

Immuno-diagnosis

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Design of a technique

Physical and chemical properties of a molecule.

- Size and shape.
- Ionic charge.
- Hydrophobic and hydrophilic properties.
- Interaction with an adsorbents.
- Behavior in an electrical field / magnetic field.
- Specific interactions.

Specific reactions

- Enzyme - Substrate / Substrate analogues / Cofactors
- Antigen - Antibody
- Hormone - Receptor
- DNA - Complementary strands
- DNA - mRNA
- Lectins - Carbohydrates

Based on Specific reactions

- Immuno assay: RIA, ELISA, Western blot.
- Replication: Southern blot
- transcription: Northern blot
- Complementation: PCR.

Immunotechniques

- RIA - ELISA
- Direct, Sandwich and competitive ELISA
- Precipitation reaction - Immunodiffusion

- Western blot

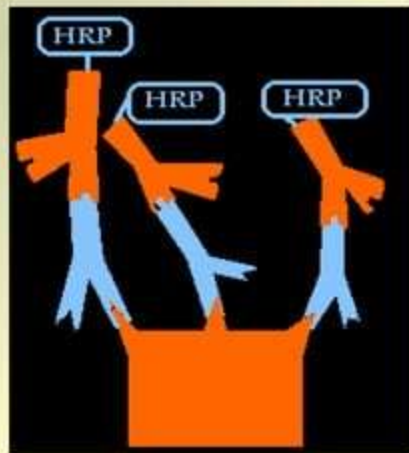
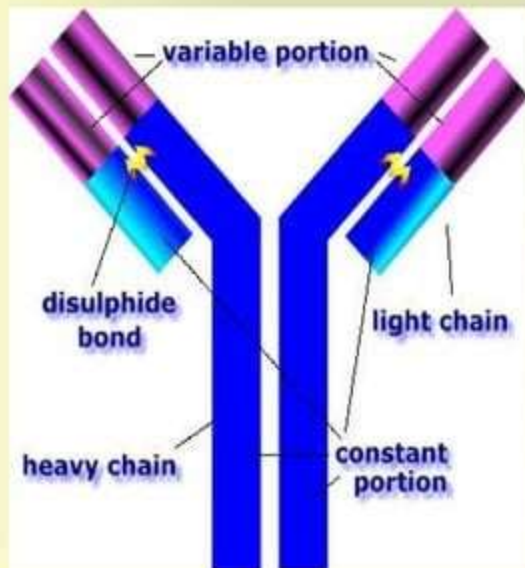
Immunodiagnosis

- ELISA - HIV
- ELISA - HbsAg
- Westernblot for HIV
- TORCH - IgM & IgG
- Competitive ELISA for Hormone analysis
- Agglutination test
- Immunodiffusion

Structure of an antibody

Primary antibody

Secondary antibody



Direct ELISA for HIV

Antigen coating

Blocking

Primary Ab

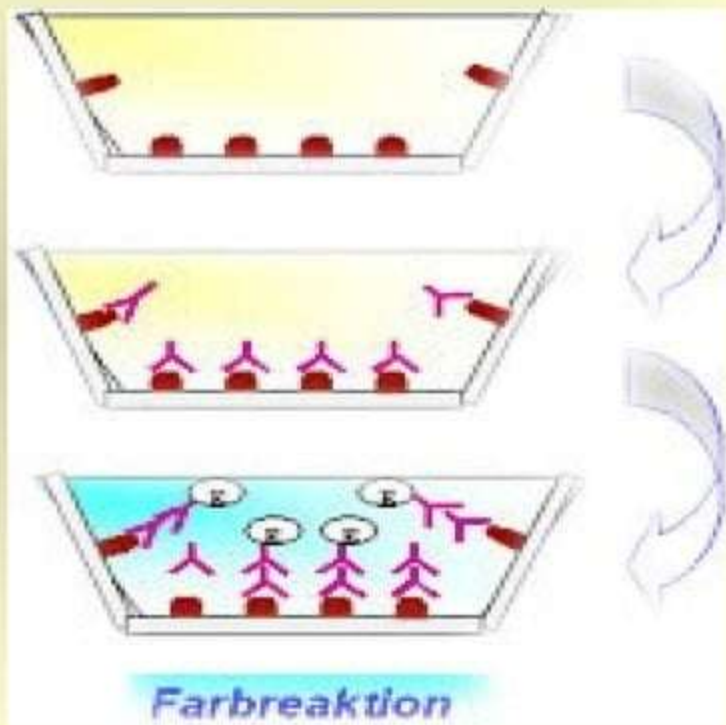
Washing

Secondary Ab

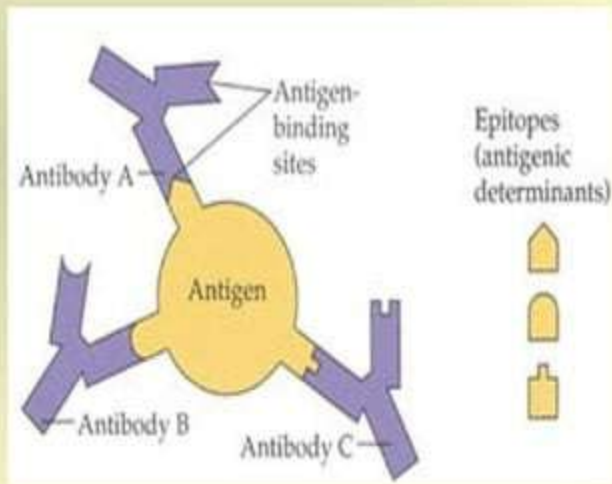
Washing

Substrate addition

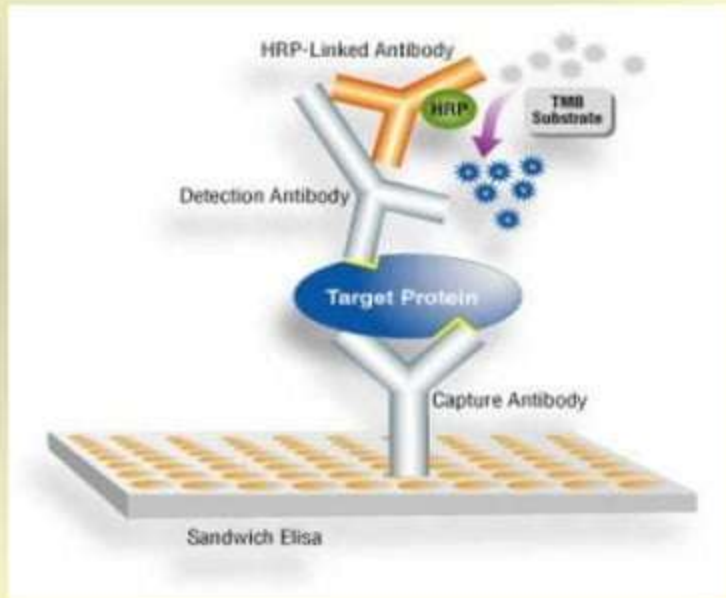
Colour development



Monoclonal antibody binding with antigen



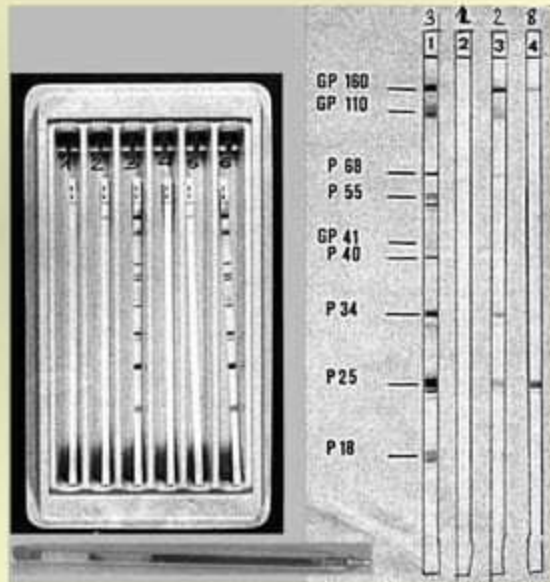
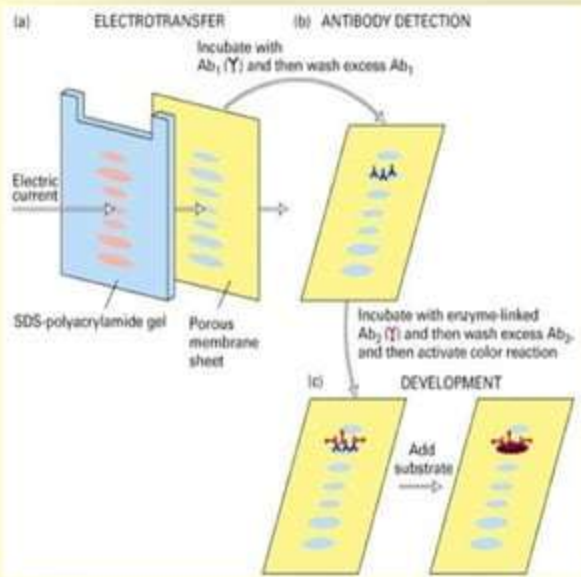
Sandwich ELISA for HBsAg



Western blot

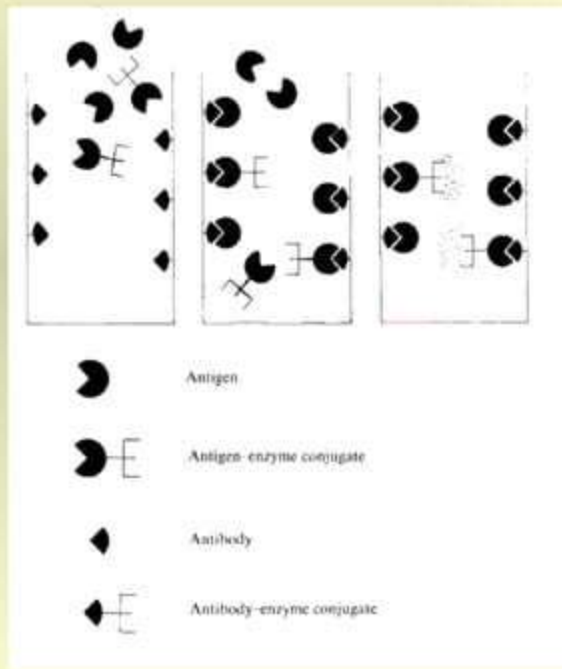
- ELISA is similar to Western blot but is more informative.
- HIV detection by ELISA and Western blot
- HIV - I and HIV - II

Western blot

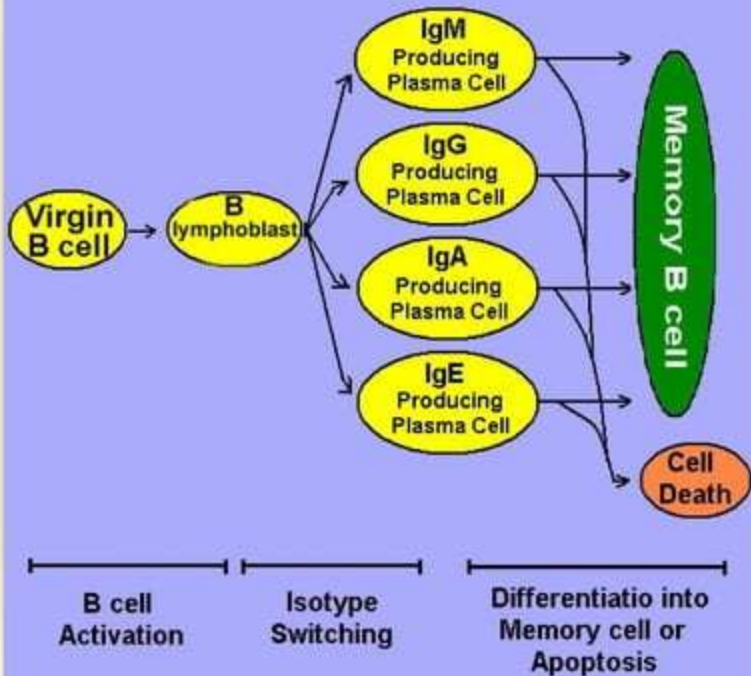


Competitive ELISA for T3

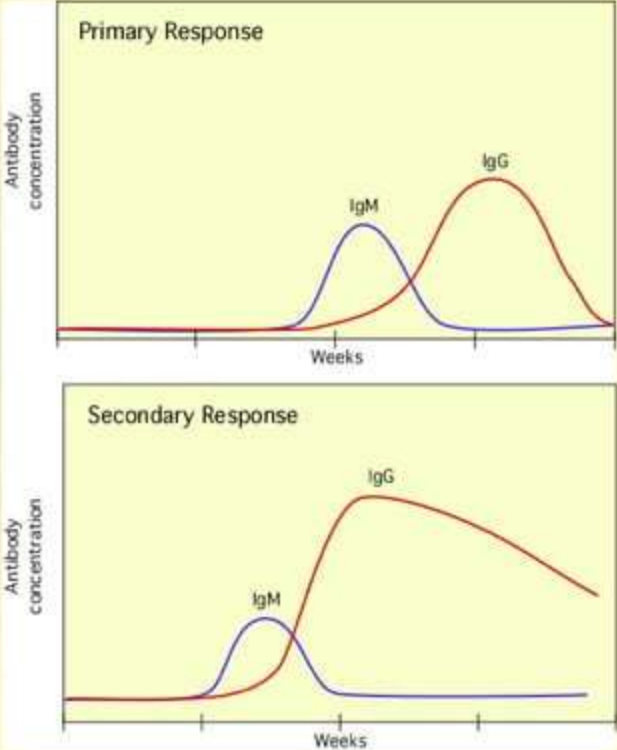
Ab-AgEC	Ag	OD
10	0	1.00
10	2	0.80
10	4	0.60
10	6	0.40
10	10	0.10



B cell Differentiation Upon Activation



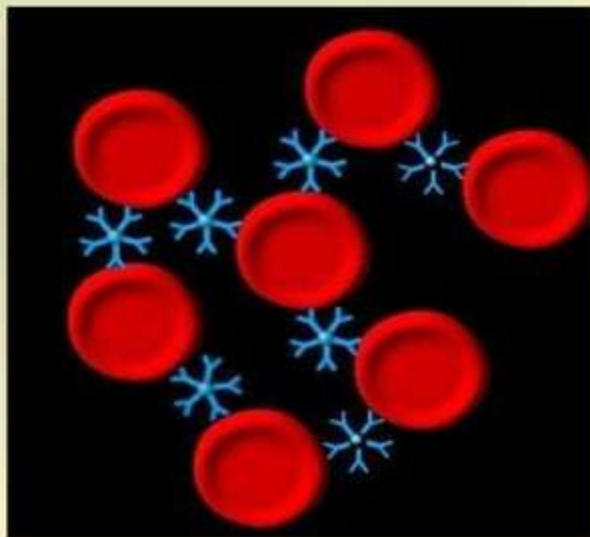
Antibody response to infections










TORCH - panel (IgM & IgG)

- Toxoplasma
 - Rubella
 - Cytomegalo virus
 - Herpes
-
- IgM - Acute or Recent infection
 - IgG - Chronic infection

Agglutination reaction

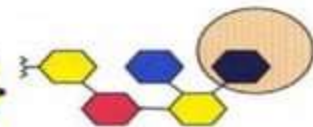
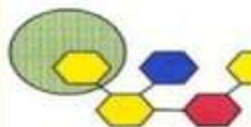
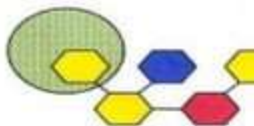


The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 <p>A agglutinogens only</p>	 <p>B agglutinogens only</p>	 <p>A and B agglutinogens</p>	 <p>No agglutinogens</p>
Plasma Antibodies (phenotype)	 <p>b agglutinin only</p>	 <p>a agglutinin only</p>	<p>NONE.</p> <p>No agglutinin</p>	 <p>a and b agglutinin</p>

O

H antigen

A**B****AB**

- | | |
|--|------------------------|
| | Galactose |
| | Fucose |
| | N acetyl-galactosamine |
| | N acetyl-glucosamine |

ABO Blood Reactions

Blood type

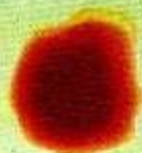
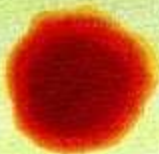
A

B

AB

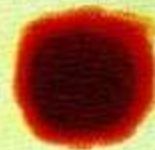
O

Anti-A



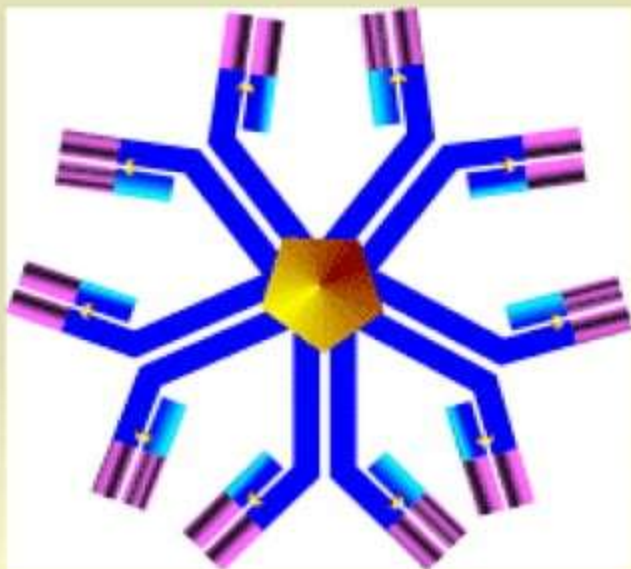
Anti-A

Anti-B

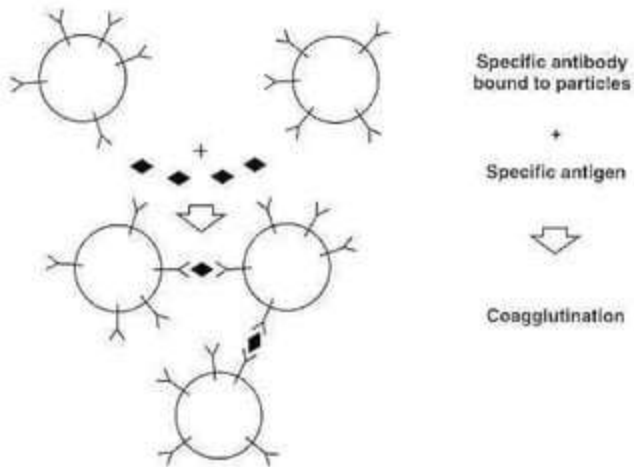


Anti-B

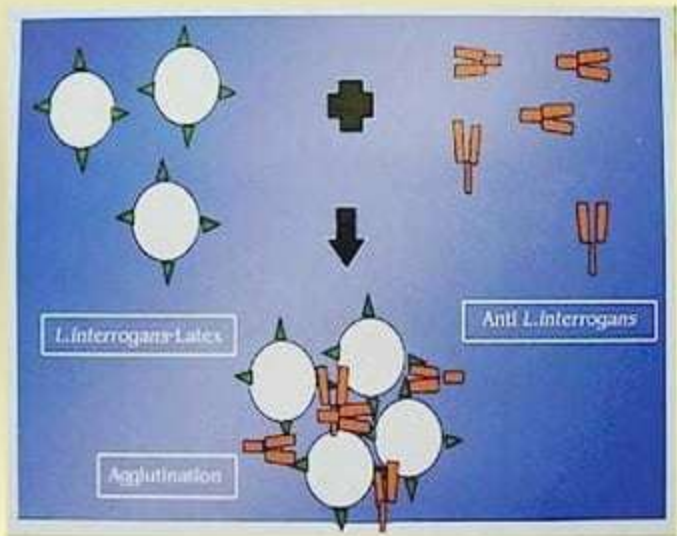
Structure of an IgM antibody



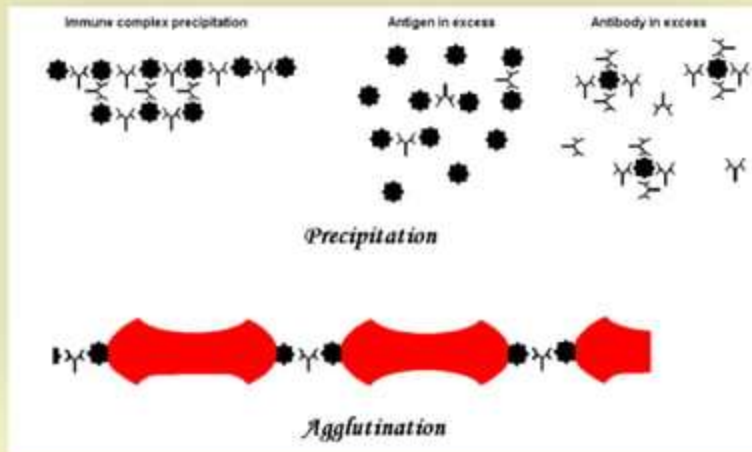
Agglutination with IgG antibody using latex beads



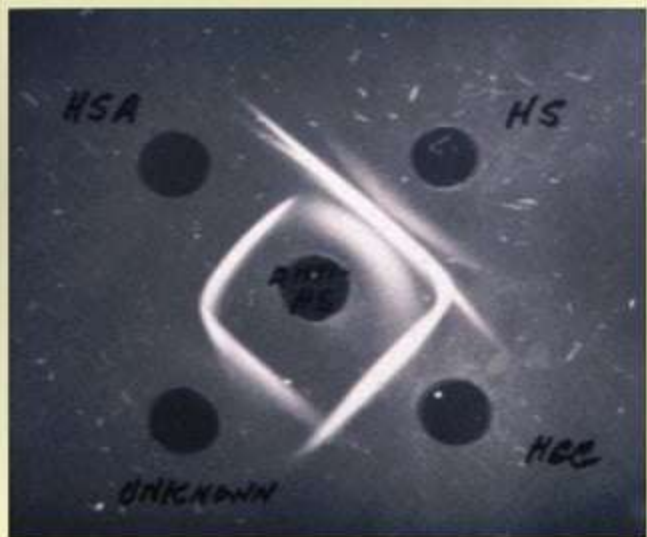
Agglutination with antigen using latex beads



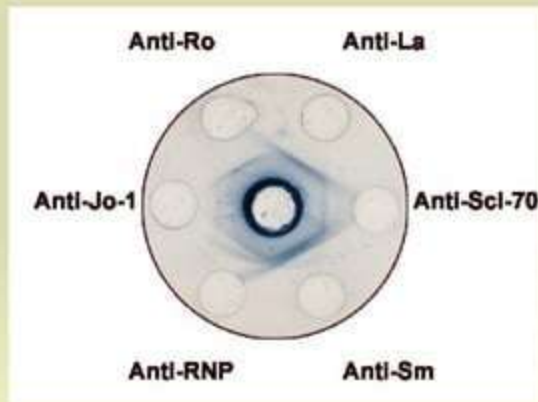
Agglutination reaction: Concentration dependent



Immunodiffusion - precipitin formation

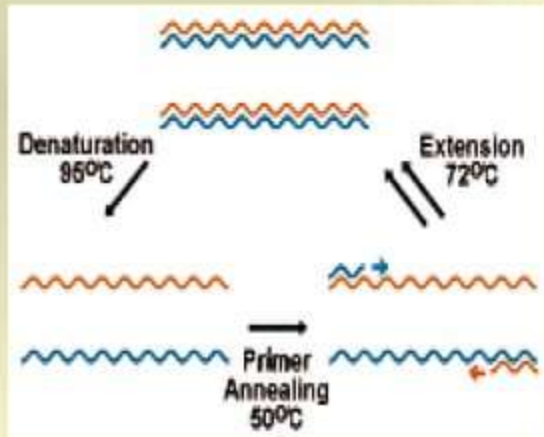


Diagnosis of autoimmune reactions

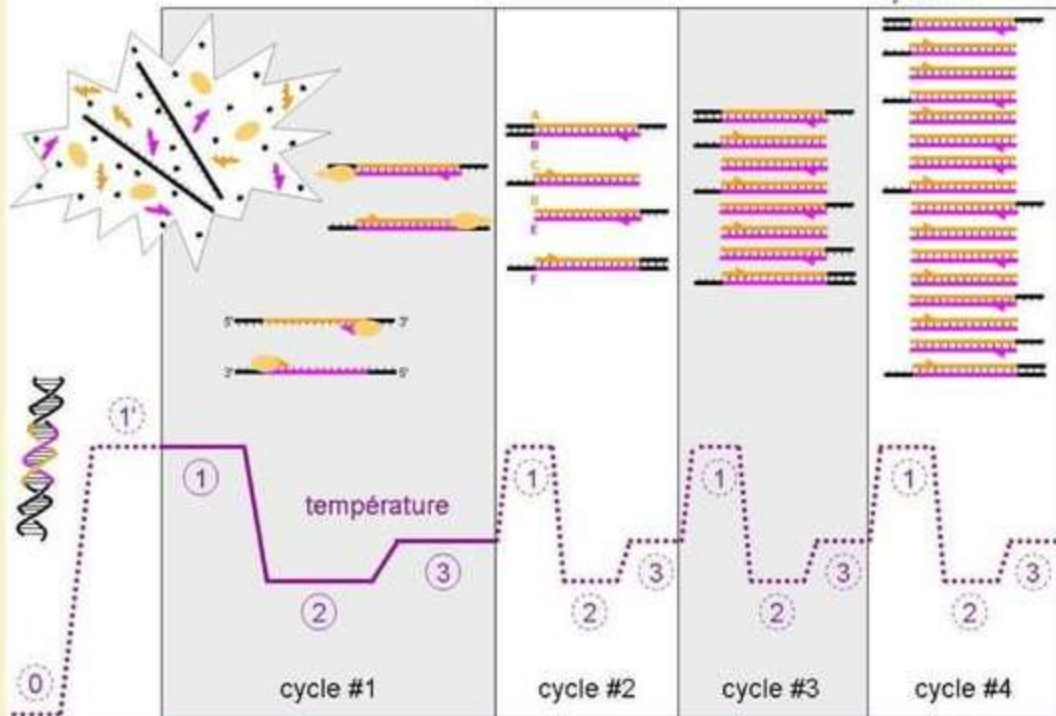


A PCR cycle

In vitro enzyme
amplification of DNA
to million folds



cycle #n.....→



Sequence to amplify



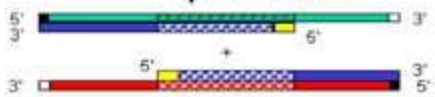
POLYMERASE CHAIN REACTION - PCR



Heat to separate
Cool, add primers



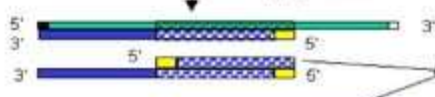
Add Taq polymerase



Heat to separate
Cool, add primers



Add Taq polymerase



From these, get amplifications of the specific target sequence

Repeat cycles

THANK YOU

Acknowledgements

- Images from Goggle search