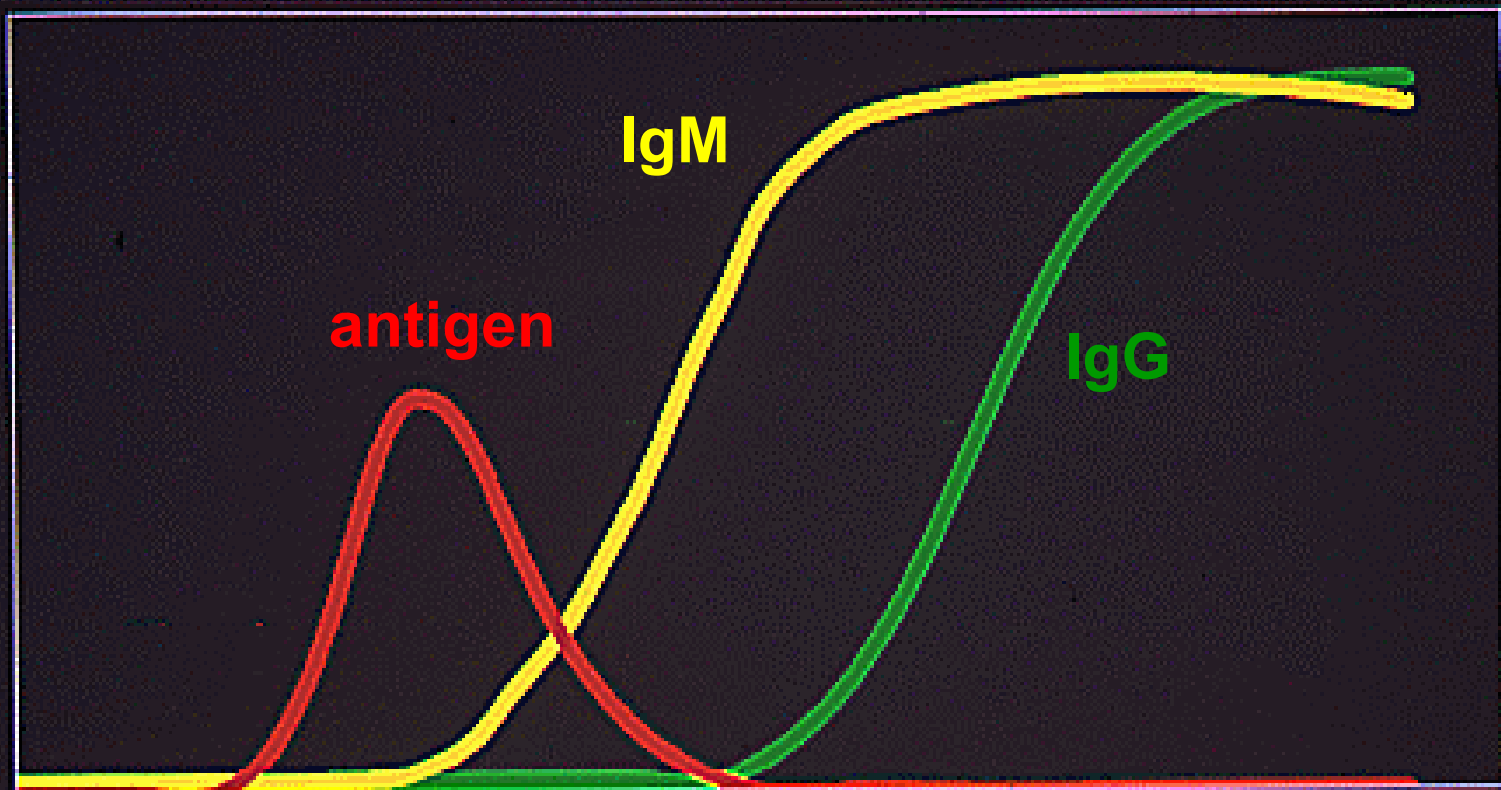


Viral Serology

Viral Serology

- ☺ IgM antibody during acute phase
- ☺ IgG antibody during convalescence
- ☺ IgA mucosal antibody
(respiratory infections, GI)



0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Days

Non-specific
Symptoms

Rash & Arthralgia

Viral Serology

- ☺ Serum sample within 7 days of onset
- ☺ Convalescent sample 10-14 days after the first sample
- ☺ 4-fold rise in IgG
- ☺ Negative to positive IgM

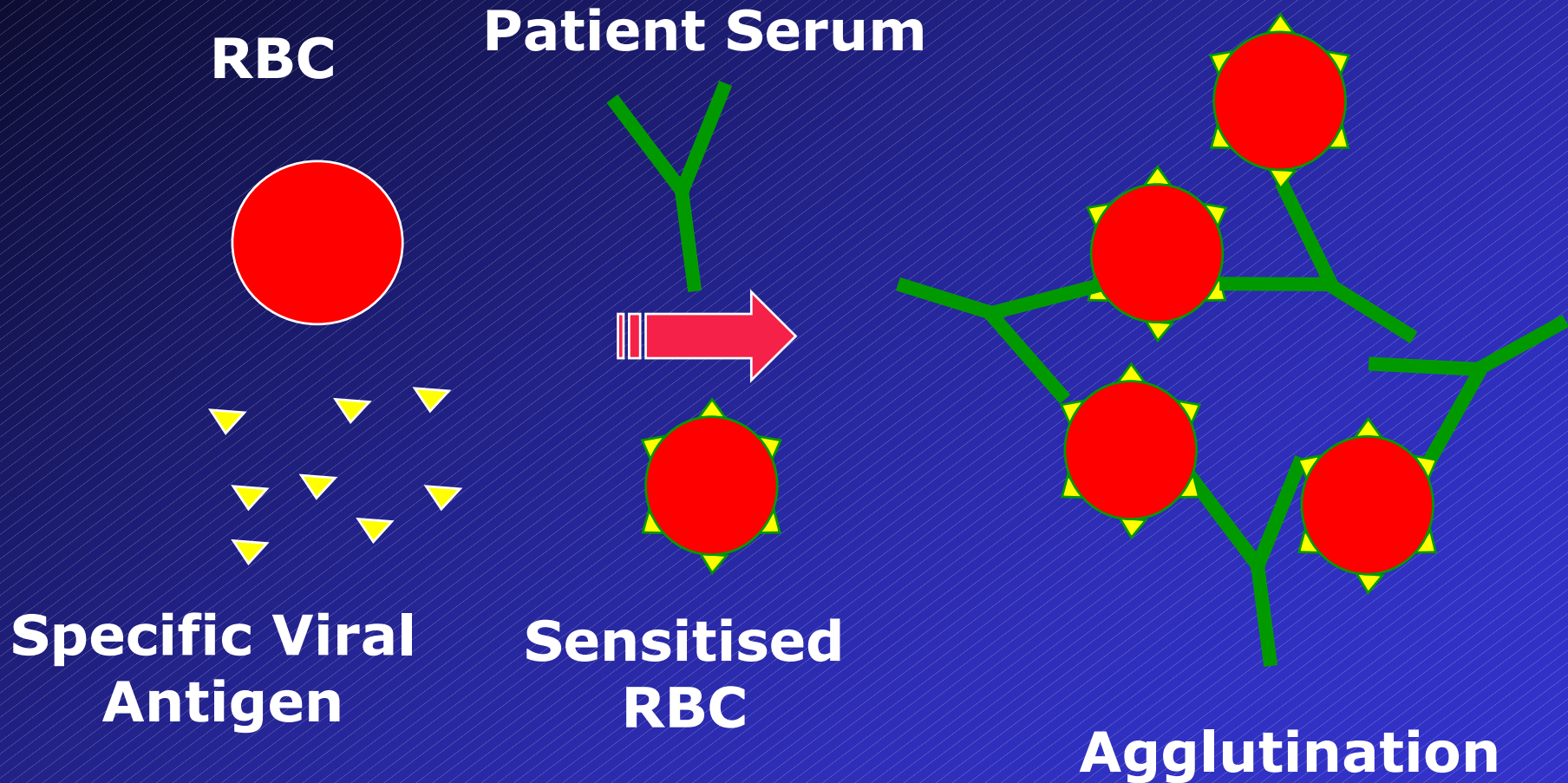
Serology Tests

- ☺ Haemagglutination
- ☺ Haemagglutination Inhibition
- ☺ Complement fixation
- ☺ Indirect IFA
- ☺ Indirect EIA (ELISA)

Haemagglutination

- ☺ Used with specific viral antigens
- ☺ Red cells are first sensitized
- ☺ Reacted with patient serum
- ☺ Agglutination of Ab to antigens produces agglutination
- ☺ Agglutination indicates presence of specific antibody in the patient

Haemagglutination

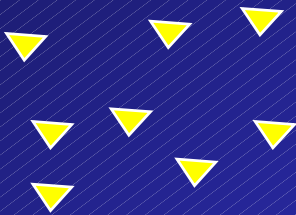
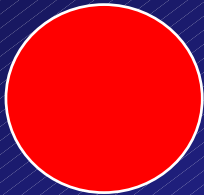


Haemagglutination Inhibition

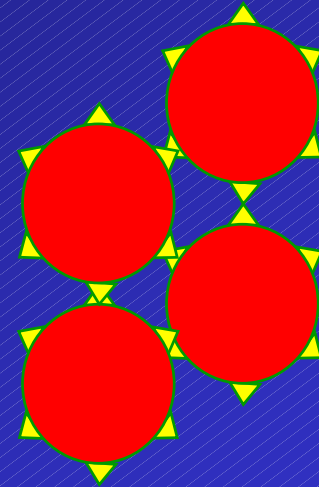
- ☺ Used with viruses that haemagglutinate
- ☺ Incubation of virus with RBC causes haemagglutination

Haemagglutination Inhibition

RBC



Haemagglutinating
Viral
Antigen

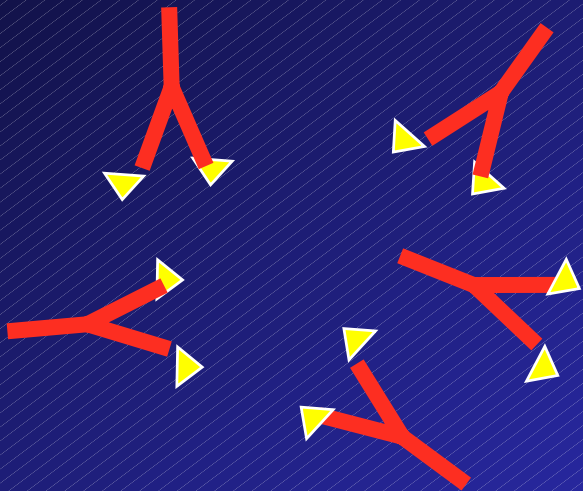


Haemagglutination

Haemagglutination Inhibition

- ☺ Used with viruses that haemagglutinate
- ☺ Incubation of virus with RBC causes haemagglutination
- ☺ Preincubation of patient serum with antigen inhibits ag-RBC agglutination
- ☺ Inhibition of agglutination indicates presence of antibodies to the viral antigen (virus) used.

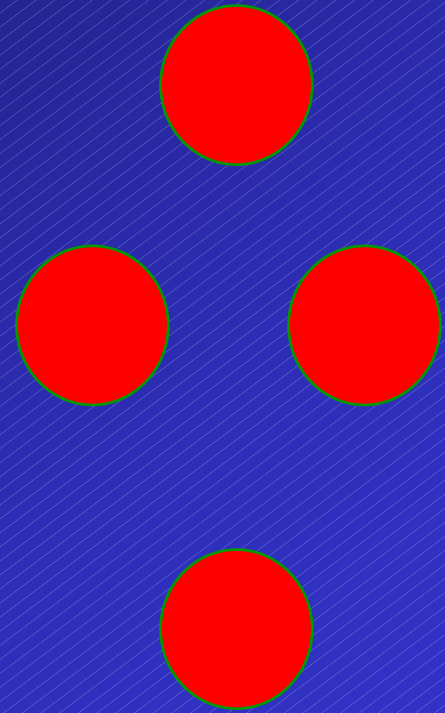
Haemagglutination Inhibition



**Haemagglutinating
Viral Antigen
is immobilised**



RBC

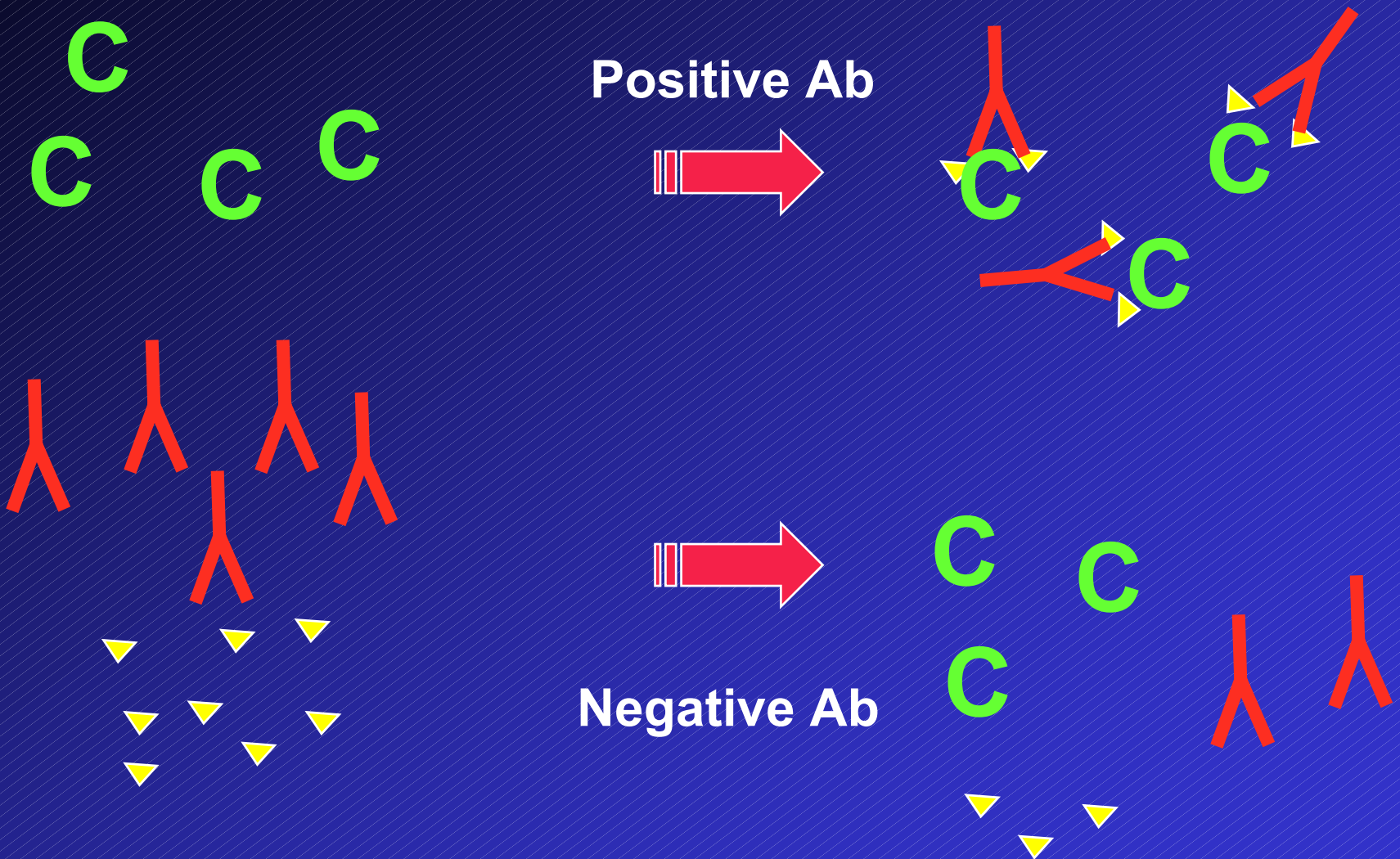


No agglutination

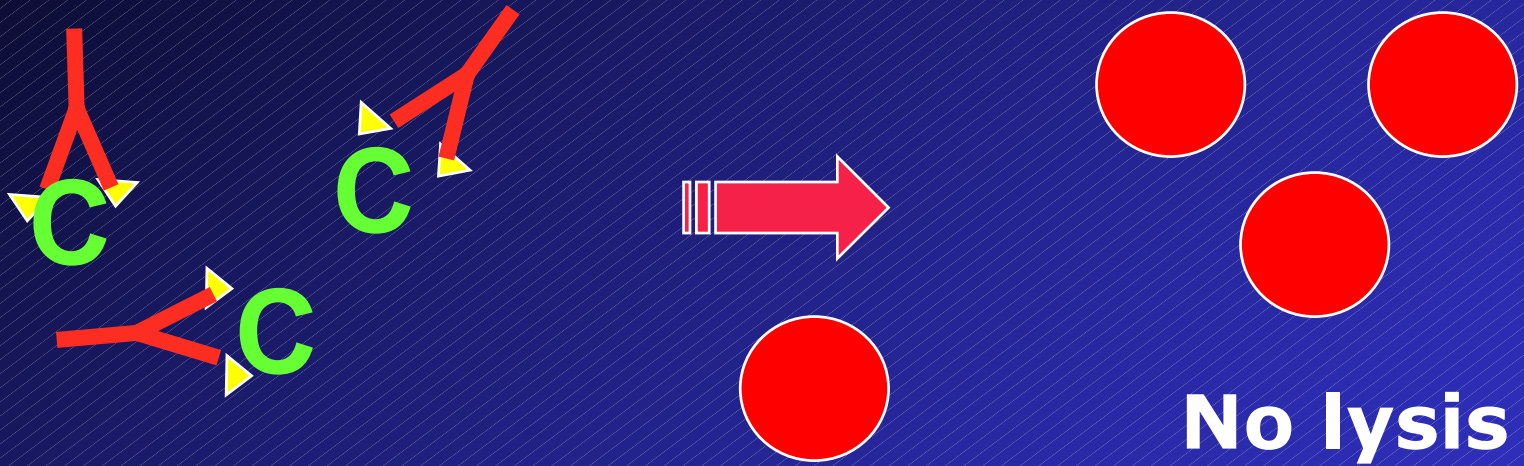
Complement fixation

- ☺ Heat inactivated patient serum mixed with specific virus (antigen) and complement
- ☺ Sheep RBC & Haemolysin added
- ☺ Incubate 37C / 45 minutes
- ☺ Absence of Ab = lysis

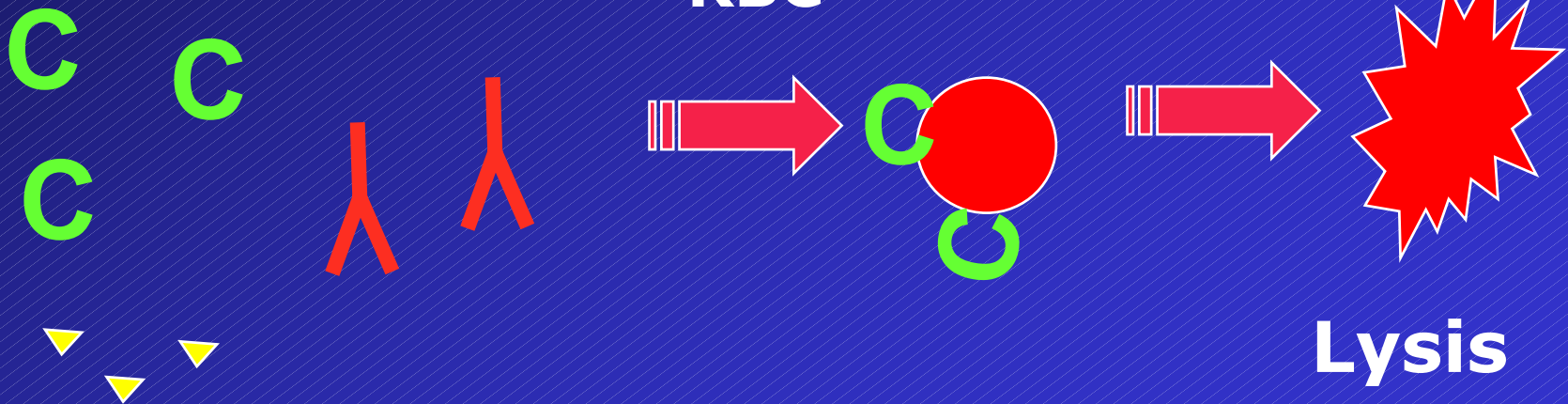
Complement Fixation



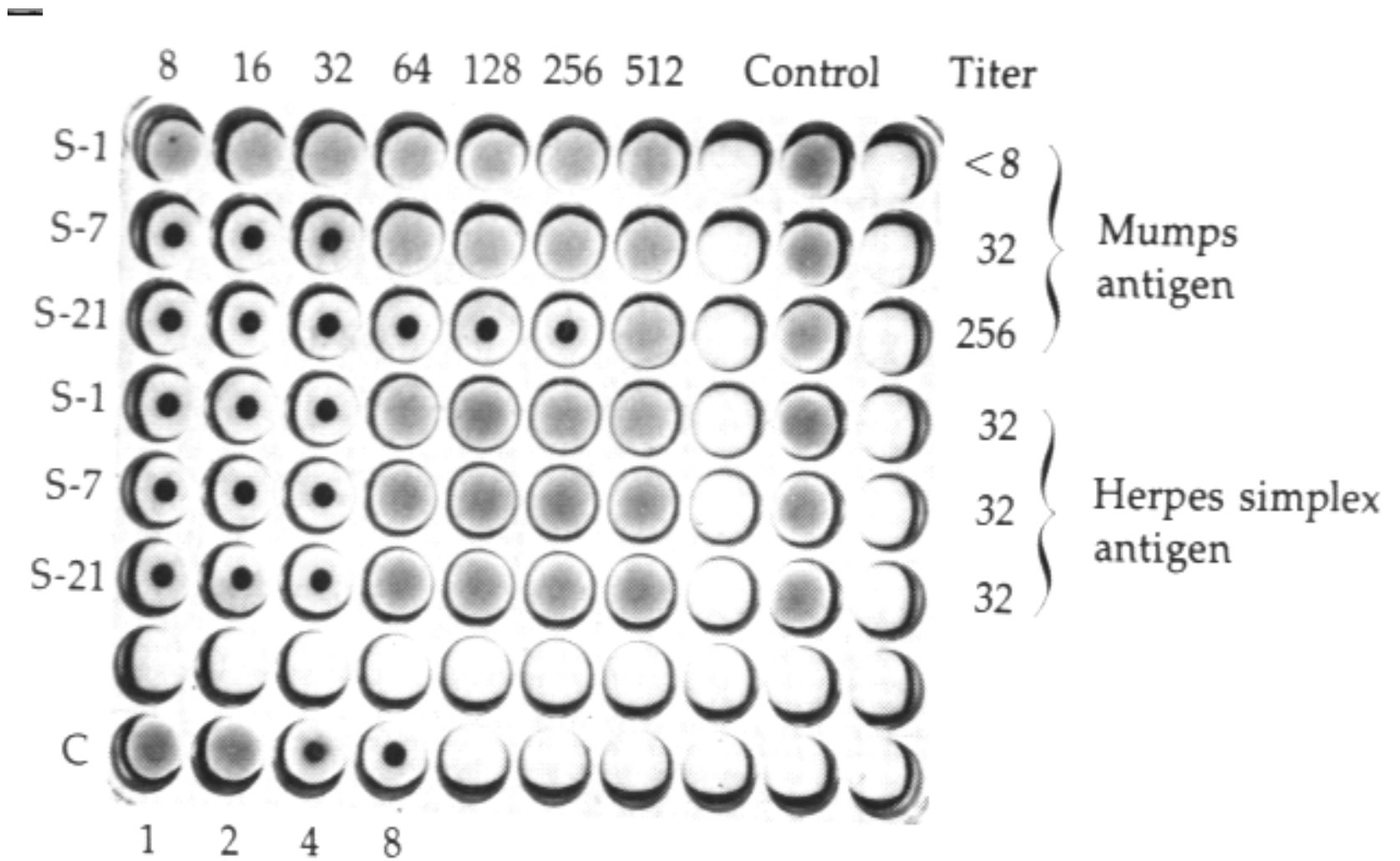
Complement Fixation



Activated
RBC



Lysis



Complement Fixation test

Viral Serology

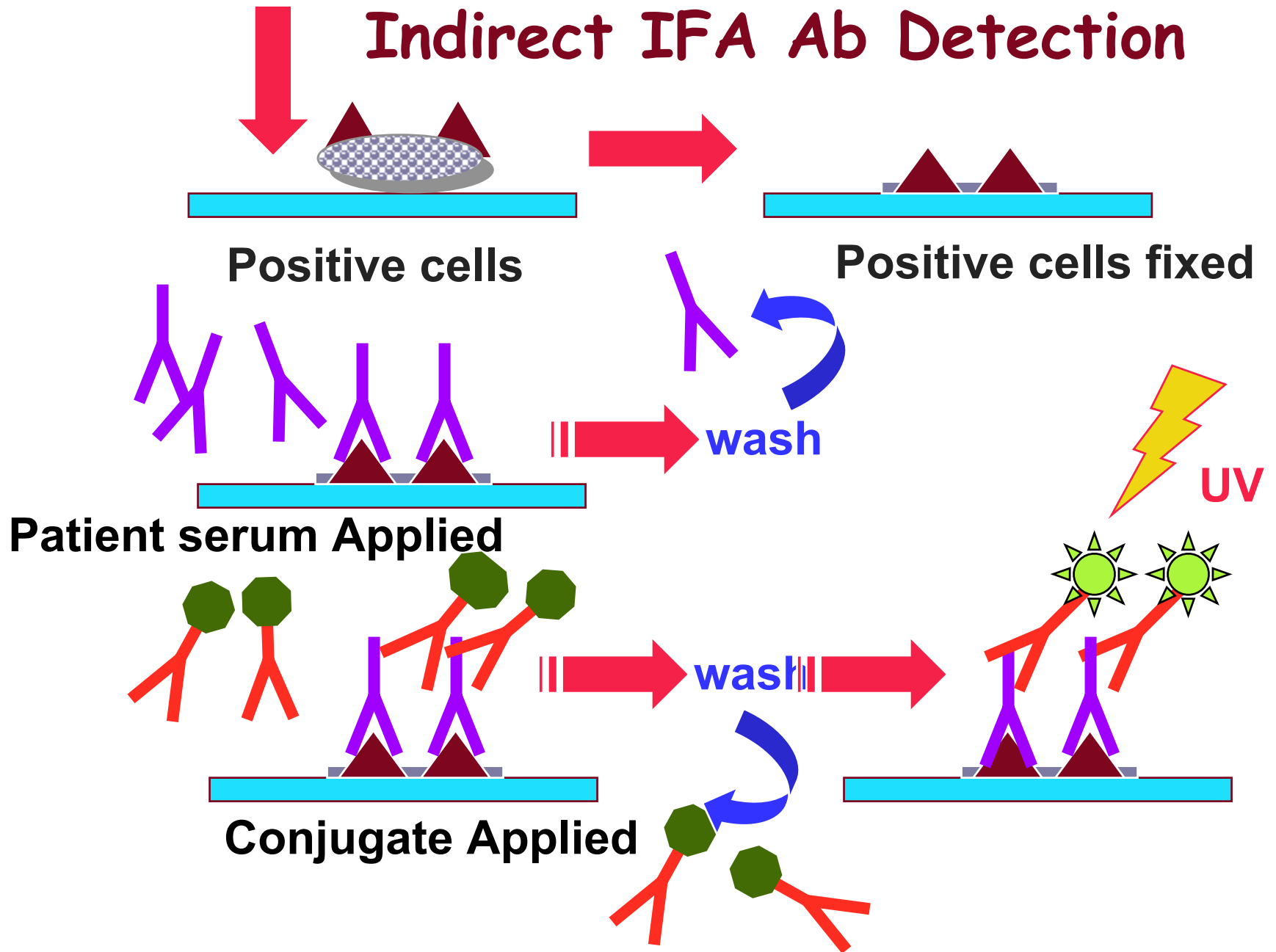
Detection of patient antibodies to a specific virus

- ☺ **Indirect Immunofluorescence Assay (IFA)**
- ☺ **Indirect Enzyme Immuno Assay (ELISA)**

Indirect IFA

- ☺ Apply cells infected with a known virus to a microscope slide
- ☺ Acetone fix
- ☺ Add patient serum, incubate
- ☺ Wash 3x
- ☺ Add secondary Ab conjugate (anti-human antibody), incubate
- ☺ Wash 3x
- ☺ Dry & examine

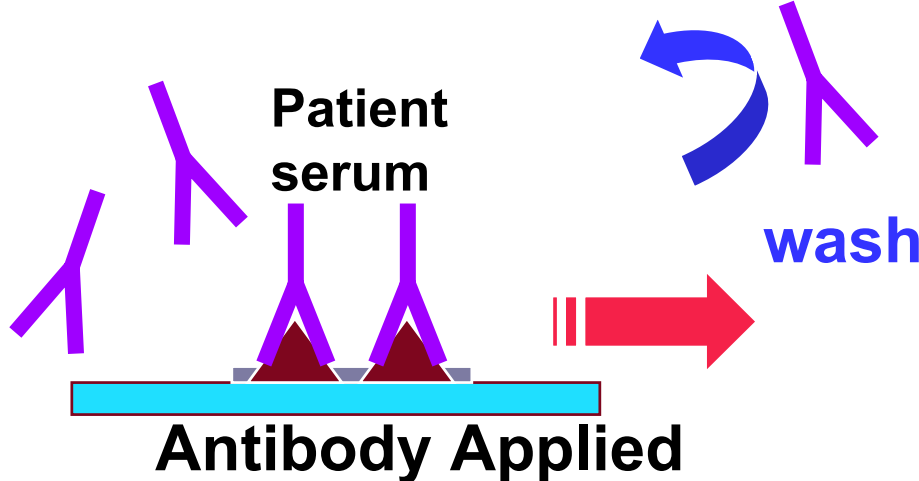
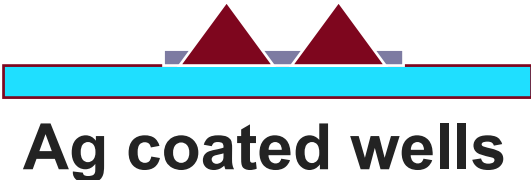
Indirect IFA Ab Detection



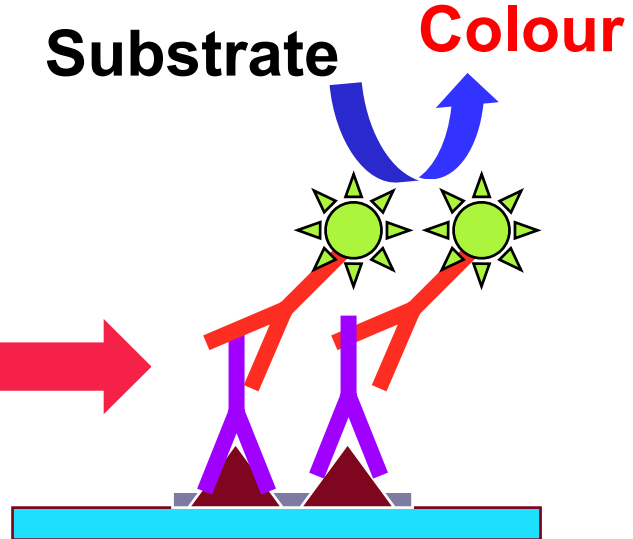
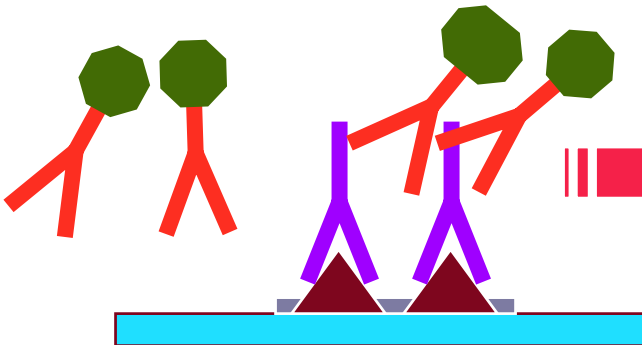
Indirect EIA -IgG

- ☺ A specific antigen is coated onto polystyrene microtitration plate
- ☺ Block uncoated sites
- ☺ Add primary Ab (patient serum) incubate
- ☺ Wash 5x
- ☺ Add anti-human antibody-enzyme conjugate, incubate
- ☺ Wash 6x
- ☺ Add substrate (TMB, DNPP)
- ☺ Read colour on spectrophotometer

Indirect ELISA



Anti-hu IgG antibody conjugate

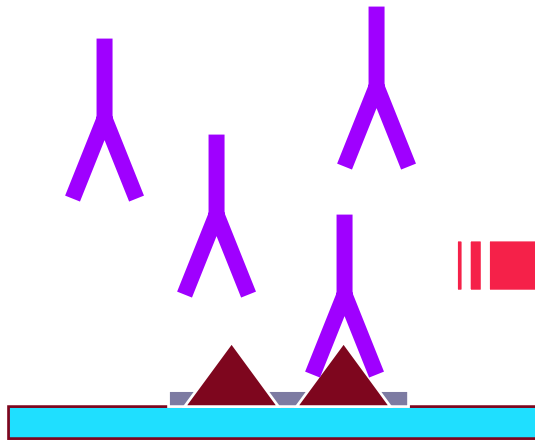


Variations of EIA method to detect antibodies

- IgM antibodies - direct detection
 - indirect detection
- Non human antibodies

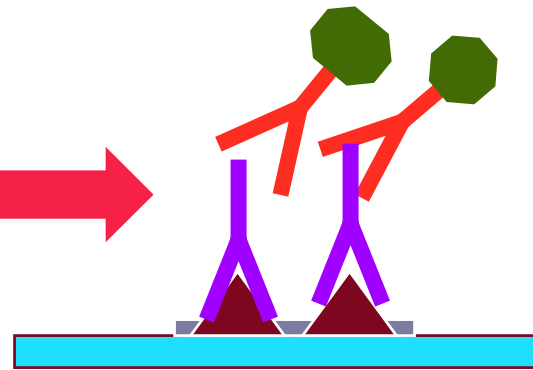
IgM Ab Detection - Indirect Method

IgM
antibody



||| → wash ||| →

Anti-hu IgM conjugate



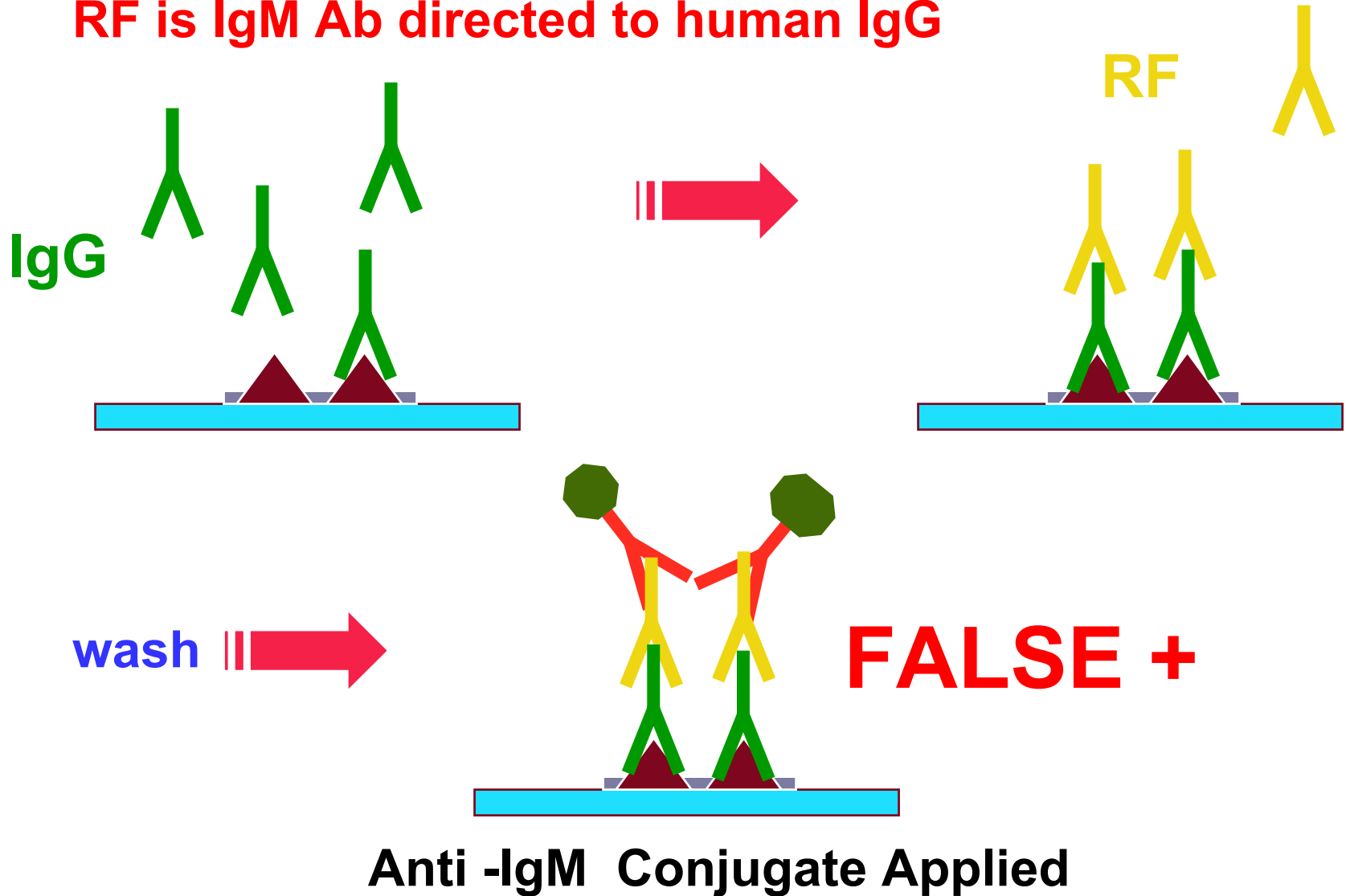
Anti -IgM Conjugate Applied

IgM Ab Detection - Indirect Method

- ☺ False Positives due to rheumatoid factor.
- ☺ False negatives due to IgG
- ☺ IgM capture is superior.

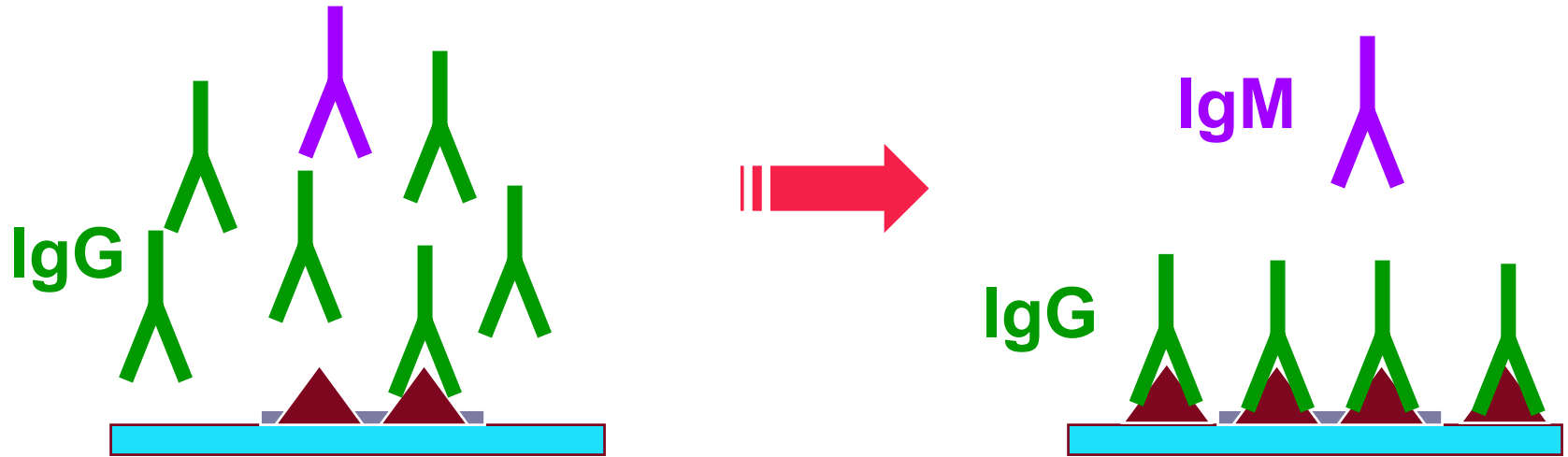
False positives - Rheumatoid Factor

RF is IgM Ab directed to human IgG

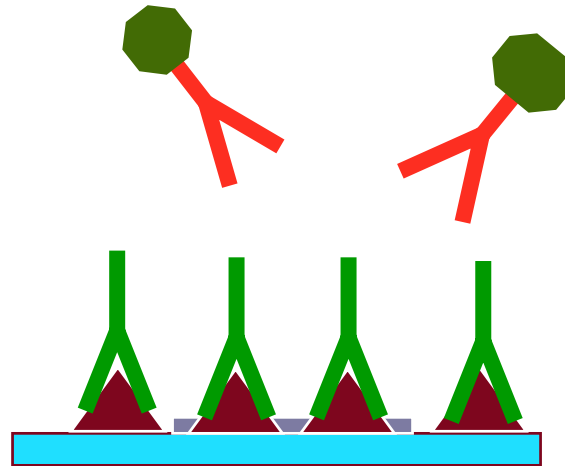


False negatives - IgG

Early convalescence \Rightarrow high levels of IgG



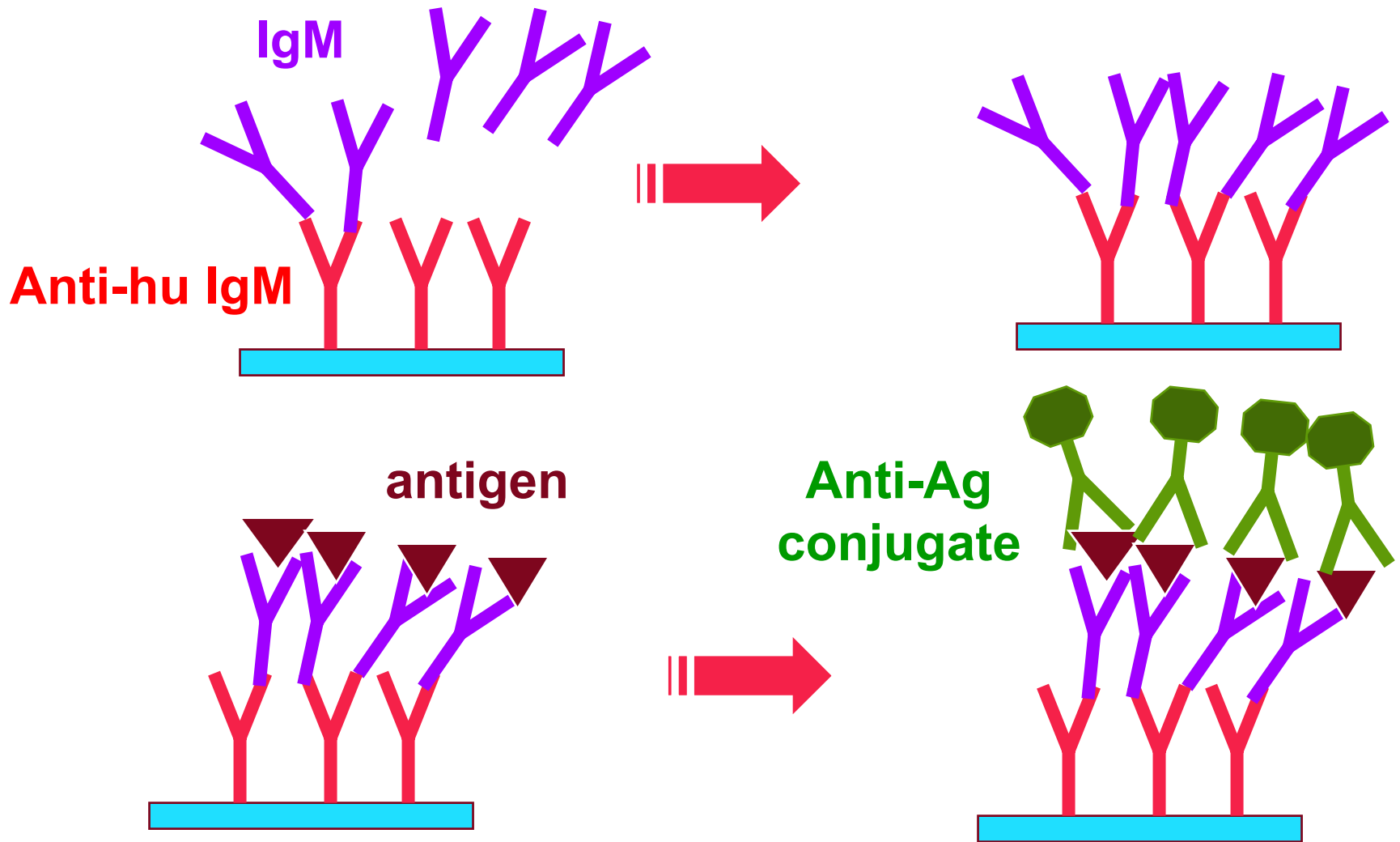
wash \Rightarrow



FALSE -

Anti-IgM Conjugate Applied

IgM Ab Detection - Capture Method



Competition ELISA

