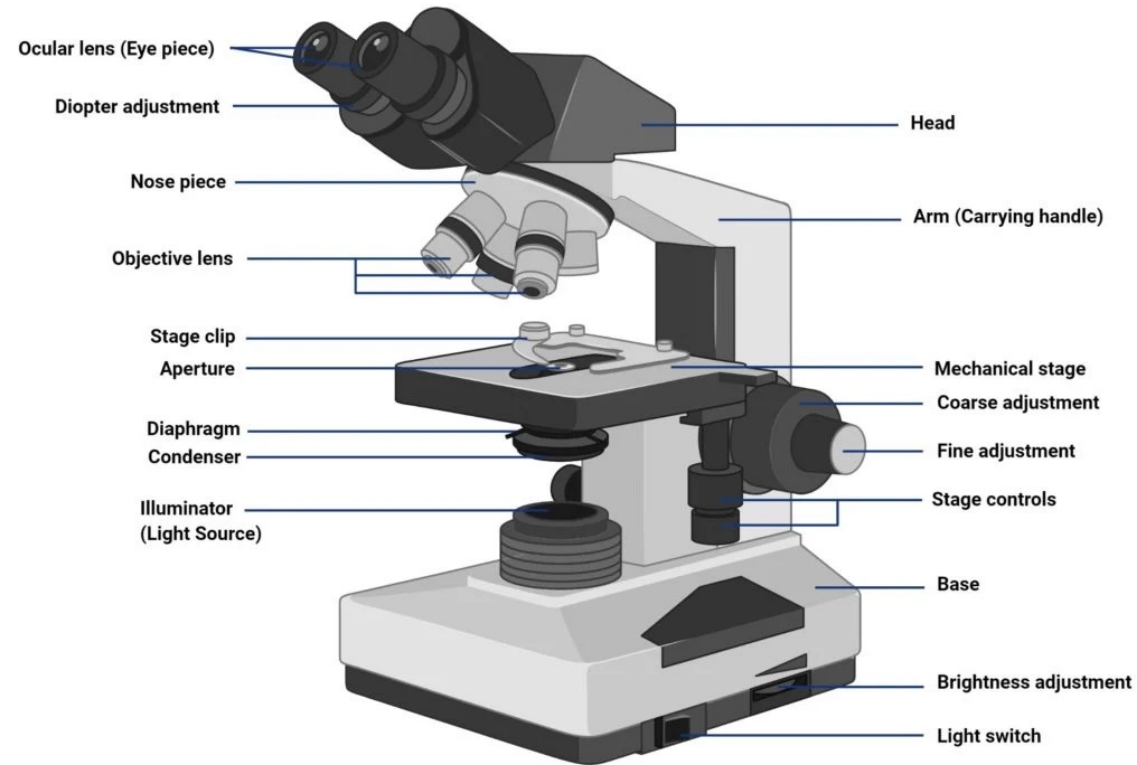


BONUS BTR

MICROBIOLOGY

MICROSCOPES



Fixative: **10% NEUTRAL BUFFER SALINE**

Slide: **GLASS**

Resolution: **0.2 μ m**

Magnification: **OBJECTIVE - 4x**

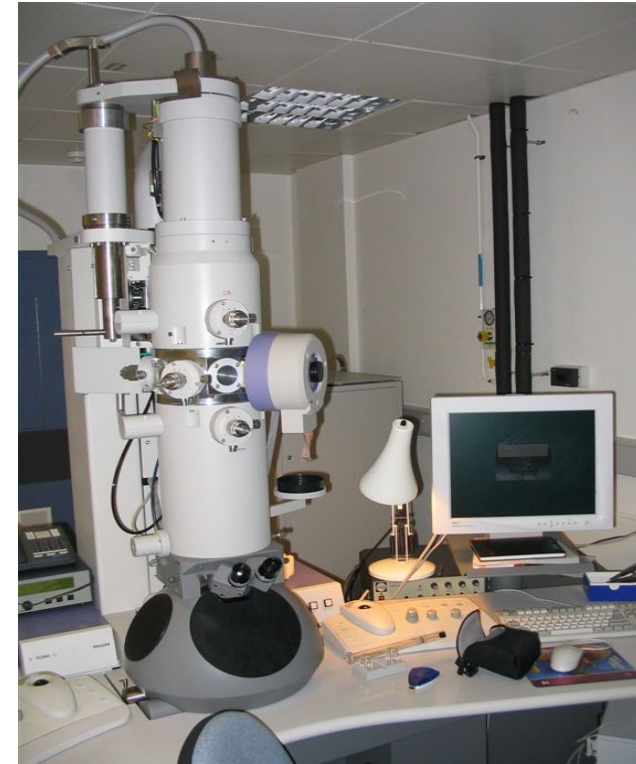
LOW POWER - 10x

EYEPIECE : 10x

HIGH POWER - 40x

OIL IMMERSION - 100x

Max magnification: **1000x**



ELECTRON MICROSCOPE

Fixative: **2-2.5% GLUTARALDEHYDE**

Slide: **COPPER METAL SLIDE**

Resolution: **2.5 nanometer**

Internal structures: **TRANSMISSION EM**

3D image: **SCANNING EM**

MICROSCOPES



DARK FIELD
MICROSCOPY

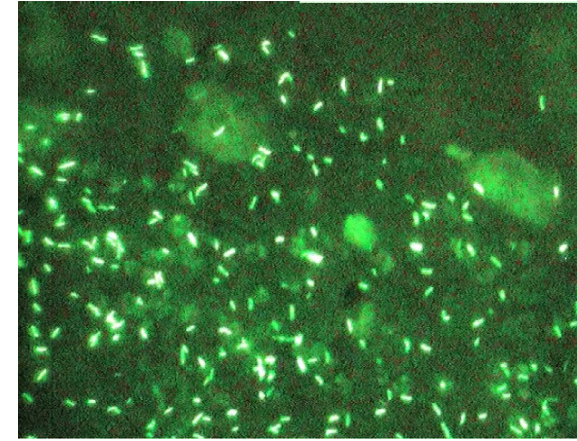
REFLECTED LIGHT
↳ CONDENSER



PHASE CONTRAST

↳ MOTILITY
INTERNAL CONTENT
HAIRY CELL

REFRACTED LIGHT



UV light $\downarrow \lambda \rightarrow \uparrow \lambda$

Dichroic mirror

Auramine/Rhodamine: MYLOBACTERIUM

Acridine orange: MALARIA

Calcofluor white: FUNGI-CHITIN

FITC: FLOW CYTOMETRY

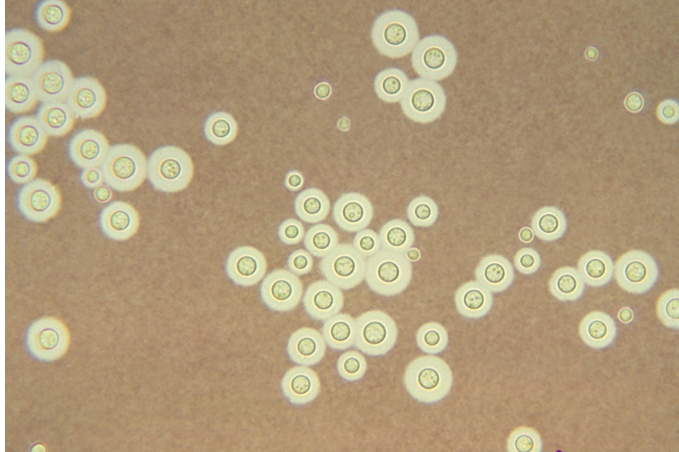
Auto-fluorescence:

Cyclospora

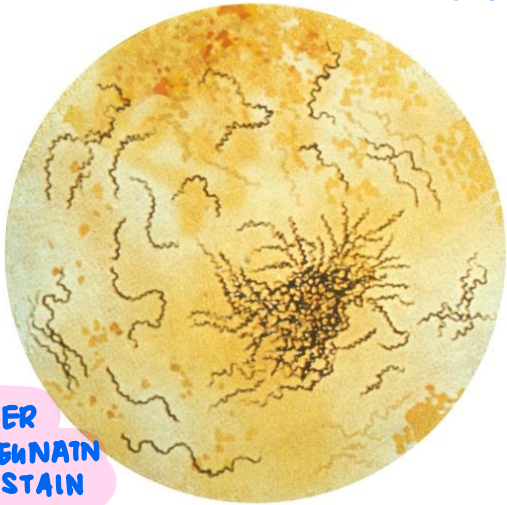
NADPH



STAINS

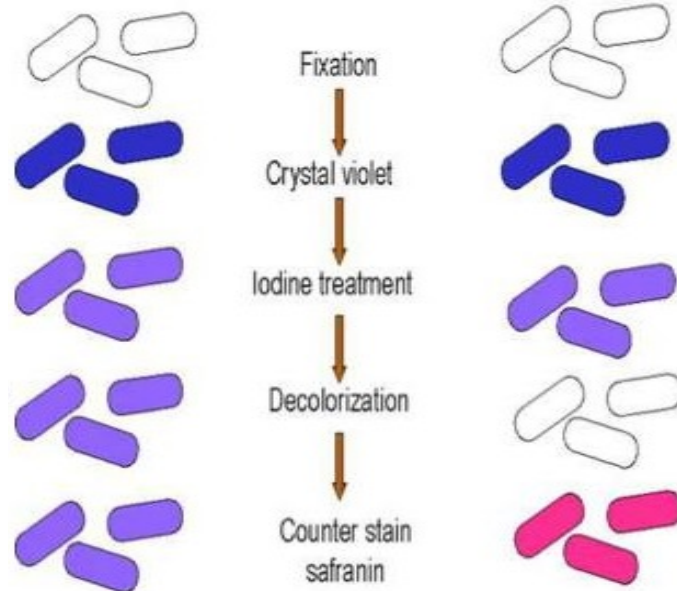


NEGATIVE STAIN - INDIA INK / NIGROSIN
↓
CRYPTOCOCCUS

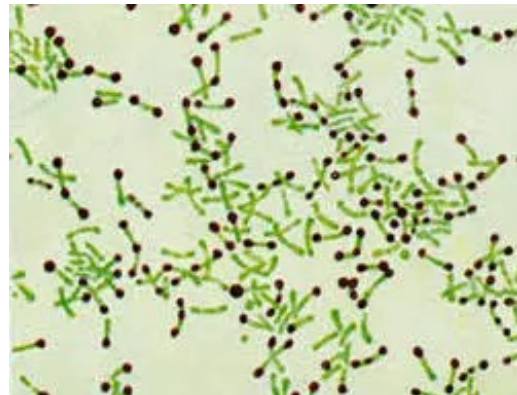


SILVER IMPREGNATION STAIN

Levaditi → TISSUE
Fontana → FLUID

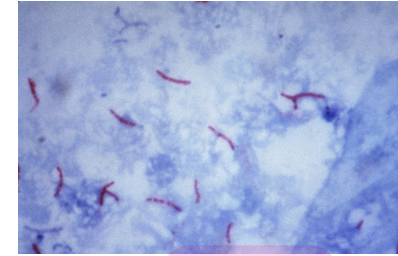


GRAM STAIN
[Peptidoglycan $\begin{matrix} \uparrow - + \\ \downarrow - \ominus \end{matrix}$]



ALBERT'S STAIN

← TOLUIDINE BLUE - also for MAST CELL STAINING
← IODINE
← MALACHITE GREEN



ZN STAIN

Primary: CARBOL FUSCIN
Mordant: HEAT
Decoloriser: 20% H₂SO₄
Counterstain: METHYLENE BLUE

ACID FAST-

TB: 20% H₂SO₄

Leprosy: 5% H₂SO₄ [FITE FORALD STAIN]

Nocardia, Legionella: 0.5% H₂SO₄

Crypto/ Isospora/ Bacterial spores/ Head of sperm: 0.25%

COLD: KINYOUN'S stain for COLLIDIAN PARASITES

SPORES:

Schaeffer and Fulton stain

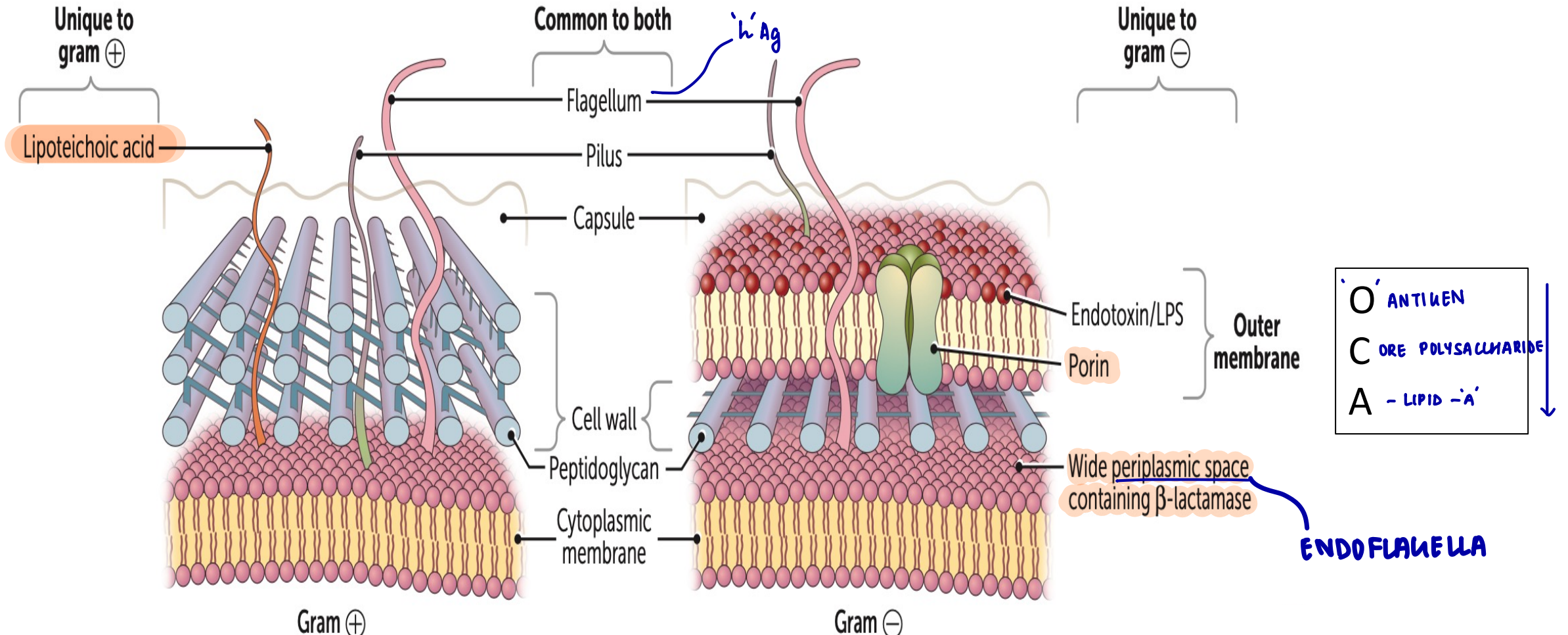
Ashby stain

FLAGELLA: Leifson and Ryu

[METACHROMATIC GRANULES]

SPORES: Ponder Albert Neisser

BACTERIAL WALL



DIAMINOPIMETIC ACID
AA unique to bacteria

Linulus amebocyte assay (LAL): FOR ENDDTOXIN TESTING
 \ominus

BACTERIAL WALL

Capsule: Polysaccharide

SHIN } *S. PNEUMONIAE*
M. INFLUENZAE
NEISSERIA MENINGITIDIS } all have IgA PROTEASE
+ ALL 3 UNDERGO TRANSFORMATION → VIRULENCE FACTOR

Klebsiella

C. perfringens

B. pertussis

Y. pestis



WAYSON STAINING

V. parahemolyticus

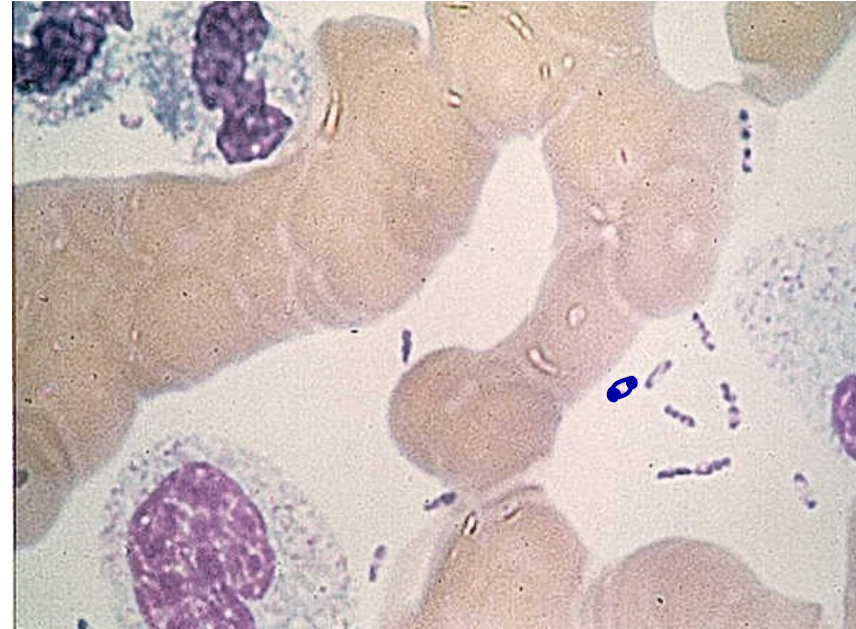


also

[DONOVAN BODIES]
KLEBSIELLA GRANULOMATIS
HEMOPHILUS DULREYI
BURKHOLDERIA PSEUDOMALLEI

Polypeptide: *B. ANTHRACIS* [D-GLUTAMATE]

Hyaluronic acid: *S. PYOGENES*



Cell wall deficient

Protoplast μ^+

\oplus

UNSTABLE FORMS

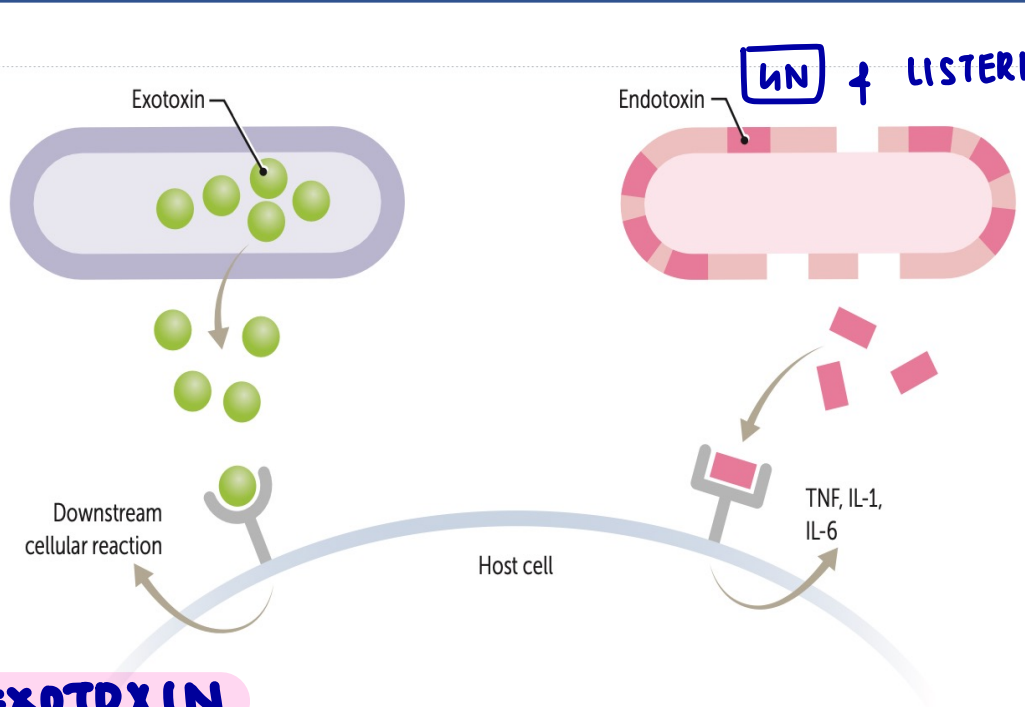
Spheroplast μ^-

\ominus

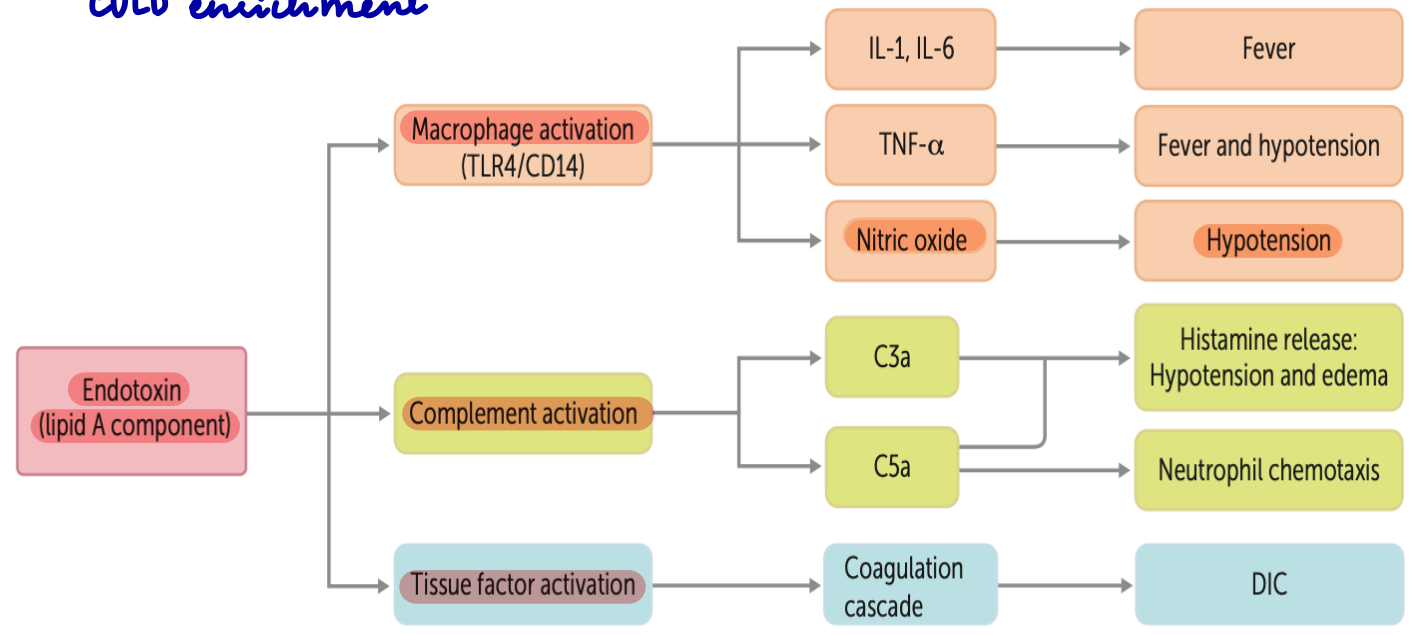
[L-FORMS]

Stable: *MYCOPLASMA*

ENDOTOXIN-EXOTOXIN



↳ *Tumbling motility*
COLD enrichment



EXOTOXIN
 all HEAT LABILE
 EXCEPT

- ST ↳ ETEC
 CUMP

ENDOTOXIN

Low antigenicity. Toxicity
 No toxoid, vaccine
 Heat stable
 Released by lysis

NO — ARGININE
 acts by cAMP

FLAGELLA → FOR LOCOMOTION

Flagella Type



Monotrichous

Vibrio - **DARTING**
Pseudomonas



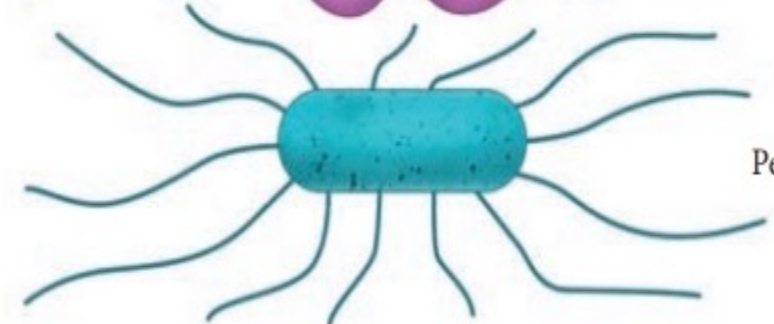
Lophotrichous

H. Pylori, Bartonella bacilliformis



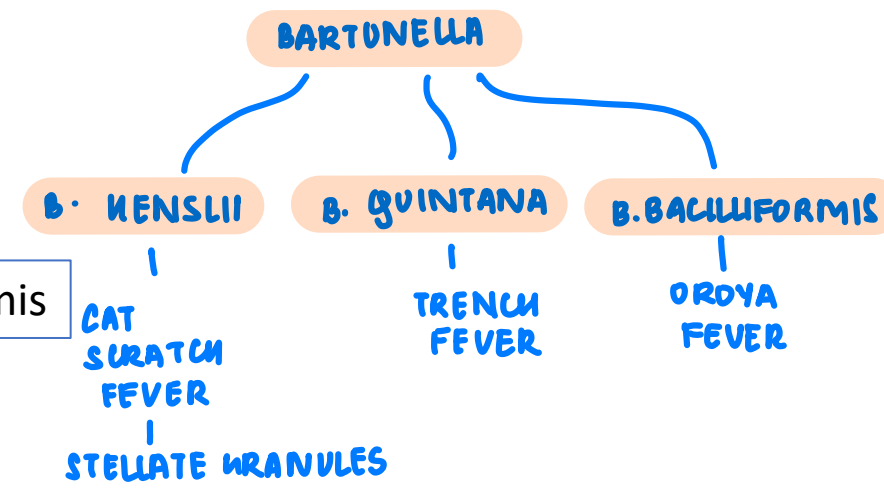
Amphitrichous

Spirilla



Peritrichous

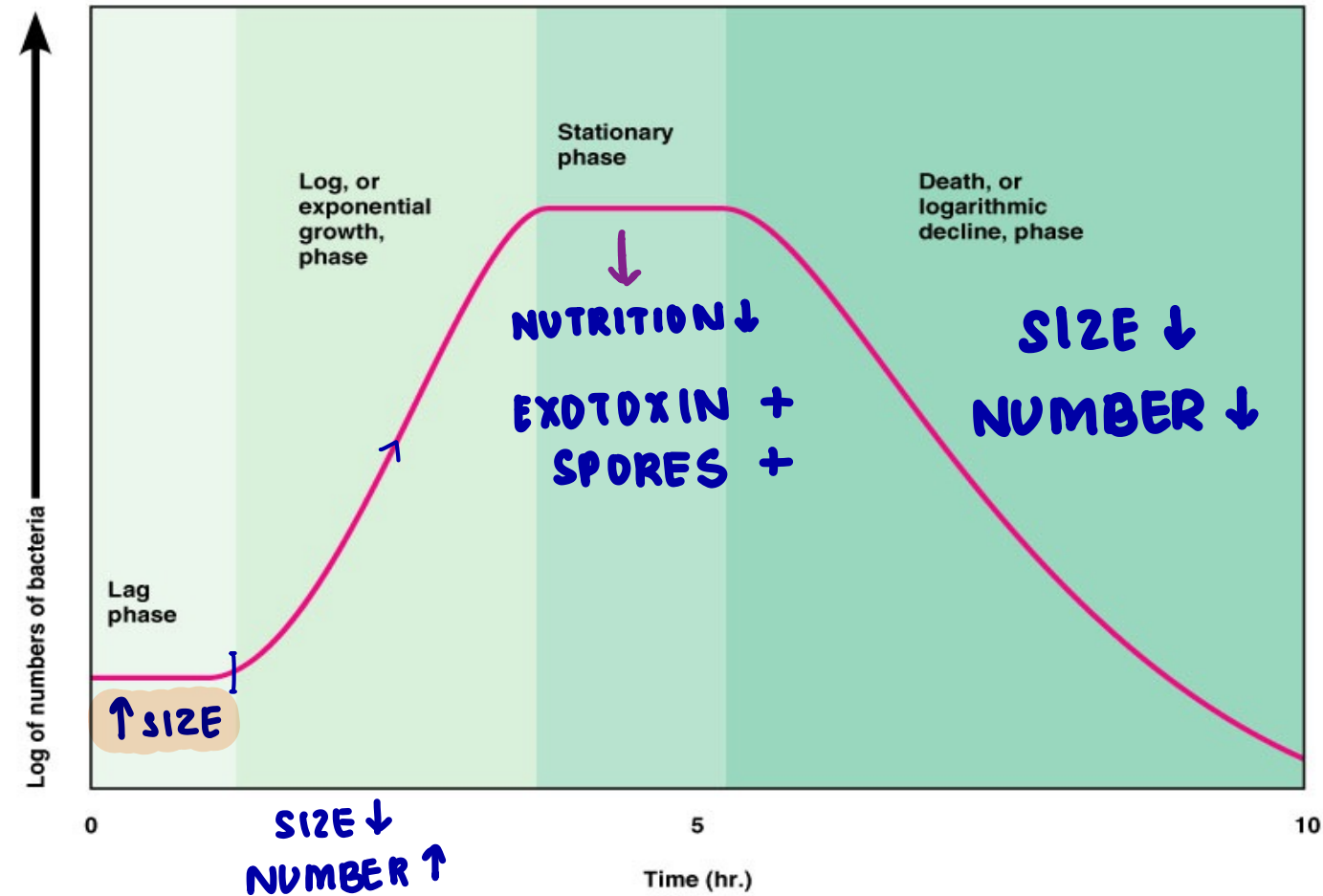
Listeria - **TUMBLING**
E. Coli
Clostridium } **SWARMING**
B. cereus
Proteus



AGAR:
Solid: **2%**
Prevent swarming: **6%**
Nutrition? **⊖**

**SEMI-SOLID : 0.5%
[10 SEE MOTILITY]**

BACTERIAL GROWTH



SIZE \downarrow
NUMBER \uparrow
METABOLISM - MAX
AB_x
SUSCEPT. MAX

CULTURE MEDIA

ENRICHED (Solid)

Blood 5% SHEEP RBC → 50°C STREPTOCOCCI

Chocolate BLOOD AGAR HEATED AT 70°C

LSS Euk C-DIPHTHERIA H-INFLUENZA

BHI

LJ medium: TB

ENRICHMENT (Liquid)

Selenite F, Tetrathionate broth < SALM. SHIGELLA

Alkaline peptone water - VIBRIO

SELECTIVE

Crystal violet S. PYOGENES

LJ M-TB (MALAKITE GREEN) - ROUGH BUFF TROUGH COLONIES

Thayer martin NEISSERIA

Cetrimide PSEUDOMONAS

PLET B. ANTHRAX

MYPA B. CEREUS

Deoxycholate citrate, XLD < SALMONELLA SHIGELLA

Potassium tellurite (McLeod) C. DIPHTHERIA

Maconkey (S,D,I)

CLED (S, D)

Mannitol salt (S,D, I)

TCBS (S,D)

TRANSPORT

Cary-Blair, VR VIBRIO

Sach's buffered glycerol saline

< SALMONELLA SHIGELLA

Pike - S. PYOGENES

Stuart, Amies - NEISSERIA

ROBERTSON'S COOKED MEAT BROTH

PROTEOLYTIC CLOSTRIDIUM TETANI

SACCHAROLYTIC CLOSTRIDIUM PERFRINGENS

WASPAK



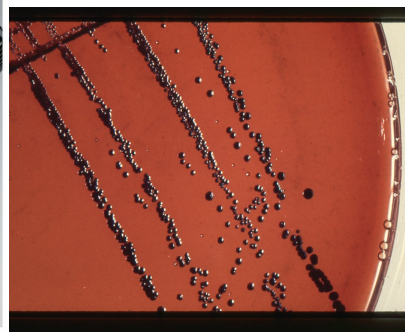
ANAEROBIC

RCM

STERILISATION BY INSPISSATION

LSS

LJ



POTASSIUM TELLURITE AGAR

CULTURE MEDIA



MAC CONKEY AGAR

LF

PINK

I

NEUTRAL
RED



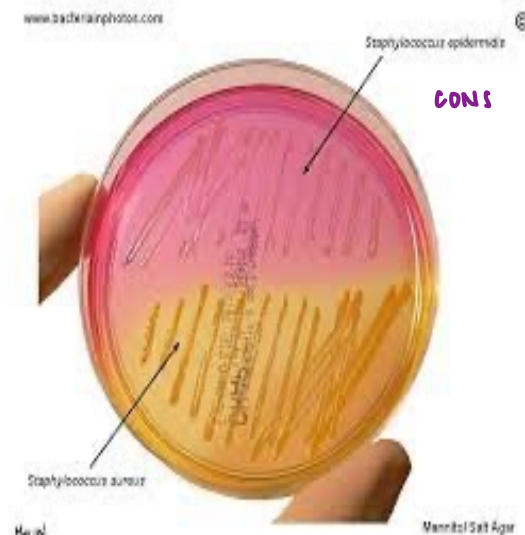
CYSTEINE LACTOSE
ELECTROLYTE DEFICIENT

LF

YELLOW

I

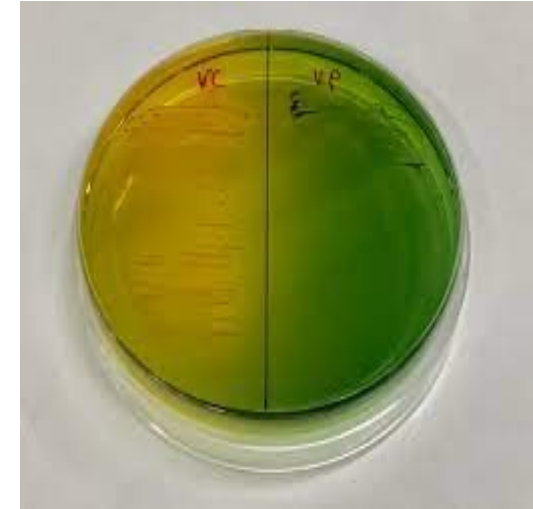
BROMOTHYMOLO
BLUE



MANNITOL
SALT AGAR

I

PHENOL
RED



TCBS

I

SUCROSE

SF

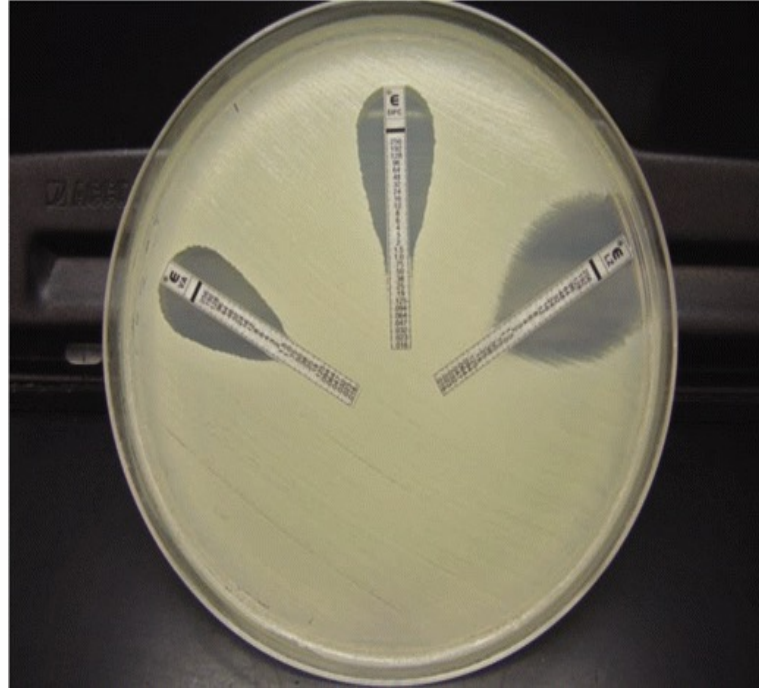
VIBRIO

YELLOW

CULTURE MEDIA



KB DISC DIFFUSION TEST



EPSILOMETER

QUANTITATIVE
[MIC]

* 6 strips can be used at once

Lawn culture
Stab
Streak

Colony morphology:

Fish eye- **SALMONELLA**

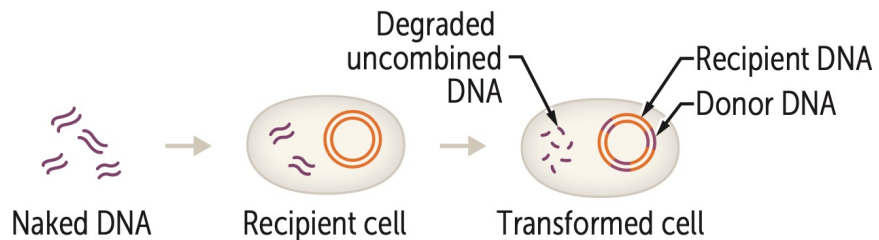
Dinger's ring (EMJH)- **LEPTOSPIRA**

Bisected pearl- **BORDATELLA**

Fried egg- **MYCOPLASMA**

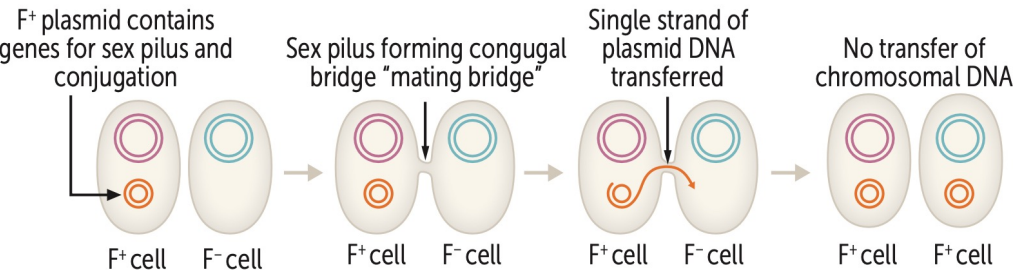
* MUELLER-HINTON AGAR USED FOR AST

BACTERIAL GENETICS

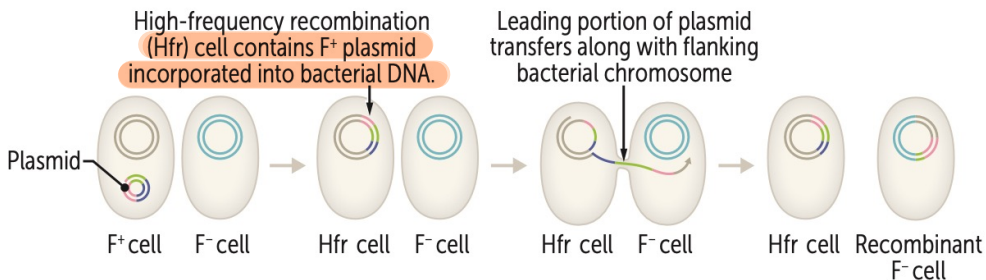


TRANSFORMATION GRIFFITH EXPERIMENT

CONJUGATION

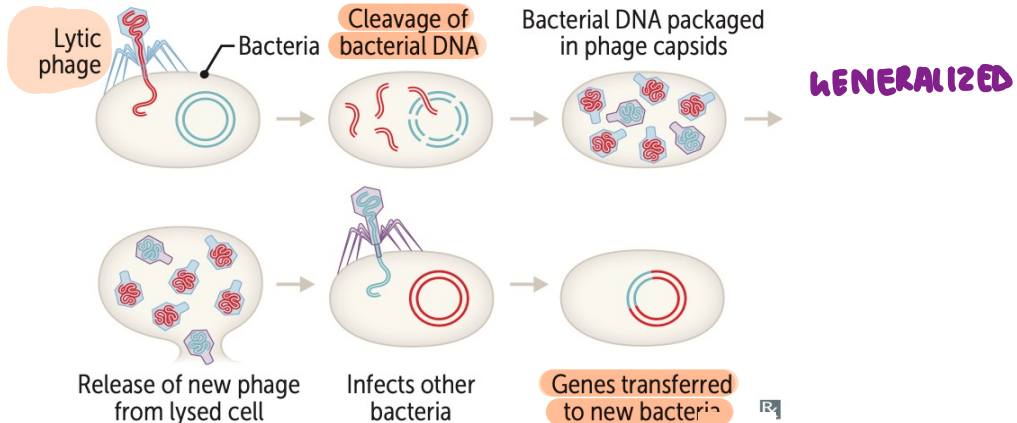


Antibiotic resistance
Bacteriocins *SELF GROWTH ↑*
Enterotoxin production *OTHER ↓↓*

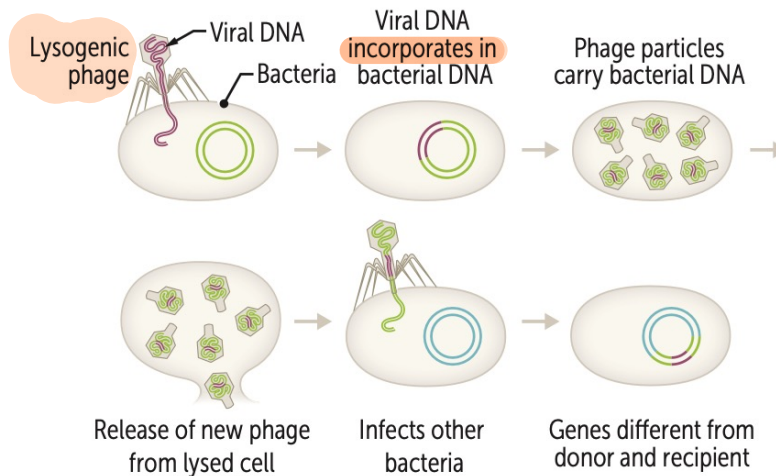


F⁻ cell bacterial DNA + F⁺ cell bacterial DNA + plasmid copy

TRANSDUCTION



SPECIALISATION



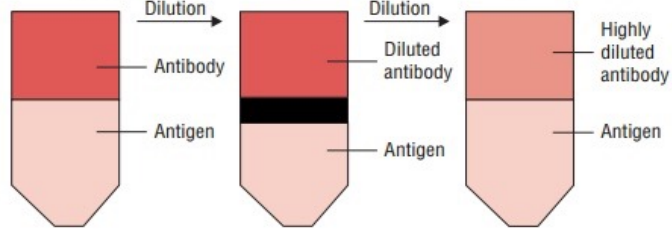
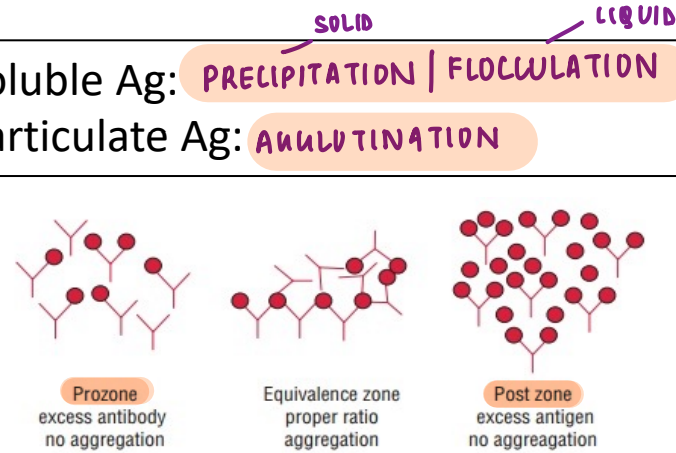
Virulent phage: **LYTIC**
Temperate phage: **LYTIC / LYSOGENIC**

TOXINS CODED BY LYSOGENIC PHAGE

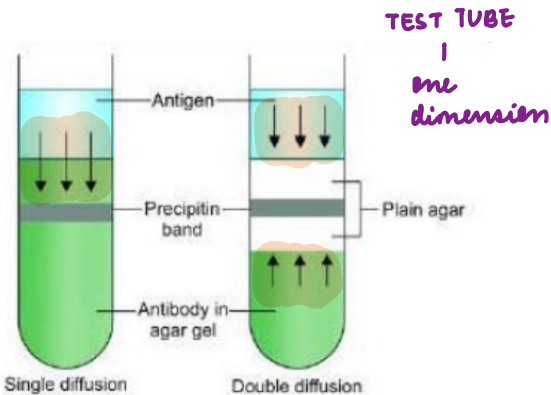
- A - GROUP A STREPTOCOCCI
- B - BOTULINUM TOXIN
- C - CHOLERA TOXIN
- D - DIPHTHERIA TOXIN
- S - SHIGA | SHIGA LIKE TOXIN (ETEC)

Antigen-Antibody reactions

Soluble Ag: **PRECIPITATION | FLOCCULATION**
 Particulate Ag: **AGGLUTINATION**



Marrack's lattice hypothesis.



DOUBLE DIMENSION - slide

Mancini **SINGLE DIFFUSION**

Ouchterlony

DOUBLE DIFFUSION

ONE DIMENSION

Oudin

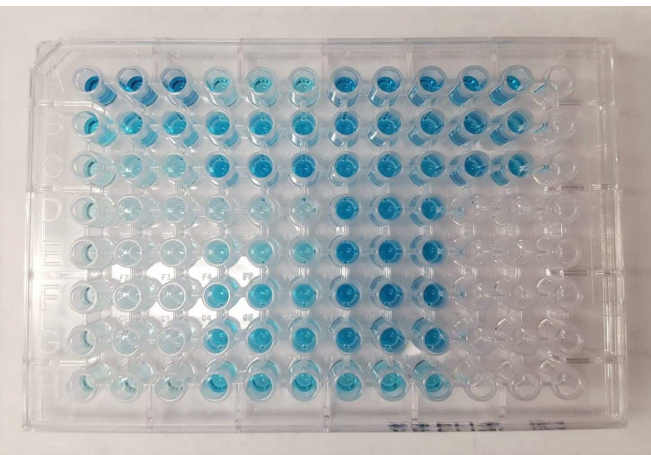
Oakley-Fulthrope

PRECIPITATION/FLOCCULATION: **GROUP A. STREP. [S. PYOGENES]**
 Ring test: Ascoli **ANTHRAX** Lancefield
 Slide: VDRL } **SYPHILIS**
 Tube: Kahn }
 Immunodiffusion/ Gel: Elek test - **C. DIPHTHERIA**
 Rocket electrophoresis → **QUANTITATE Ag**

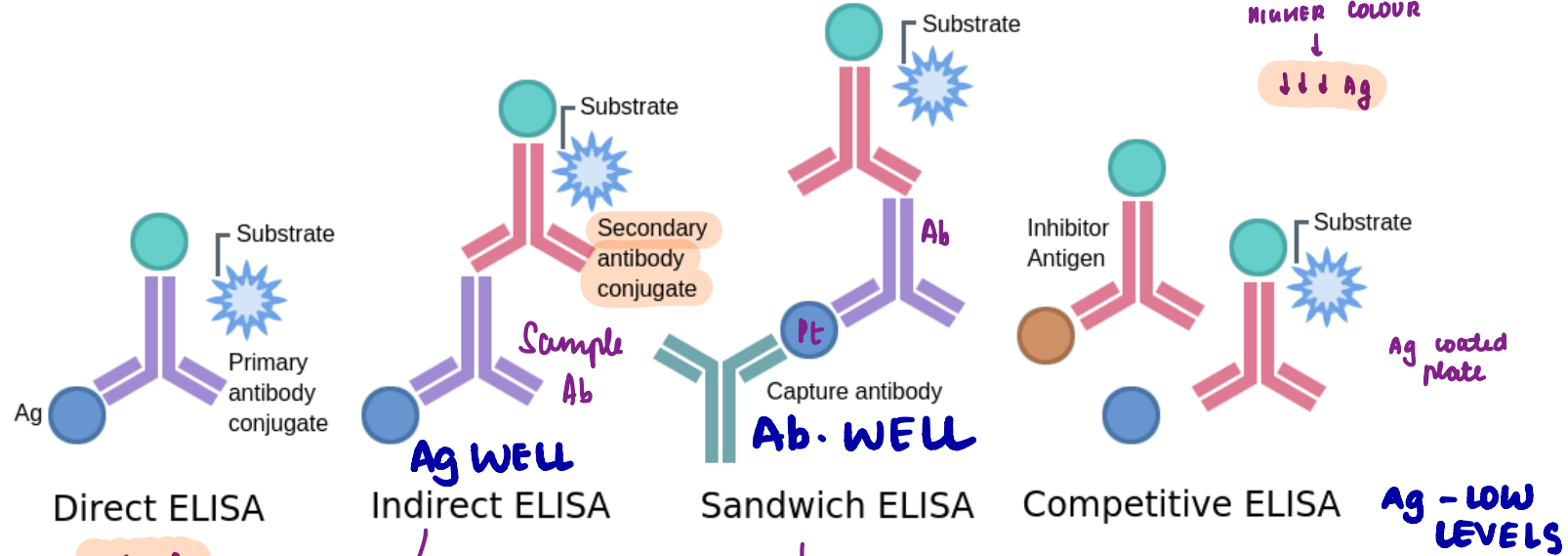
AGGLUTINATION
 Slide: Blood grouping
 Tube: **Widal** **Weil Felix** **Paul-Bunell** **CAT** **SAT** **MAT**
TYPHOID **ENTEROPHILE** **EBV + SHEEP / HORSE RBC** **MYCOPLASMA** **BRUCELLA also ROSE BENJAL TEST** **LEPTOSPIRA**
SRUB TYPHUS + PROTEUS Ag
 Coombs test
 Indirect/Passive agglutination:
 Latex-**ASO**, CRP, RF, HCG
Heme-Rose Waaler test - RA FACTOR

Complement fixation:
 Wassermann, TPI → **SYPHILIS**
 Sabin Feldman - **TOXOPLASMA**

ELISA / IF / RIA



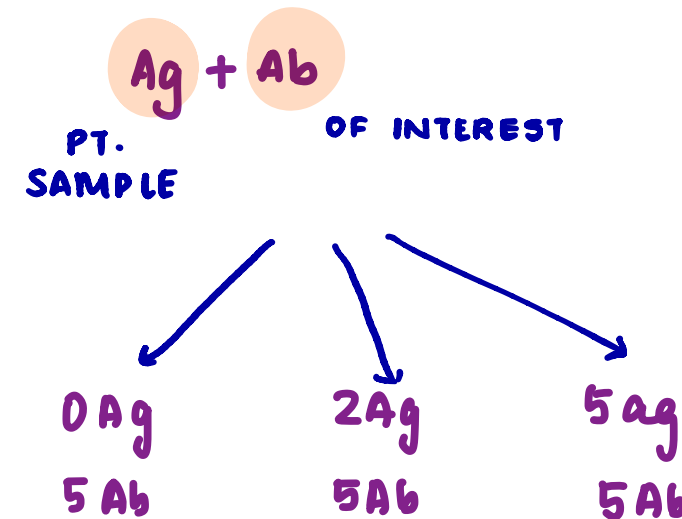
96 wells



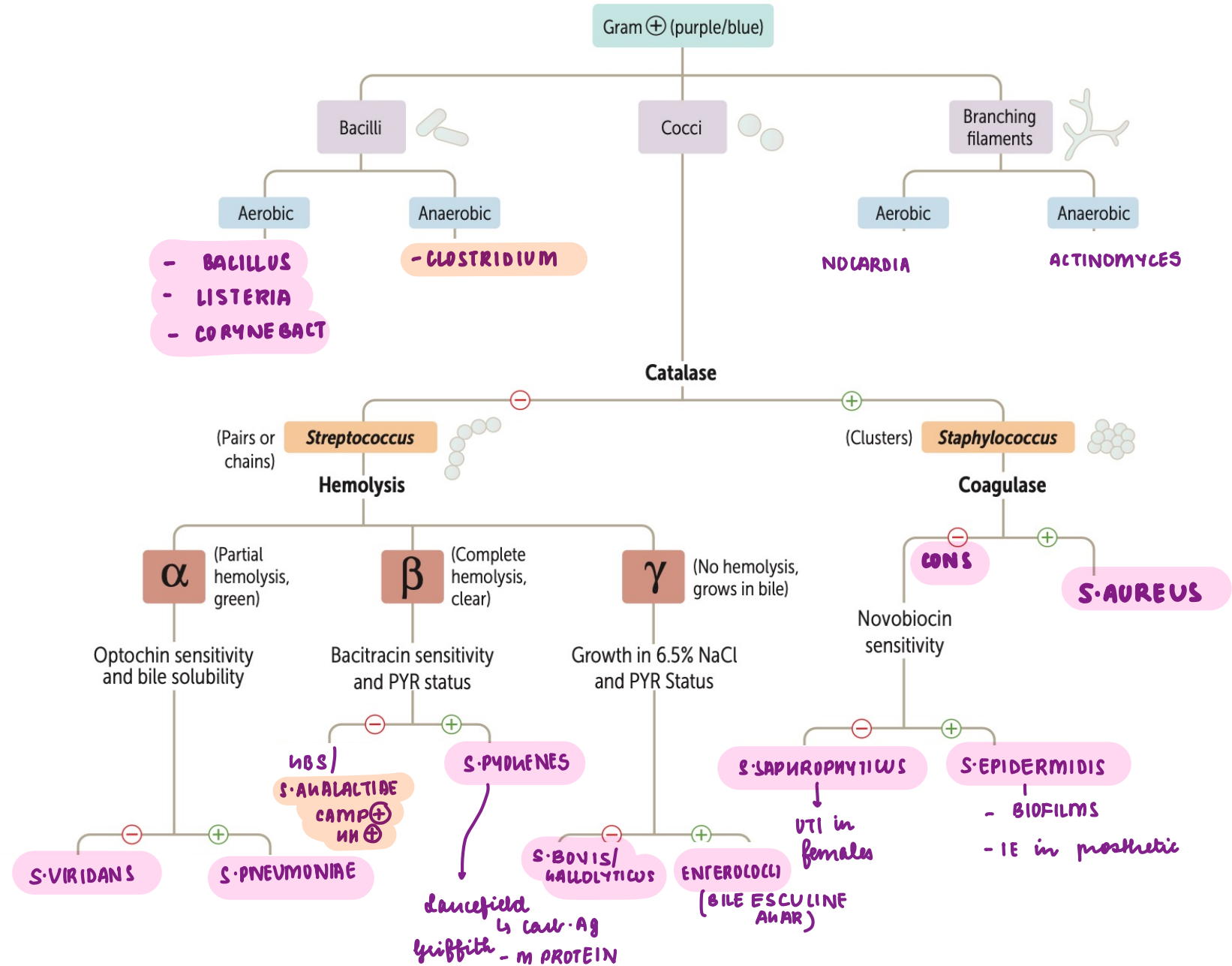
Enzyme	Substrate	Chromogen
Horseradish Peroxidase	Hydrogen peroxide	Tetramethyl benzidine (TMB)
Urease	Urea	Bromocresol
β -Galactosidase	ONPG	ONPG
Alkaline Phosphatase	pNPP	pNPP



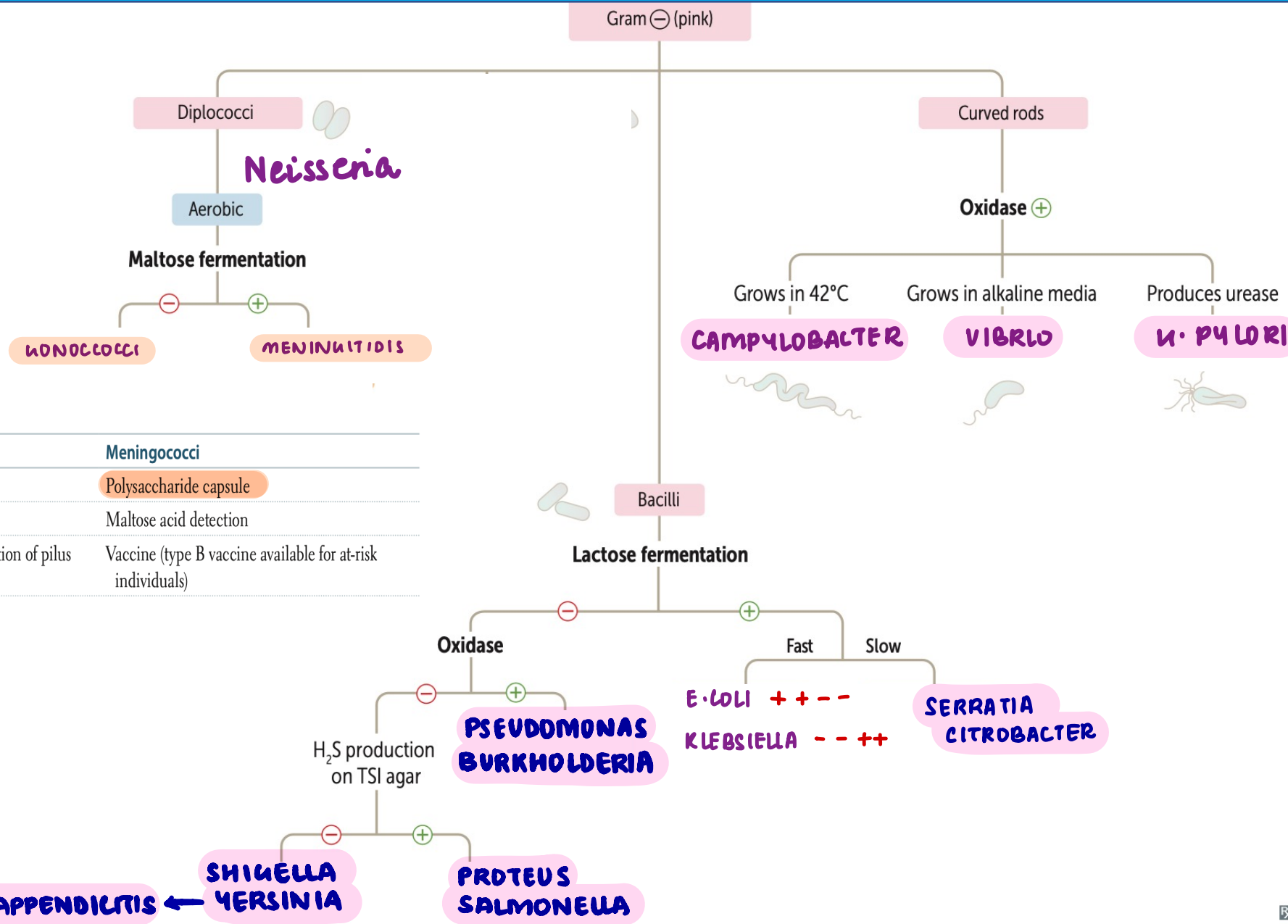
IMMUNOCHROMATOGRAPHY



BACTERIOLOGY



BACTERIOLOGY



Gonococci	Meningococci
No polysaccharide capsule	Polysaccharide capsule
No maltose acid detection	Maltose acid detection
No vaccine due to antigenic variation of pilus proteins	Vaccine (type B vaccine available for at-risk individuals)

BACTERIOLOGY

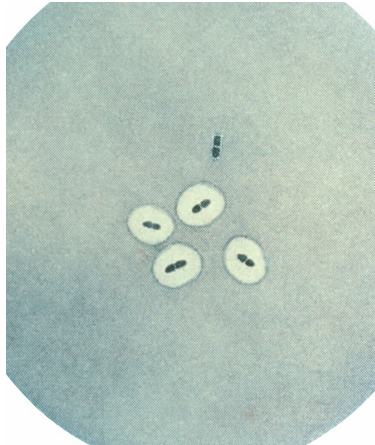


SSSS

|

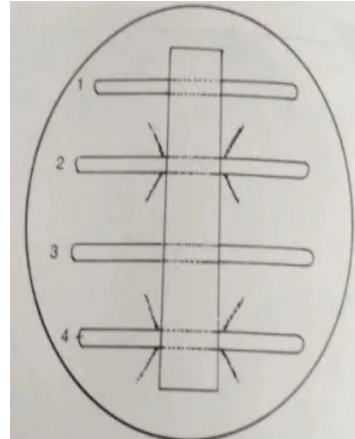
EXFOLIATIVE
TOXIN

RITTER'S DS



QUELLUNG'S RXN

S. PNEUMONIAE



C. DIPHTHERIAE

|

ELEK'S TEL
PPT

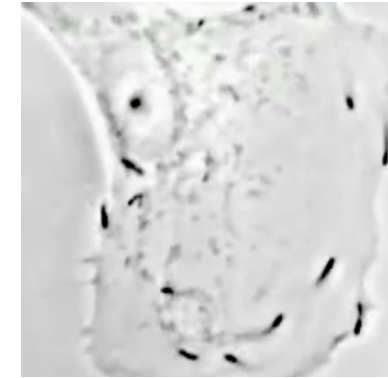


FLOPPY BABY

|

INFANT BOTULISM

HONEY - SPORES



Jet-actin
motility

|

LISTERIA

+ TUMBLING



+

Iridiscent colonies

↓

ECTHEMA

HANCRENSUM

|

PSEUDOMONAS

VIROLOGY

DNA VIRUSES

dsDNA

ssDNA

Nonenveloped

Enveloped

PARVOVIRUS

Nonenveloped
Smallest

ADENOVIRUS
(SPACESHIP SHAPED)

HERPES
VZV
EBV
CMV
HHV-6/7/8

POX VIRUS

Largest
Complex
symmetry
Cytoplasm - REPLICATION

Replicates
through an RNA
intermediate

HEPADNA/
HEP-B

PAPOMA (HPV)

POLYOMA
(JC/BK)

SIMIEN
POX VIRUS
(CV-40)

BAD
KIDNEY
|
DECOY
CELLS

SMALL POX
MONKEY POX
FOWL POX
MOLUSCUM CONTAGIOSUM

RNA VIRUSES:

ssRNA EXCEPT ROTAVIRUS (dsRNA)
NSP-4
INTUSSUSCEPT

SINGLE COPY except RETROVIRUS (2 copies)
(HIV/HTLV)

UNSEGMENTED

BUNYA — ③
INFLUENZA — ⑧
ROTA — ⑪
ARENA — ②

SEGMENTED

Nucleic acid replication

DNA

|
NUCLEUS
except
POX

RNA

|
CYTOPLASM
except
RETROVIRUS
INFLUENZA
MEASLES