoresis
fluorescent method.

NA : Nucleic acid

Modifications :

	Characteristics
Conventional PCR	Detects only DNA
Real time RT PCR	• Detects DNA & RNA • Uses reverse transcriptase (RT)
multiplex realtime RT PCR	• Detects multiple organisms • For syndromic approach
Automated realtime RT PCR	• Cartridge based : CBNAAT • Chip based : TrueNAT

Uses :

- Diagnosis : Detect organisms
- Prognosis (m/c) : Cycle threshold/CT value
  - $1/\propto$ → Organism load
  - $\propto$ → Prognosis

Immunological Methods

01:05:30

	Tests		Organisms
Ag detection	Nagler's reaction		Clostridium perfringens
	ELEK's gel precipitation test		Corynebacterium diphtheriae
Ab detection	Widal agglutination test (WAT)		Salmonella
	Standard agglutination test (SAT)		Brucella
	Microscopic agglutination test (MAT)		Leptospira
	Weil Felix test	OX K Ag +ve	Orientia
		OX 2 ± OX 19 Ag +ve	Rickettsia