

Heterophil Antibodies



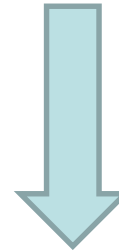
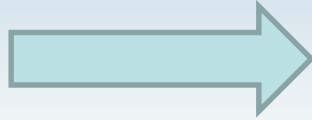
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Heterophil Antibody



- an antibody produced in response to one antigen that will react with second, genetically unrelated, antigen
- Forsmann, 1911

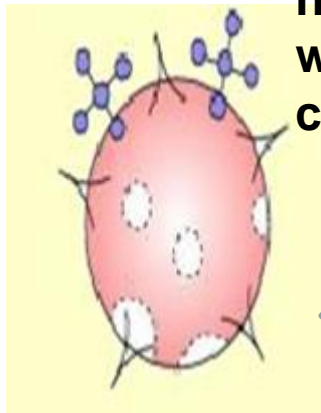
Organ emulsification from Guinea Pig



INJECTED INTO THE RABBIT



Rabbit: produced hemolysin w/ complement



Hemolysis



Can lyse sRBC

Forsmann Antigen



- found at the red cells of many species
- horse, sheep, dog, cat, mouse, fowl
- Pneumococci, Salmonella, Shigella, C. welchii and B. catarrhalis
- Absent: humans, monkeys, rabbits, rats, ducks and cow

Heterophil Antibodies in IM



- Paul and Bunnell, 1932
- antibodies were found to react with a heat stable antigen on sRBC that is shared by beef rbc but not GPK
- IM induces increase proliferation of lymphocytes and monocytes in the lymph node
- IgM observed within 2 weeks, lasting 4-8 weeks

Epstein-Barr Virus

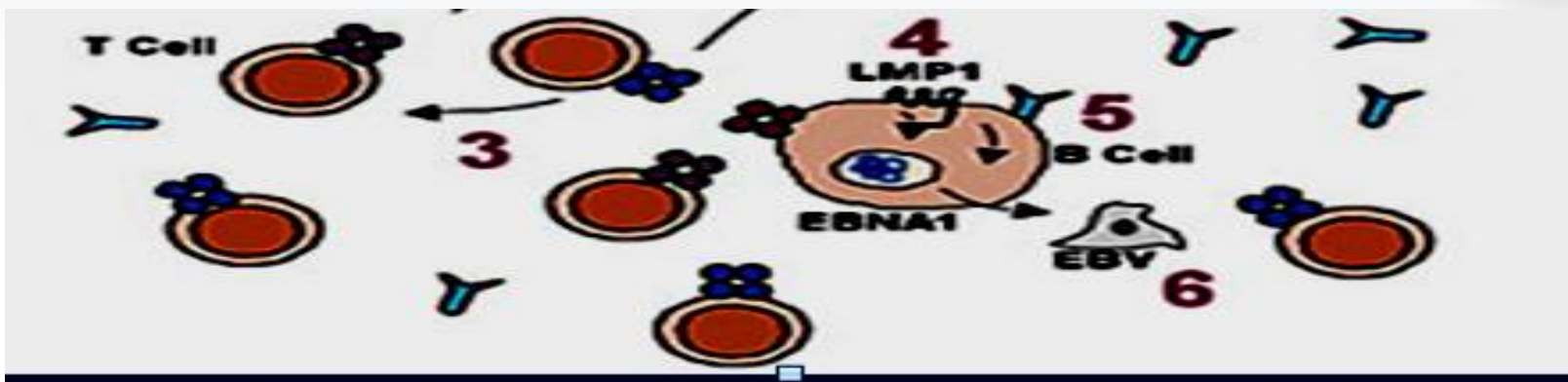


- enveloped
- DNA
- Herpesviridae
- MOT: saliva, blood transfusion, transplacental routes, and possibly by mosquitoes



EBV

- infects B cells



- once infected, we became a life long carrier
- present worldwide
- especially in low SES countries → 80% of the children were seropositive



Kissing Disease

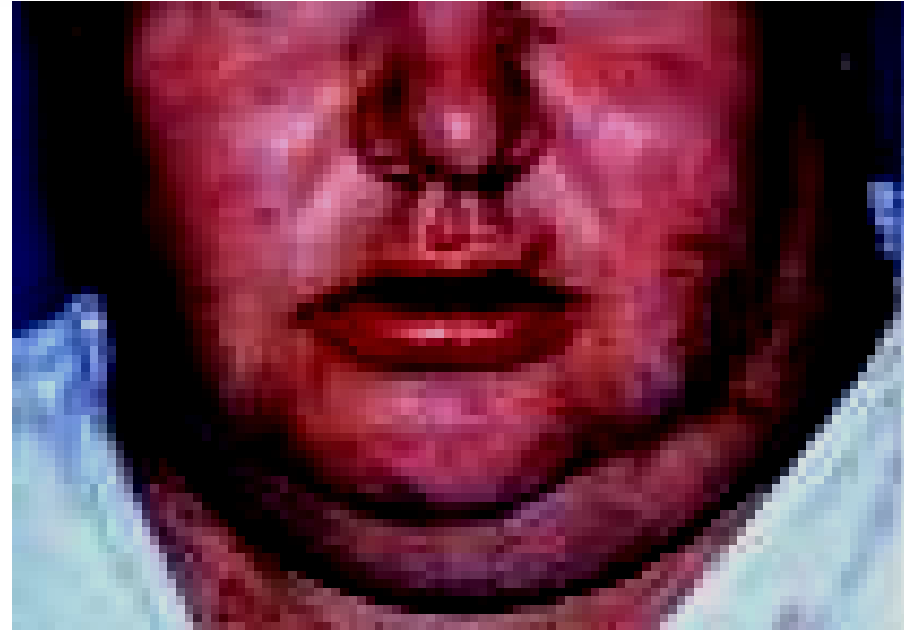
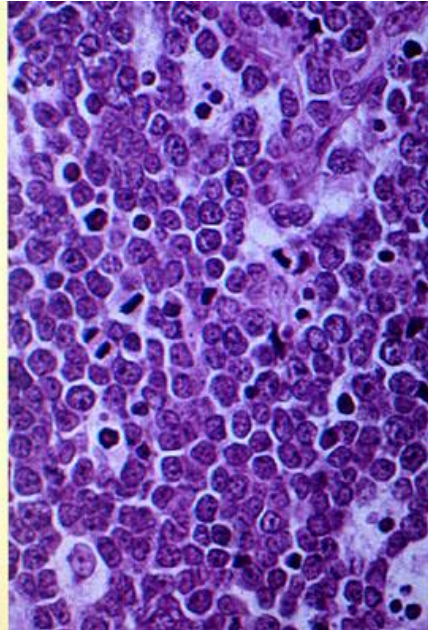
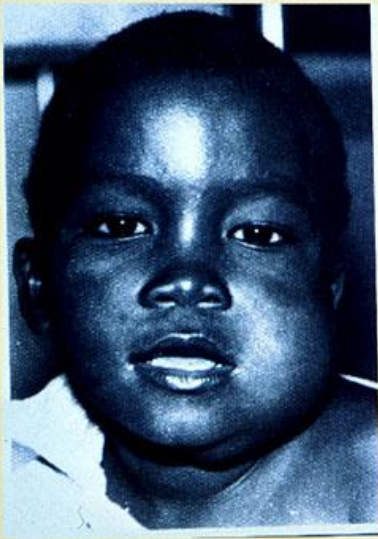
- play a risk for immunocompetent patients
- fever, malaise, lethargy, sore throat, with exudate, enlarged lymph nodes in the neck, mild hepatitis, enlarged spleen, blotchy skin rash



Diseases that are associated with IM

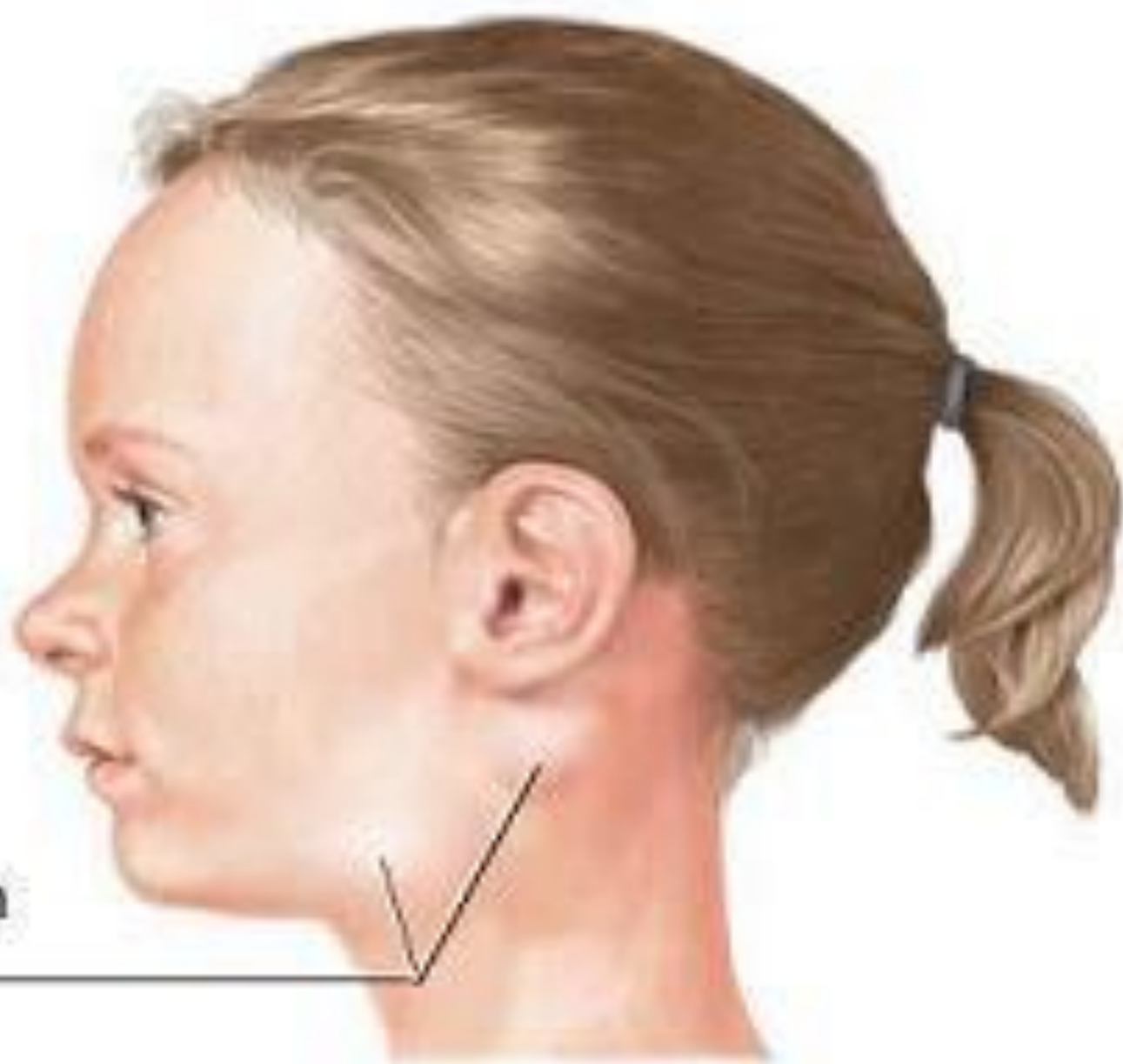
Burkitt's Lymphoma

**Nasopharyngeal
Carcinoma**



Mononucleosis causes:

- Fever
- Fatigue
- Sore throat
- Swollen lymph glands

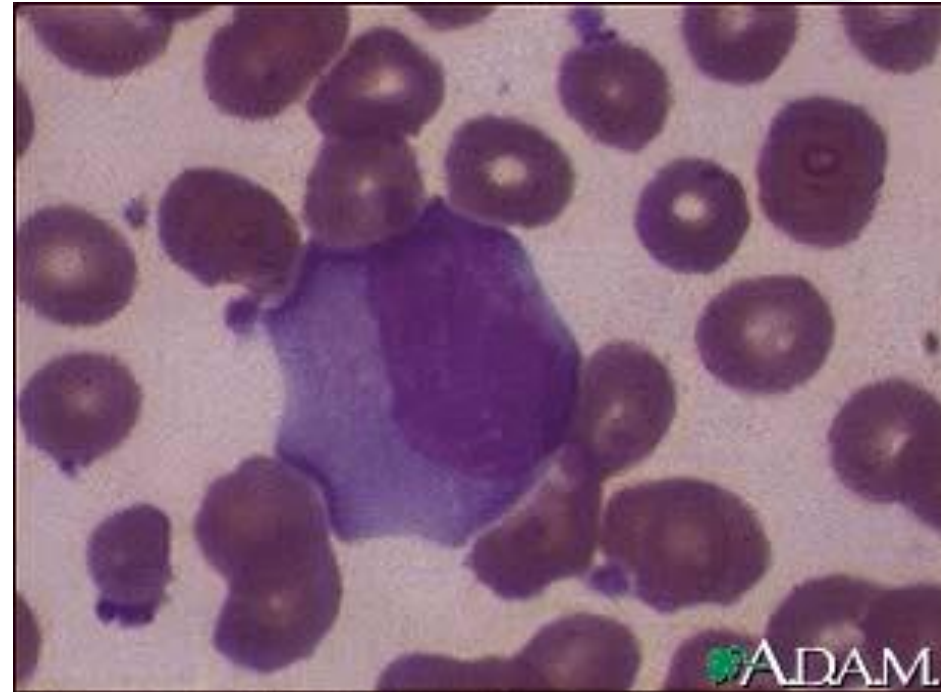


Some clinical signs of IM

sore throat w/ exudate



Downey cells



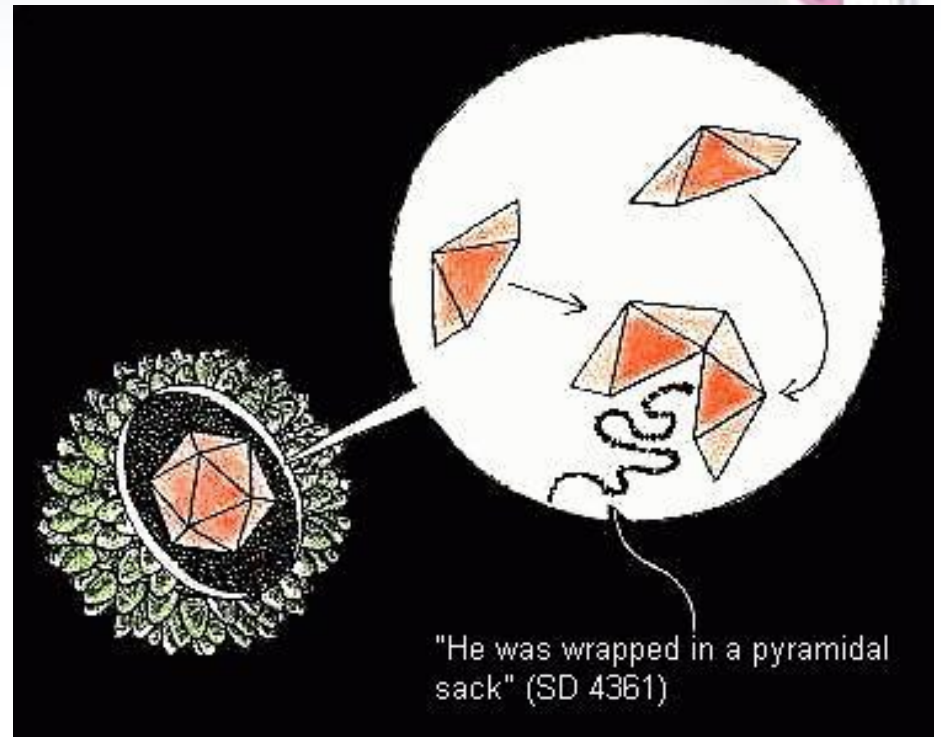
Other antigens of EBV

- viral capsid antigen
- early antigen
 1. EA-diffuse
 2. EA- restricted
- EBNA



VCA

- produced by infected B cells
- IgM detected early, disappears w/in 2-4 months
- IgG anti-VCA 4-7 days after the onset of s/s (persists longer)



EA-D and anti-EA-D



EA-D

- nucleus and cytoplasm of B cells

anti-EA-D

- IgG strongly indicates active infection
- not detectable among 10-20%
- disappears within 3 mos
- not a consistent indicator of the disease

EA-R and anti-EA-R



EA-R

- found as a mass in the cytoplasm only

anti-EA-R

- IgG detected in the serum of a very young children but not in adults during acute phase
- appears transiently in the later convalescent stage
- not an indicator of the disease stage

EBNA and anti-EBNA



EBNA

- found in the nucleus of all EBV infected cells
- synthesize before EA synthesis during the infection of B cells but does not stimulate antibody after the incubation period of IM, at which the EBV genome carrying B cells are destroyed by T cells

anti-EBNA

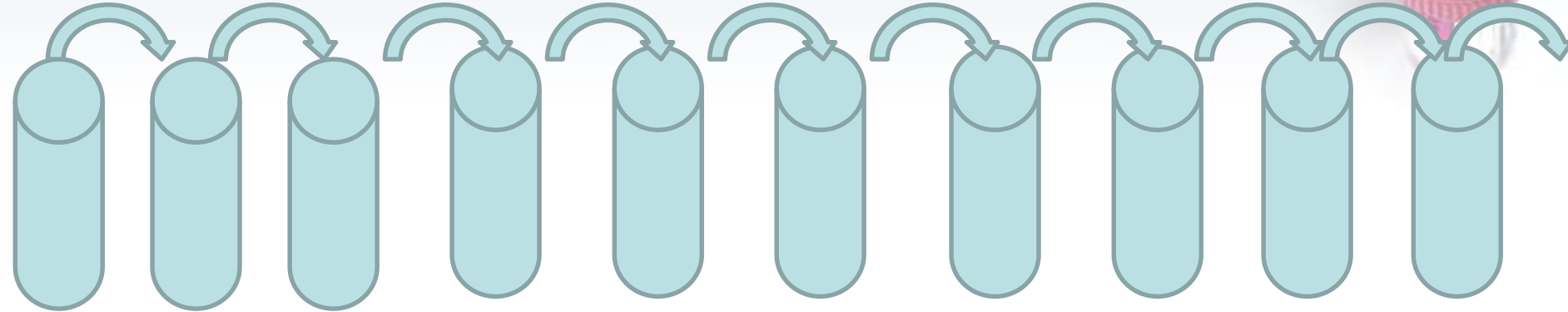
- IgG does not appear until the convalescent stage
- almost always present in sera containing IgG antibodies to VCA unless the patient is in the early acute stage
- ab titer plateau: 3-12 mos after the infection

Paul Bunnel

- requires inactivated serum



SERIALLY TRANSFER 0.25 ml from tube 1 to 10. Discard after the 10th tube



.4 ml NSS 0.25 0.25 0.25 0.25 0.25 0.25 0.25 0.25 0.25
.1 ml serum

Add 0.1 ml of 2% sRBC to each of the tube after the serial dilution. Shake incubate at 37°C overnight.

Significant titer: 1:56

Davidsohn Differential Test



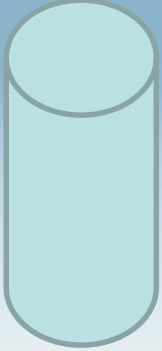
- classical test to distinguish between heterophil sheep cell agglutinins in human serum due to:
 - a. Forsmann antigen
 - b. serum sickness
 - c. IM

PRINCIPLE: some of the ag can cause agg of beef rbc but not on GPK

Absorption Patterns in Davidsohn Differential Test



Type of Heterophil Antibody	Absorbed by Guinea Pig Kidney	Absorbed by Beef Erythrocytes
Forssman	Yes	No
Infectious mononucleosis	No	Yes
Serum sickness	Yes	Yes



- Beef RBC
- Tube 1

1.0 ml beef RBC
0.25 ml serum
5 mins RT

Centrifuge 1,500 RPM 10 mins
Remove 0.25 ml clear supernatant
Place Tube 1 and 2 in Row 1 and 2, respectively
Place 7 additional tubes in each row
Add 0.25 ml of diluent to each tube
Serially dilute 0.25 ml of from tube 1 to tube 2 until tube 8
Repeat procedure for Row 2
Add 0.1ml of sRBC
Shake and leave at RT for 2 hours
Read and record results



- GPK cells
- Tube 2

1.0 ml GPK cells
0.25 ml serum
5 mins RT



Interpretation for IM

- the titer of the Paul-Bunnell test is 1:56
- The titer in row 1 is reduced > 8 fold
- The titer in row 2 is reduced > 4 fold



Serum Sickness



- Titer of 1:56 in Paul-Bunnel
- Titer in row 1 is reduced > 8 fold
- Titer in row 2 is reduced > 8 fold

SERUM SICKNESS: a reaction caused by the presence of antigen at the time the antibody is being formed

Forssman Antigen



- The titer of the Paul-Bunnet test is 1:56 or higher
- The titer in row 1 is not reduced
- The titer in row 2 is reduced > 8 fold

Rapid Differential Slide Test



Papain Treated sRBC

- Wallner's discovery: when papain is added to sRBC, receptors for IM antibody is inactivated
- Can be used for IM diagnosis

Horse RBC

- indicator
- 2 stages
 1. absorption with GPK
 2. absorption w/ ox RBC
- (+) for IM = agglutination with horse rbc and serum absorbed w/ GPK
- False (+) = pancreatic carcinoma, rubella and RA

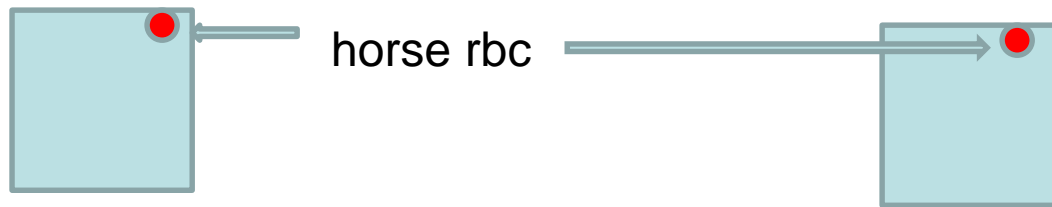
Interpretation of Rapid Differential Slide Tests Using Native and SRBC

Serum	Native SRBC	Papain Treated SRBC
Normal Serum >1:64	Agglutination	No agglutination (occ. weak clumping)
IM	Same as above	Same as above
Serum sickness or other heterophil antibodies	Agglutination	Agglutination

Monospot Test



- agglutination of horse rbc by heterophil antibody present in IM



SQUARE 1

1 drop GPK antigen
1 drop patient' s serum

SQUARE 2

1 drop of anti-GPK ag
1 drop patient' serum

Mix 10x the serum avoid horse RBC

Mix 10 x the serum with beef rbc

Blend the horse rbc over the entire surface

Start timer

Observe agglutination for not > 1 min after final mixing

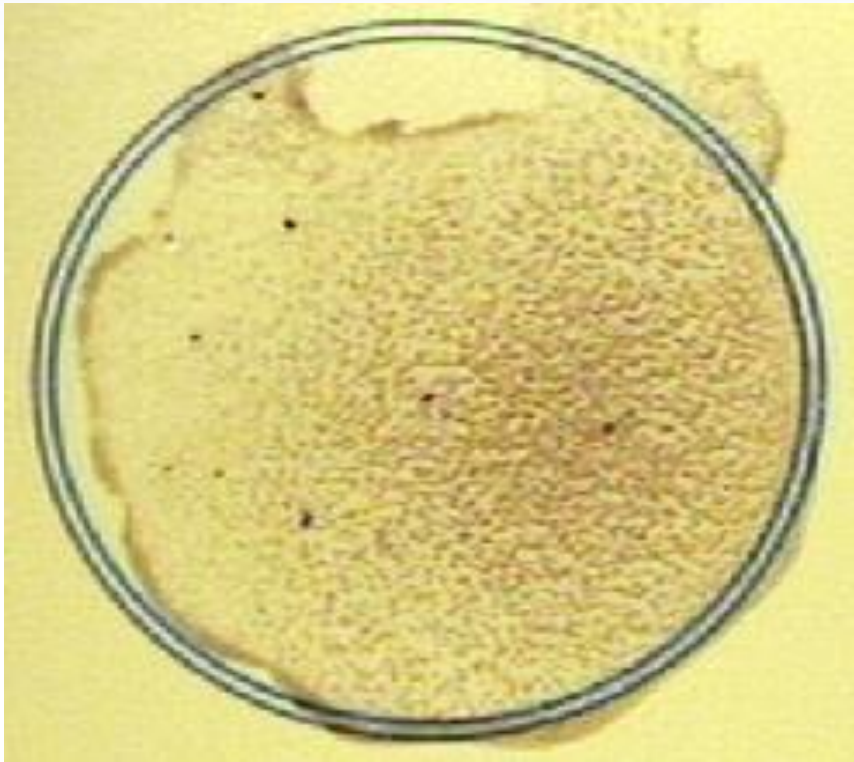
Stronger
Weaker
equal

Weaker = test is +
Stronger = test is -
equal = test is -

Monospot Test



Positive



Negative



IM Latex Test



Main Concept: Infectious Mononucleosis

Diseases

Serological Studies

Serological Tests

Burkitt's Lymphoma

Anti-VCA

Serum Sickness

Forsmann heterophil ab

Infectious mononucleosis

Absorbed by GPK not by Beef RBC

Absorbed by beef RBC not by GPK

Absorbed by GPK and beef RBC

Anti-EA-D

Anti-EA-R

Anti-EBNA

IgM disappears in 4 mos

IgG = active infection

IgM young children not adult

Convalescent ab

Paul Bunnel

Papain Treated

Monospot Test

Horse RBC in two slides

IM Latex

Davidsohn Differential Test

Agglutination Strong in Square 1