

Mucosal component

Dr.Baha. Hamdi.Al-Amiedie •

Ph. D. Microbiology •


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The mucosa – associated lymphoid tissue or (MALT)

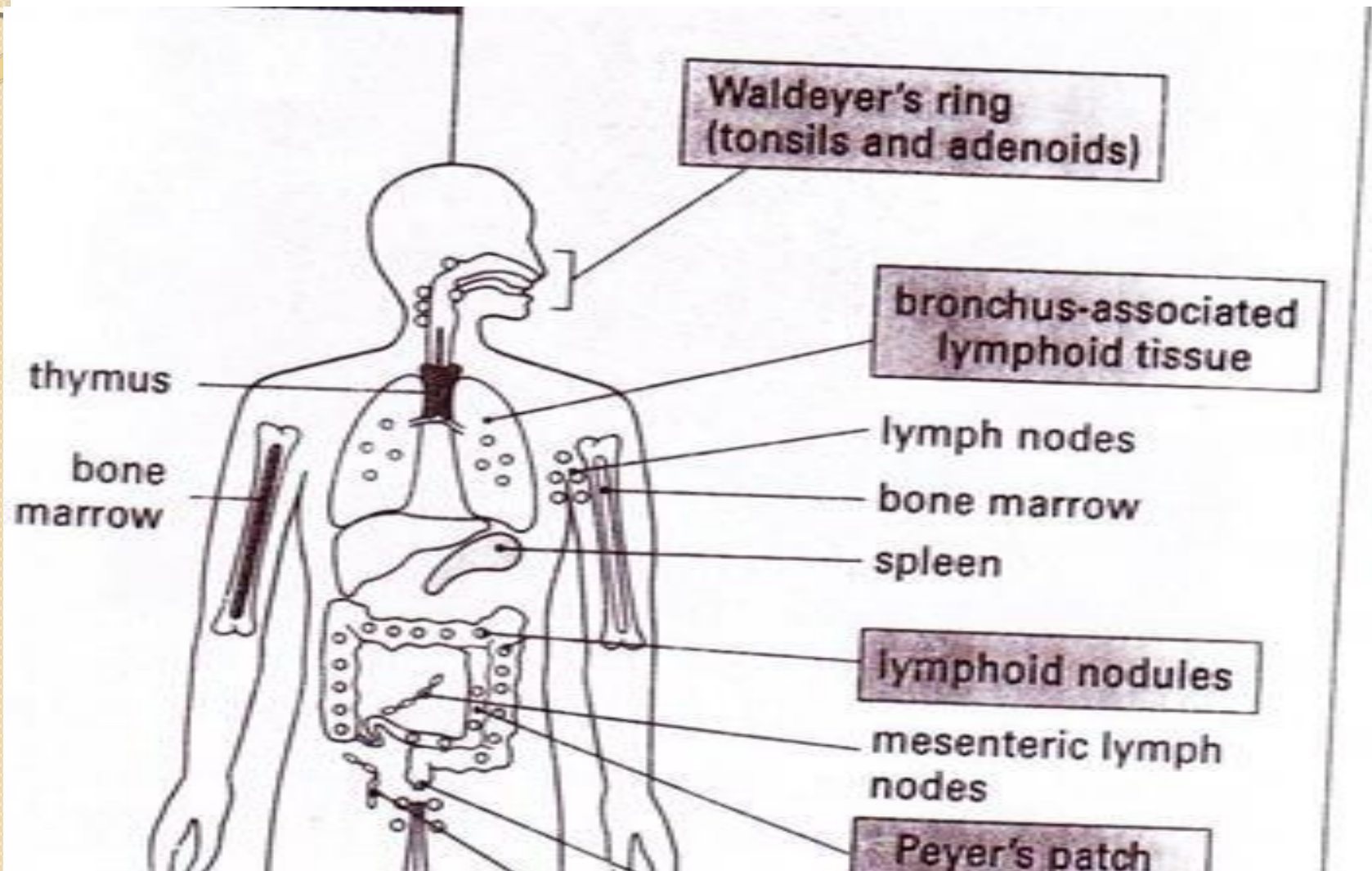
Local immune component are recently being

- talked by immunologist as an active local immune system it consist of both B- and T- Cells epithelial cell producing secretory piece they can fix up both humoral and cellular immune response in site , the mucosa –

associated lymphoid tissue or (MALT) the main mucosa- associated lymphoid tissue are the gastrointestinal tract (including oral mucosa and salivary gland , the genitourinary tract , the respiratory tract and the secretion of the mammary glands

- 
- through local immune stimulated this tissue lymphoidmucosa- associated the areas in discreteinto organizedwhich is just below patches Peyerasknowngut the epithelial cells in the lamina propria specialized Antigen travels through to the subepithelial epithelium(M Cells) antibody maintissue.The lymphoid IgA , secretoryis sites produced at these it aquireswhich immunoglobulindimeric transport secretory component during epithelium. theacross

The mucosa – associated lymphoid tissue or (MALT)



Follicle-associated epithelial & M cells

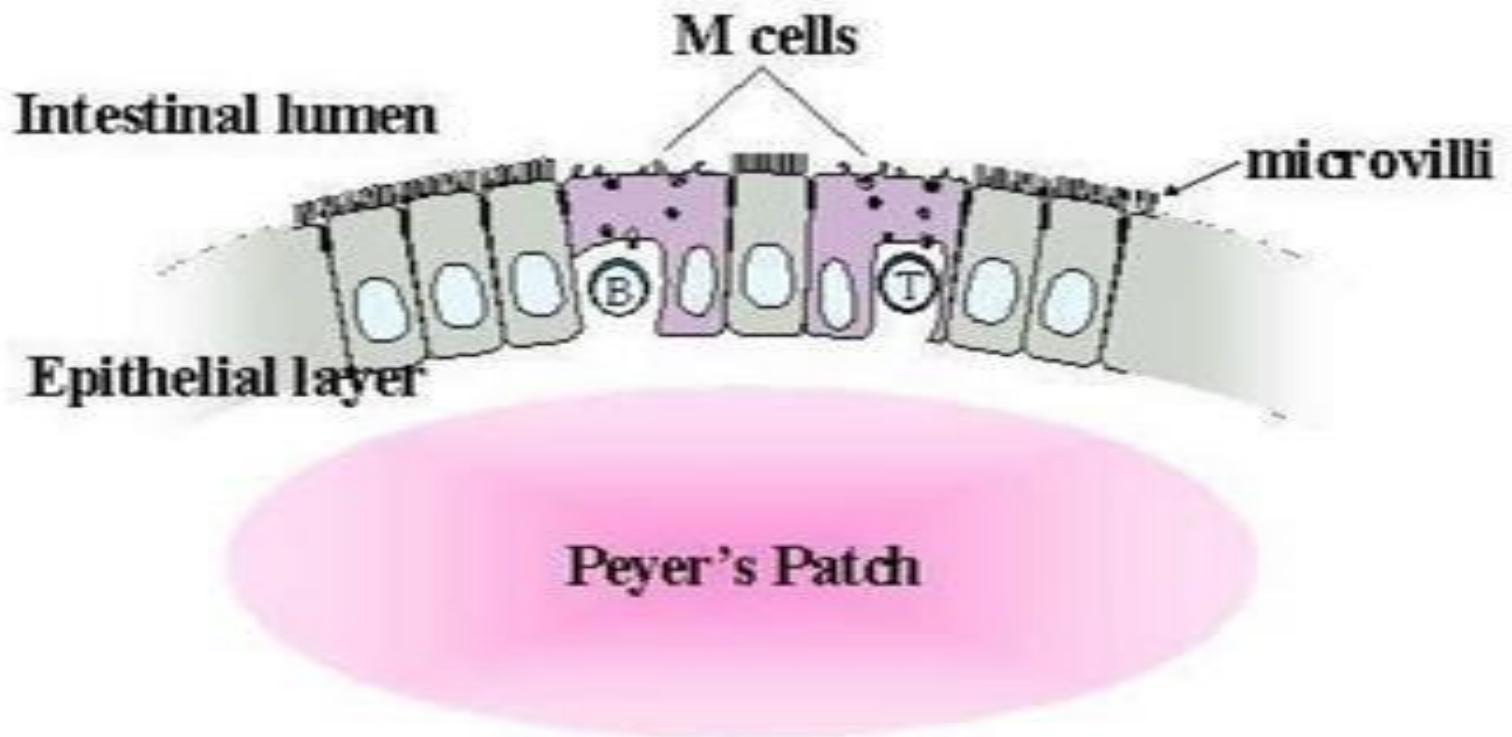
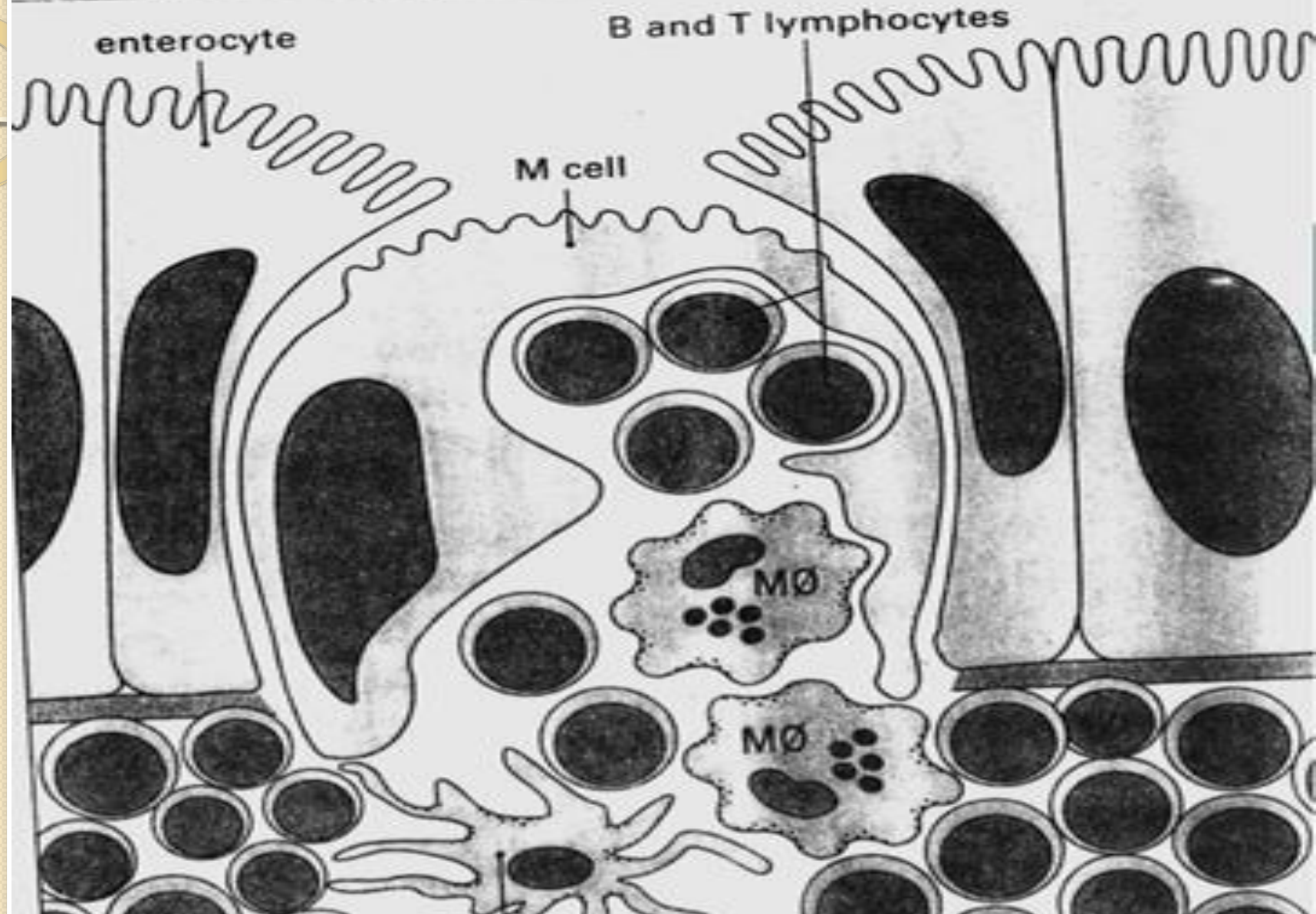
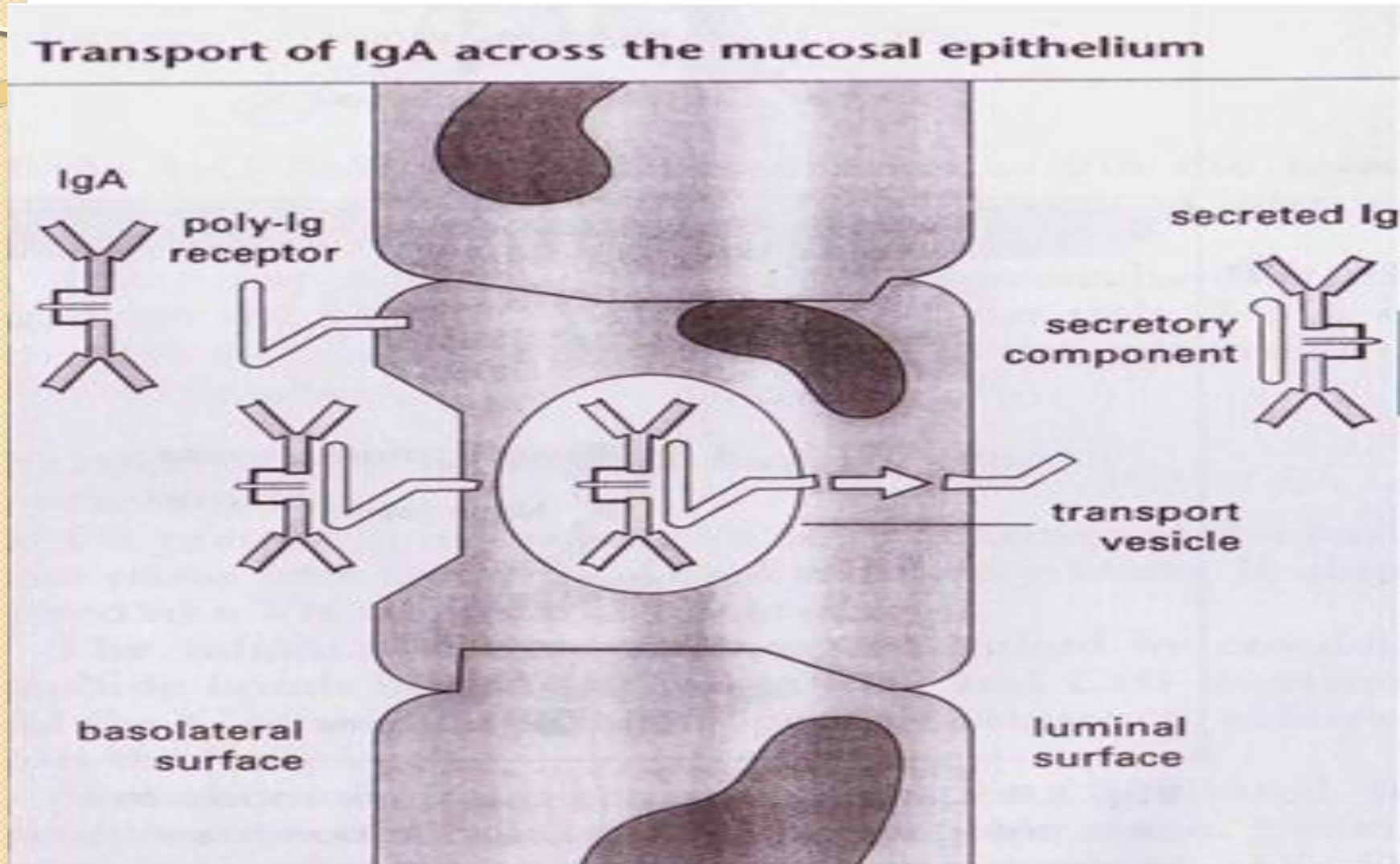


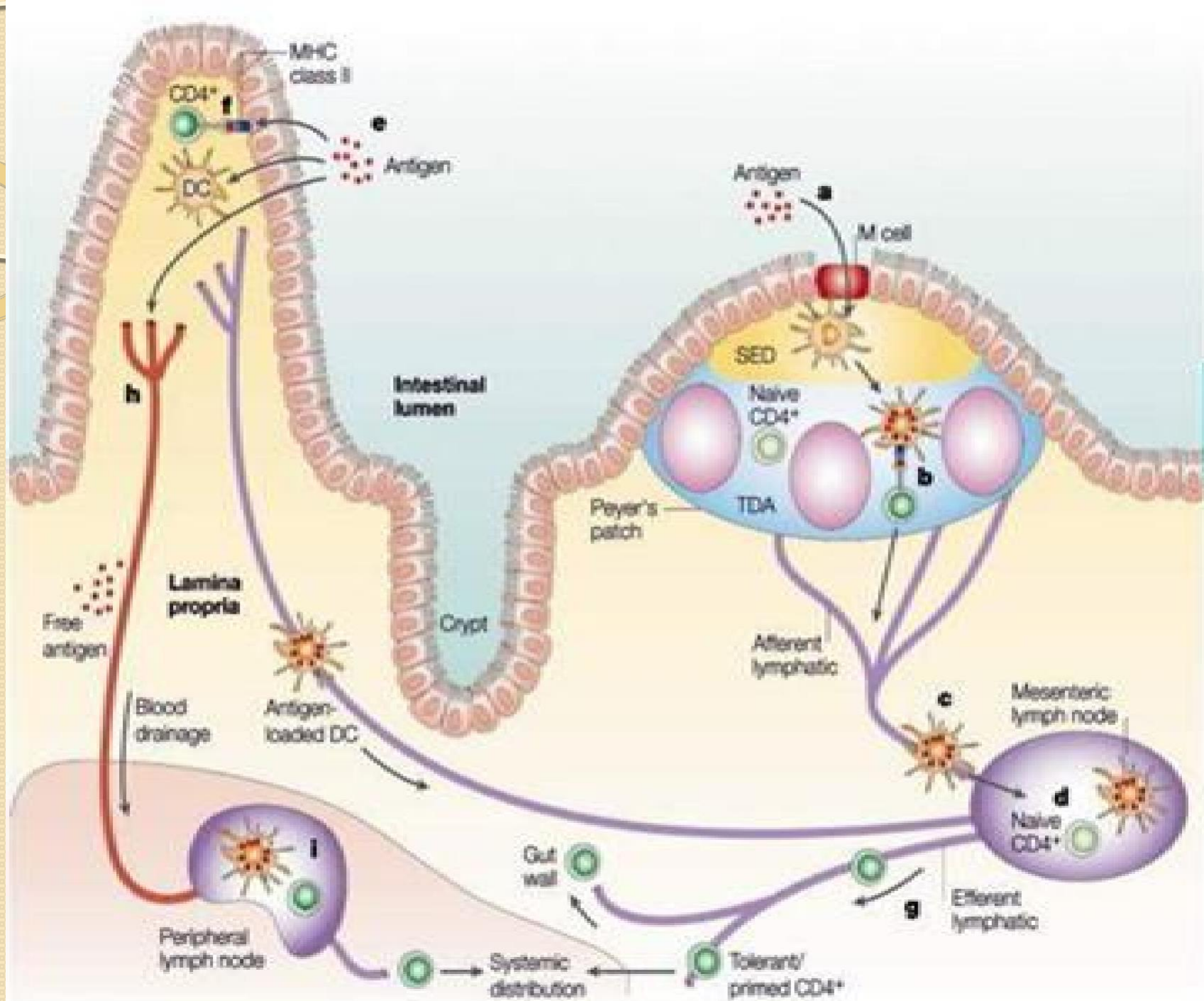
Figure. Follicle-associated epithelia (FAE) and M cells

Location of M cells

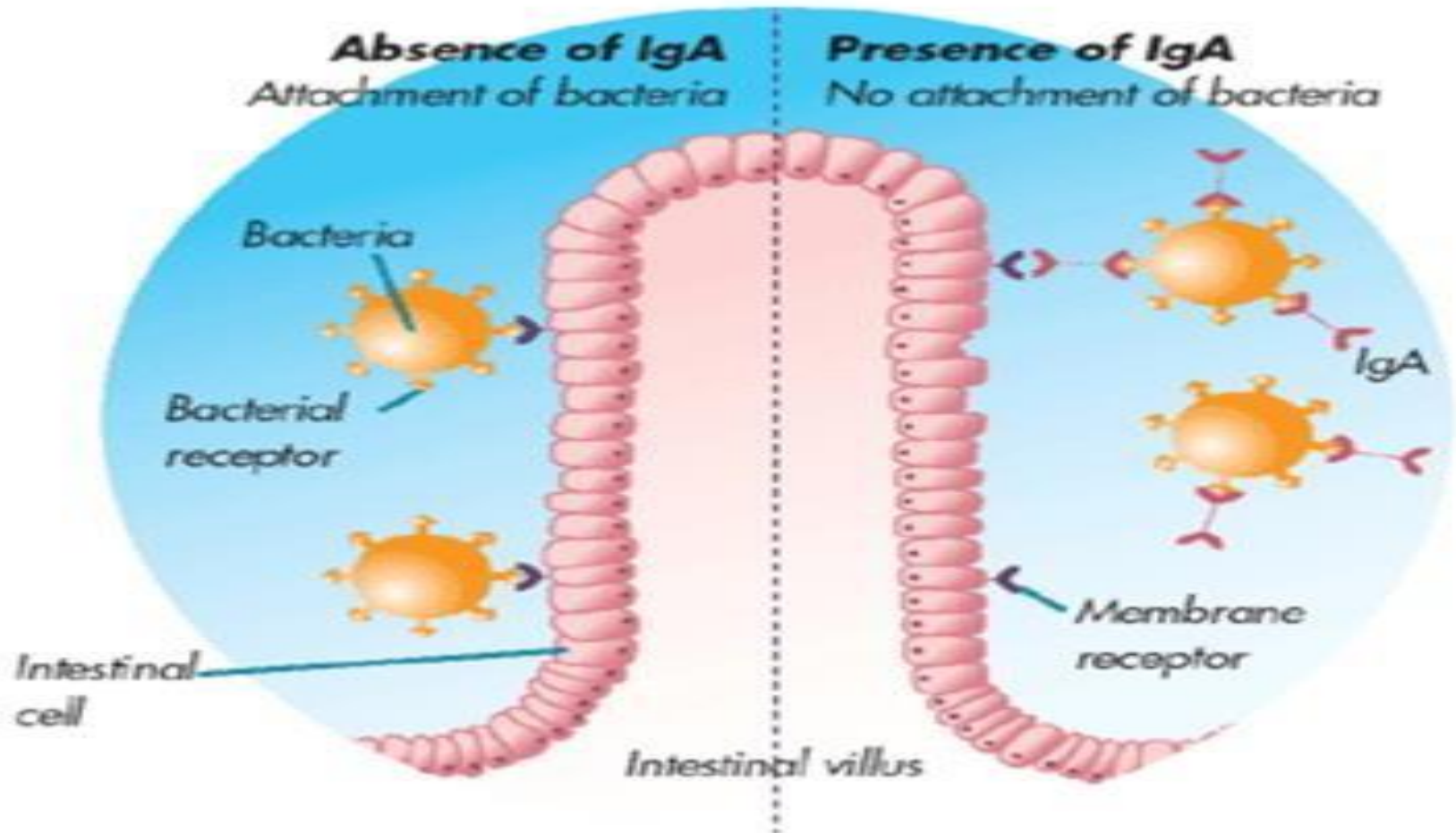


Transport of IgA across the mucosal epithelium





Role of IgA mucosal component



immunity of oral cavity

oral cavity : the oral cavity immunity of controlled primary by environment is saliva mucosal surface saliva contain several non-specific host factors (mucins, lactoferrin, lysozyme, peroxidase, Histatins, cystatins, salivary glycoprotein)

predominant is S. IgA established that it is well considered to saliva it is whole immunoglobulin mechanism in defense mainly specific be the limiting by surface oral

& tooth adherence to epithelial microbial host factors surface by neutralizing toxin & oral cavity immunity. another part of

Protective barrier of oral mucosa

There are several factors which may prevent penetration intact of oral mucosa by microorganisms:

1-saliva

2-keratin

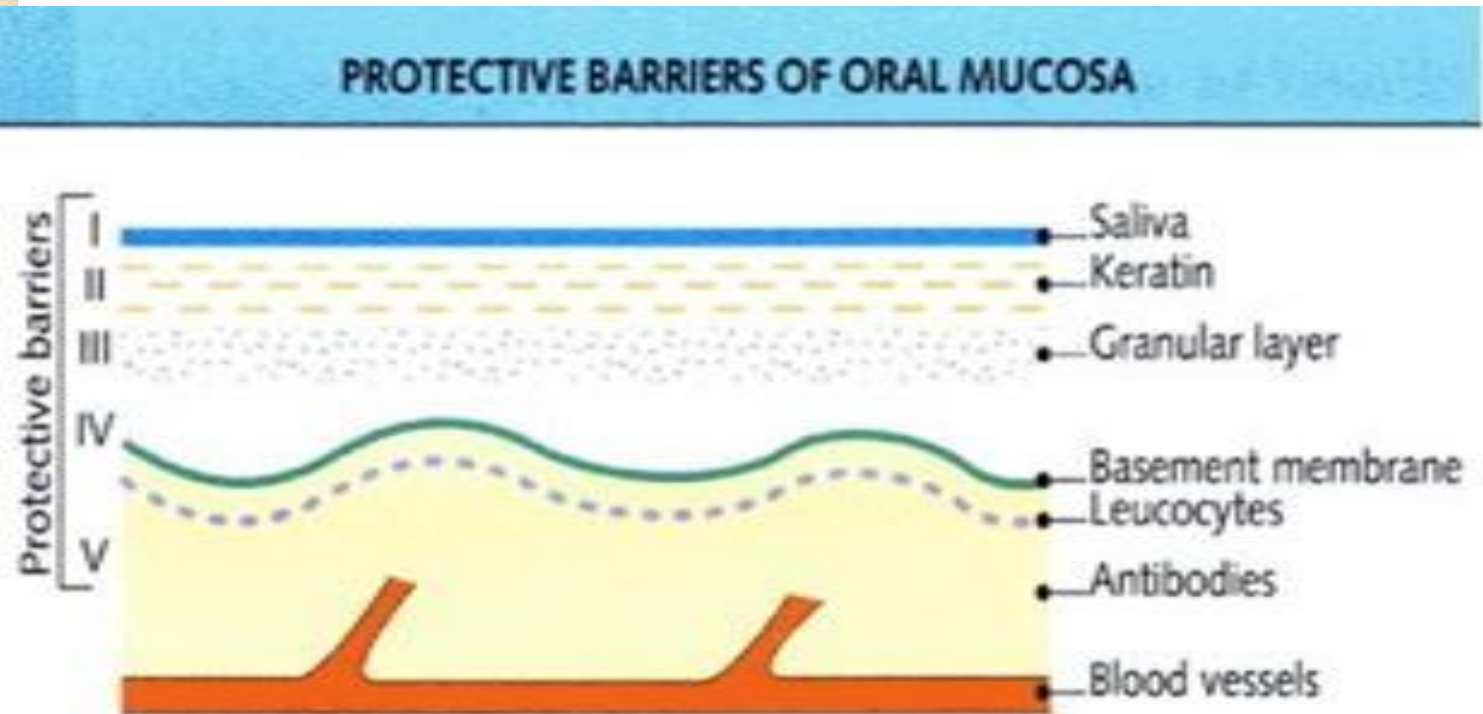
3-granular-layer

4-basment membrane

5-leucocytes

6-antibody

Protective barriers of oral mucosa





Saliva:

it is very important component of oral defenses both by mechanical washing & factors it contains:

1-mechanical cleaning

2-lysozymes

3-peroxidase

4-lactoferrine

5-leucocytes

6-secretory IgA

ANTIMICROBIAL ACTIONS OF SALIVA

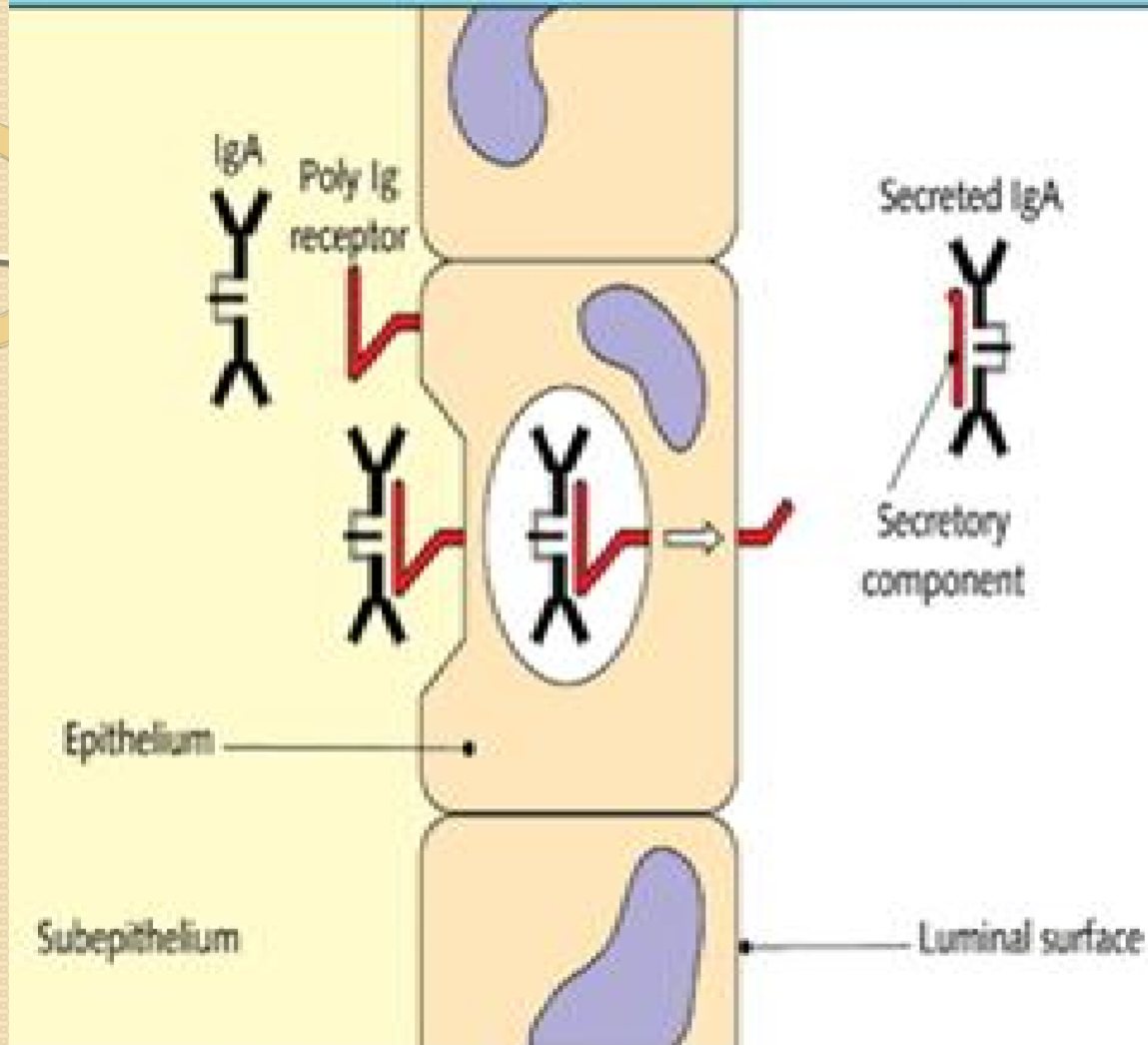
Mechanical cleansing	Muscular movements, in conjunction with saliva, maintain hygiene in accessible areas of mouth Swallowed microbes are inactivated in the stomach
Lysozyme	Bactericidal, by splitting the bond between <i>N</i> -acetyl glucosamine and <i>N</i> -acetyl muramic acid in the cell wall
Peroxidase	Heat-labile, anti-bacterial enzyme
Lactoferrin	Heat-stable protein, bacteriostatic to many micro-organisms
Leucocytes	Saliva contains many leucocytes (99% polymorphs) Migrate from blood via the gingival crevice
Secretory IgA	IgA is the predominant immunoglobulin in saliva Produced by plasma cells within salivary glands Mainly in dimeric form, complexed with secretory component Functionally, secretory IgA prevents microbial adherence to host surfaces

Role of secretory IgA in oral

Cavity

- is by far the most important immunoglobulin in Saliva IgA is secreted by gland plasma cells two molecules salivary combined by a J chain which are also secreted by local plasma cells, the result the resultant complexes by secretory diametric IgA is than component synthesized by epithelial cell of IgA salivary aching & the complete secretory mouth into the into the duct lumen & thence is more resistant to the so secretory IgA proteolysis degradation than other by immunoglobulin it function probably combining with microorganisms & prevent their adherence to the host surfaces.

SECRETION OF IgA AT MUCOSAL SURFACES

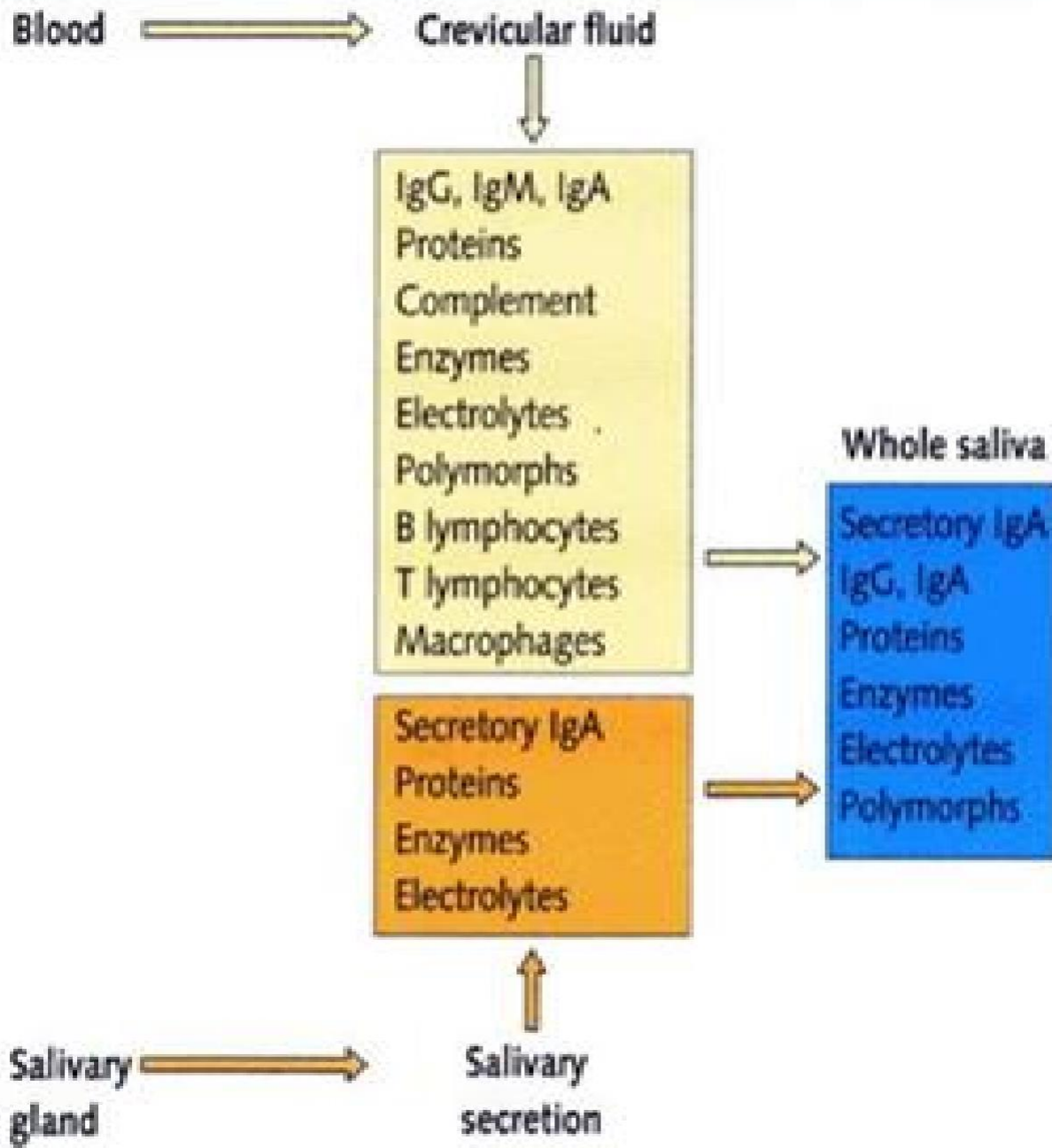



The gingival cervical fluid

- In addition to the immunoglobulins the (GCF) the Gingival Cervical Fluid: including complement system both classical & alternative pathway activated in gingival cervical fluid
- the oral cavityable to reachleucocytes are Blood including the ofthe junctional epitheliumfluid through flow ofvia the with the inflammationgreatlyincreases fluid gingival cervicalar to immunoglobulin additionaccompany periodontal disease in detected , other component suchhave been,complement system as lysozyme , protease and collegians & cervical fluid comprises and Neutrophile with some number of macrophage B- and T-Cell these cells migrate continuously from Blood epithelium into the gingival cervic over through the junctional canandare functionalNeutrophilesasof macrophage80% phagocytosis microorganism .

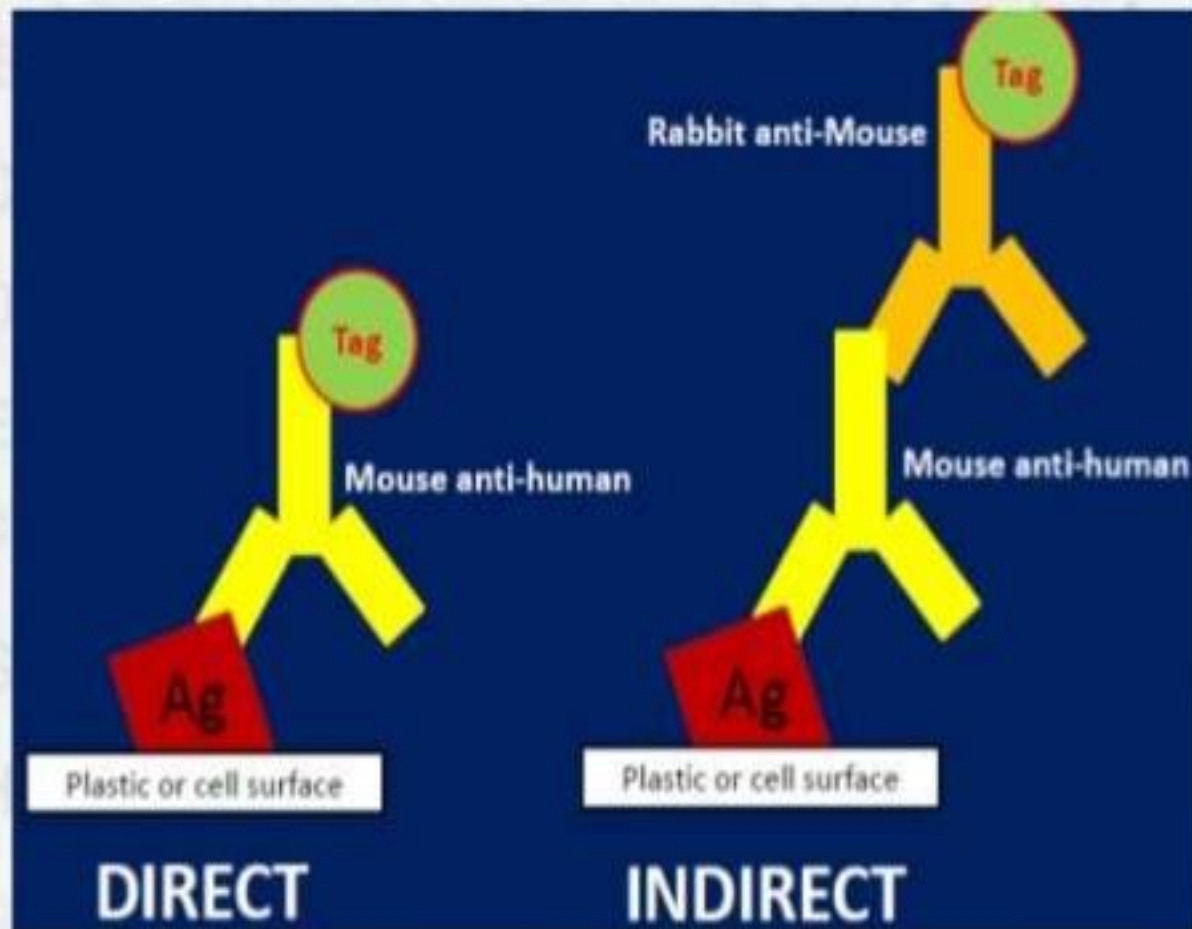
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COMPONENTS OF ORAL IMMUNITY



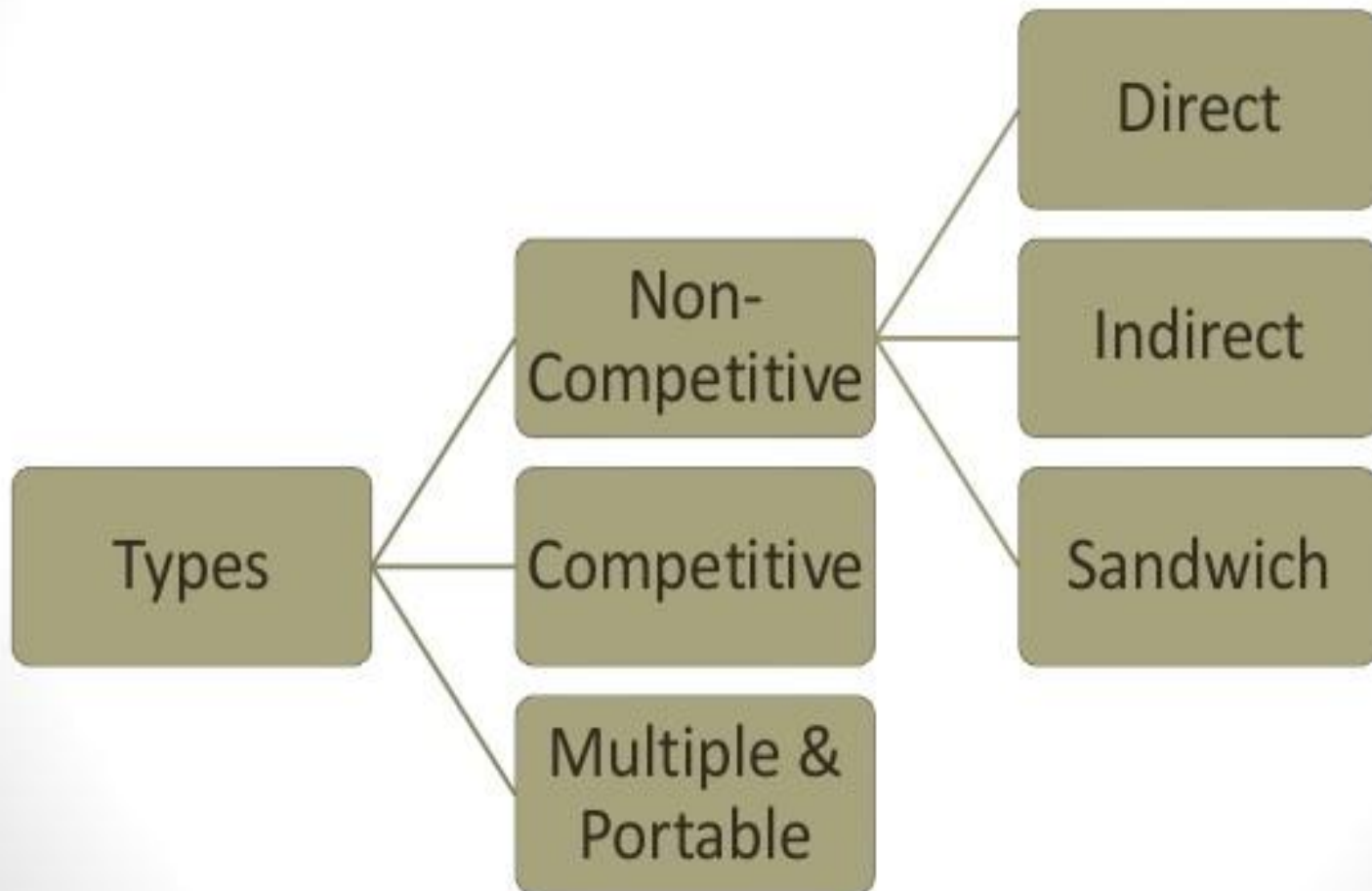
- 
- is that the tooth surface is clear therefore influence by both local (mucosa) salivary by immune mechanisms mediated largely IgA & by systemic immunity involving secretory all the varied immune components present in blood the way in which these contributing interact to provide immunity within factors the oral cavity

Direct and Indirect ELISA



Types of ELISA:

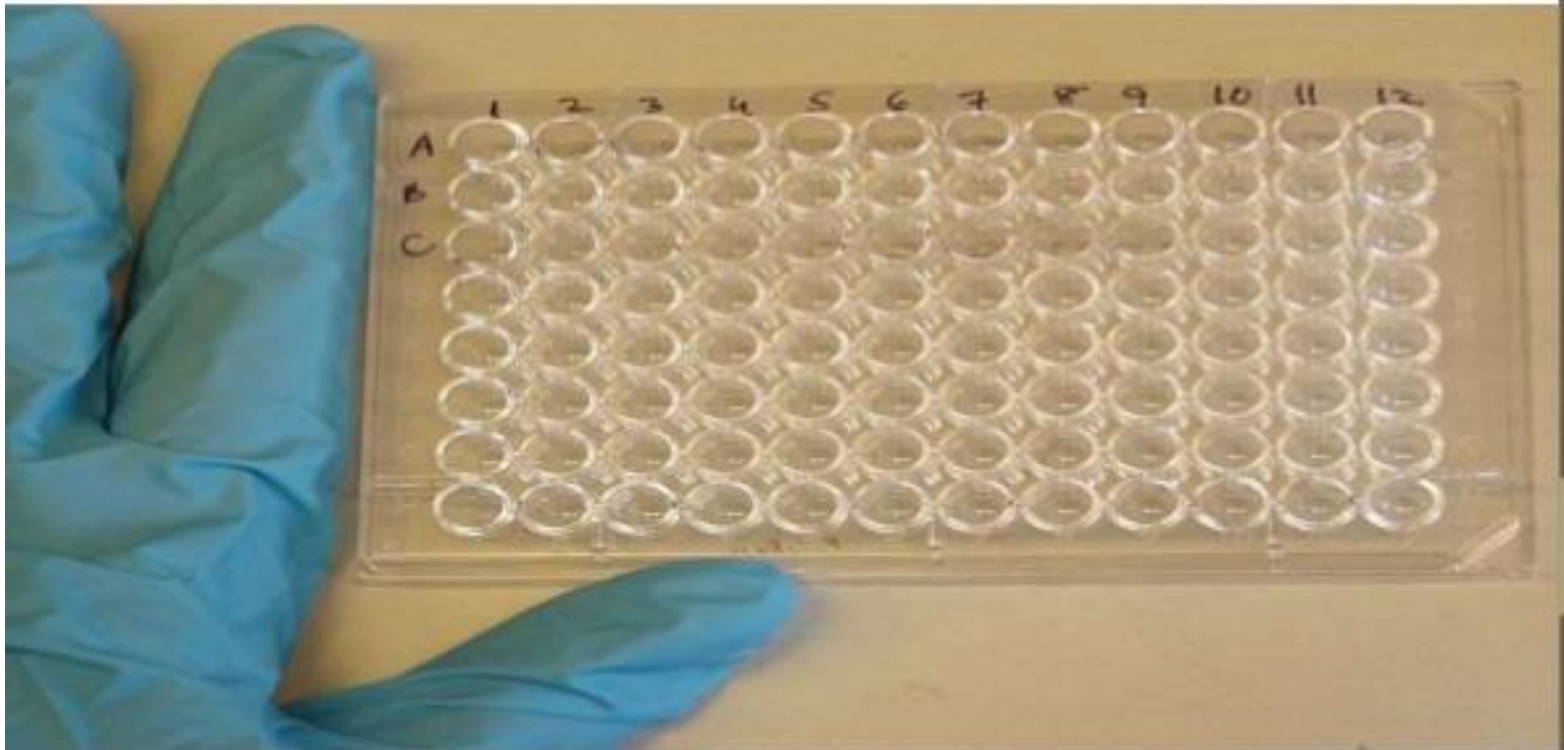
(on the basis of procedure)



Basic Terms:

- **Solid Phase:**

Usually a microtiter plate well, having 8 × 12 well format.





Basic Terms:

- **Adsorption:**

The process of adding an antigen/antibody, diluted in buffer, so it attaches to the solid phase on incubation.

- **Washing:**

The simple flooding & emptying of wells with a buffered solution to separate bound from un-bound reagents in ELISA.

Basic Terms:

- **Chromogen:**

A chemical alters color as a result of an enzyme interaction with substrate (color reaction used as signal) e.g Trimethyl benzidine (TMB).

- **Stopping:**

The process of stopping the action of an enzyme on a substrate.

- **Reading:**

Spectrophotometric measurement of color developed in ELISA.



Principle of ELISA:

- ❖ **Based on Basic Immunology Response**

- ❖ Lock and Key Concept:

1) Antigen (key) 2) Antibody (lock):

–Key fits into the lock

- ❖ Enzyme conjugate substrates

- Bound to a secondary antibody that binds with the antibody-antigen complex.

Equipments:

1) Microwell Plate:

Flat bottom
polystyrene
plate,
contains 8 x 12
wells holding
350 μL each.



Equipments:

2) Multipipette :

An 8-channel 100 μ L pipette is a good help for even small-scale work.



Equipments:

3) Washing Device:

- manually operated washing devices.
- may be of use particularly when there is a risk that the samples tested in ELISA contain infectious material, so must be collected for subsequent disinfection.



Equipments:

4) Microplate washer:

- These are very efficient with unusually low carry-over contamination.

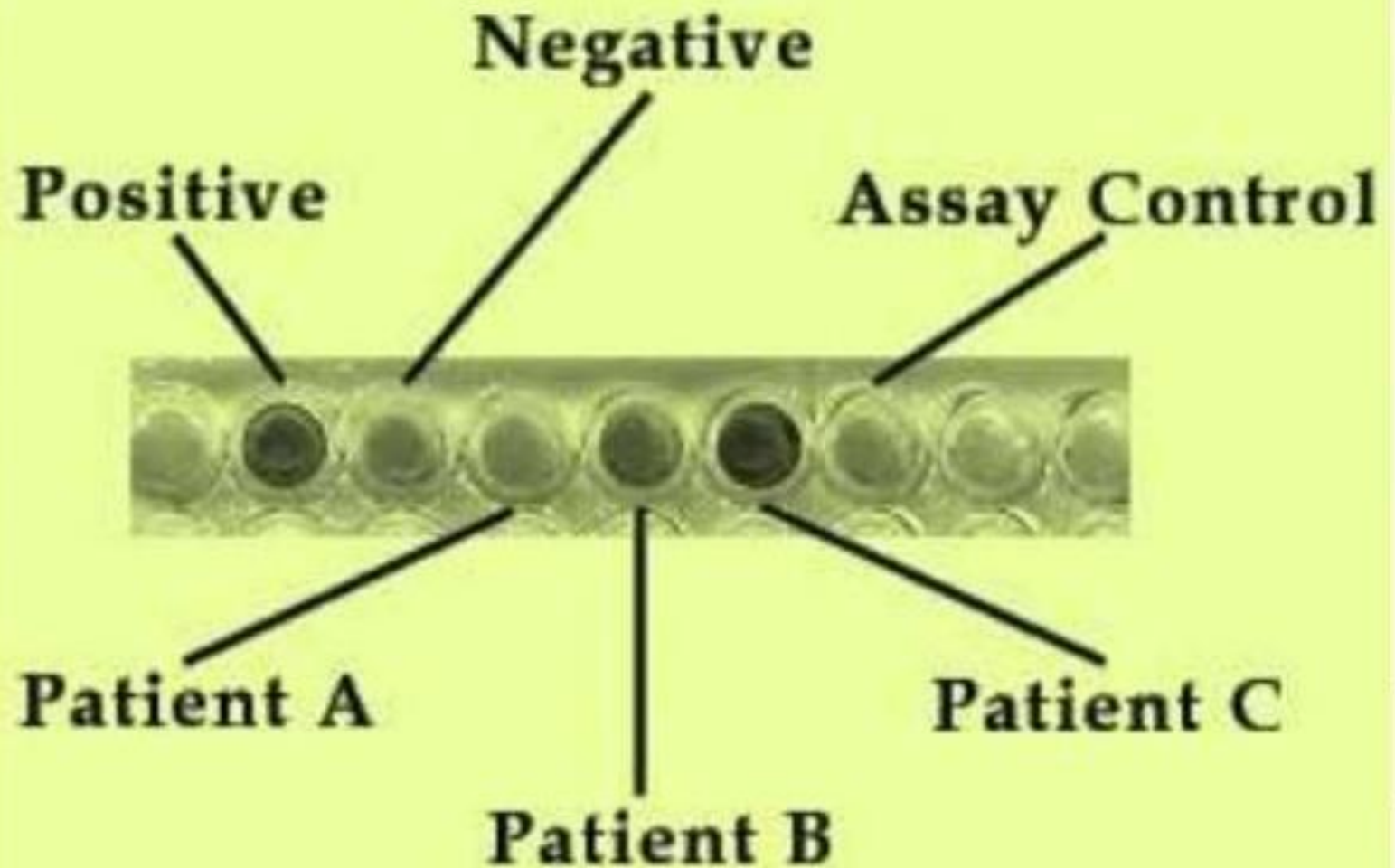




Reagents Used:

Reagent	Composition
Coating Buffer	0.01 M Phosphate Buffer + 0.15 M NaCl (PBS)
Diluting/Washing Buffer	0.01 M Phosphate Buffer + 0.50 M NaCl + 0.1% Tween 20
Blocking Buffer	Bovine Serum Albumin (BSA)
Enzyme	Horse-radish peroxidase (HRPO)
Chromogenic Substrate	Trimethyl benzidine (TMB)
Stop Solution	0.5 M H ₂ SO ₄

Results:

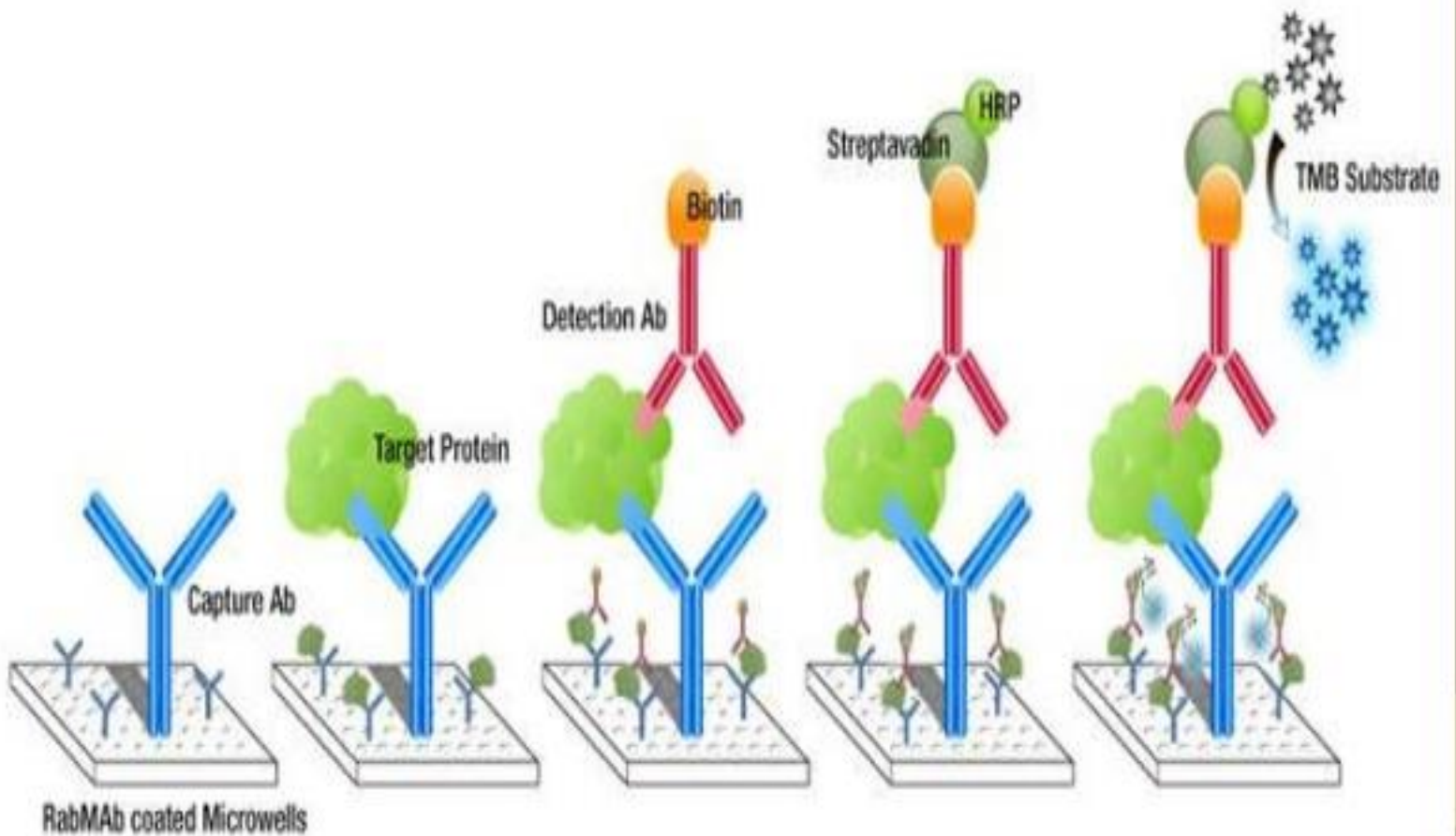


Reading:

- Measure the absorbance at 450nm with the help of ELISA reader.
- Calculate the absorbance for each sample and reference.
- Ascent software for the calculation of results can be used.



Modified ELISA:





THANK YOU