University of AL-Mustaqbal
Medical Laboratories
Techniques Department

Systemic lupus erythematosus , C-Reactive Protein (CRP) and Rheumatoid factor (RF)

Lect- 3

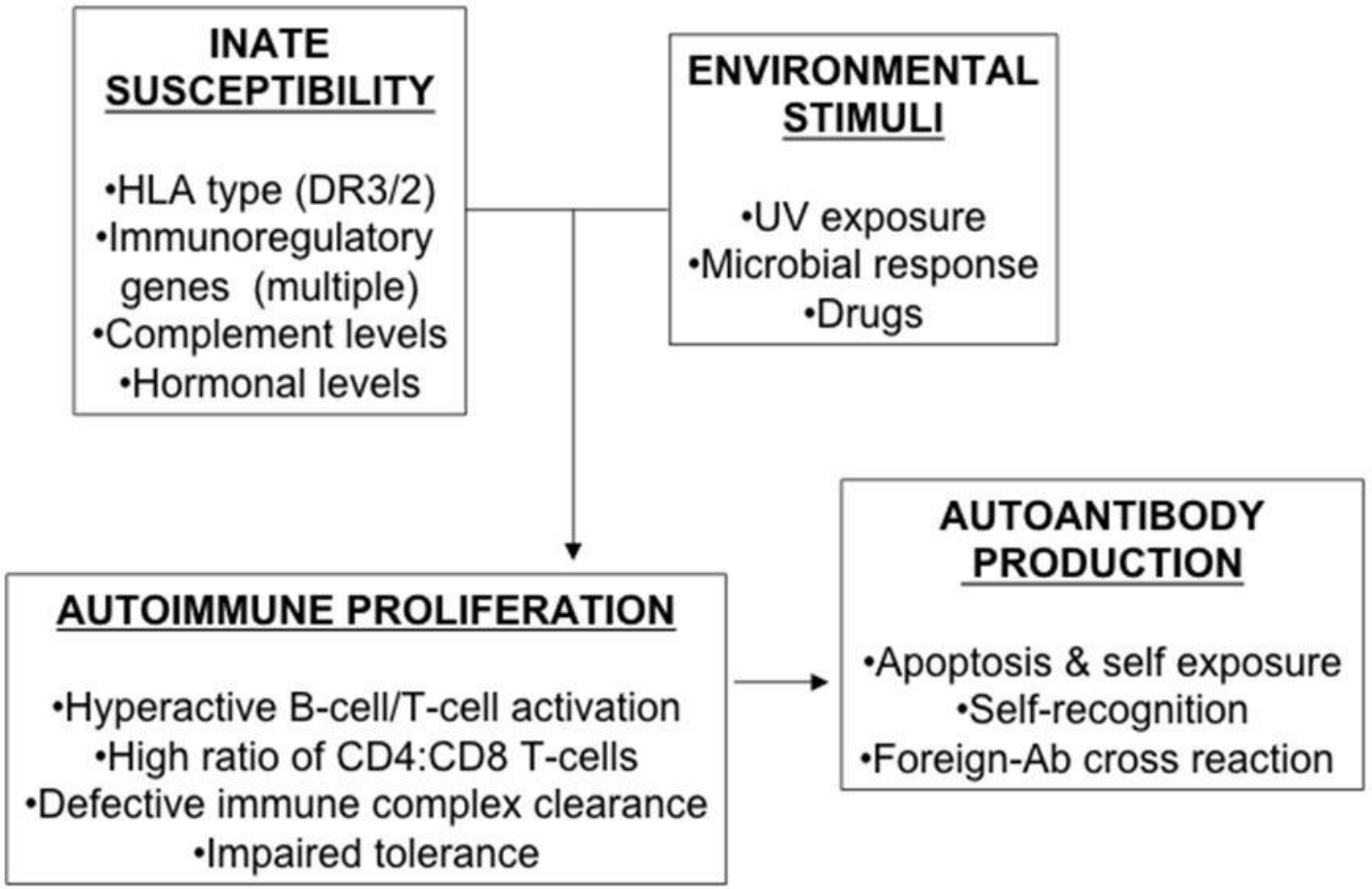
Systemic lupus erythematosus

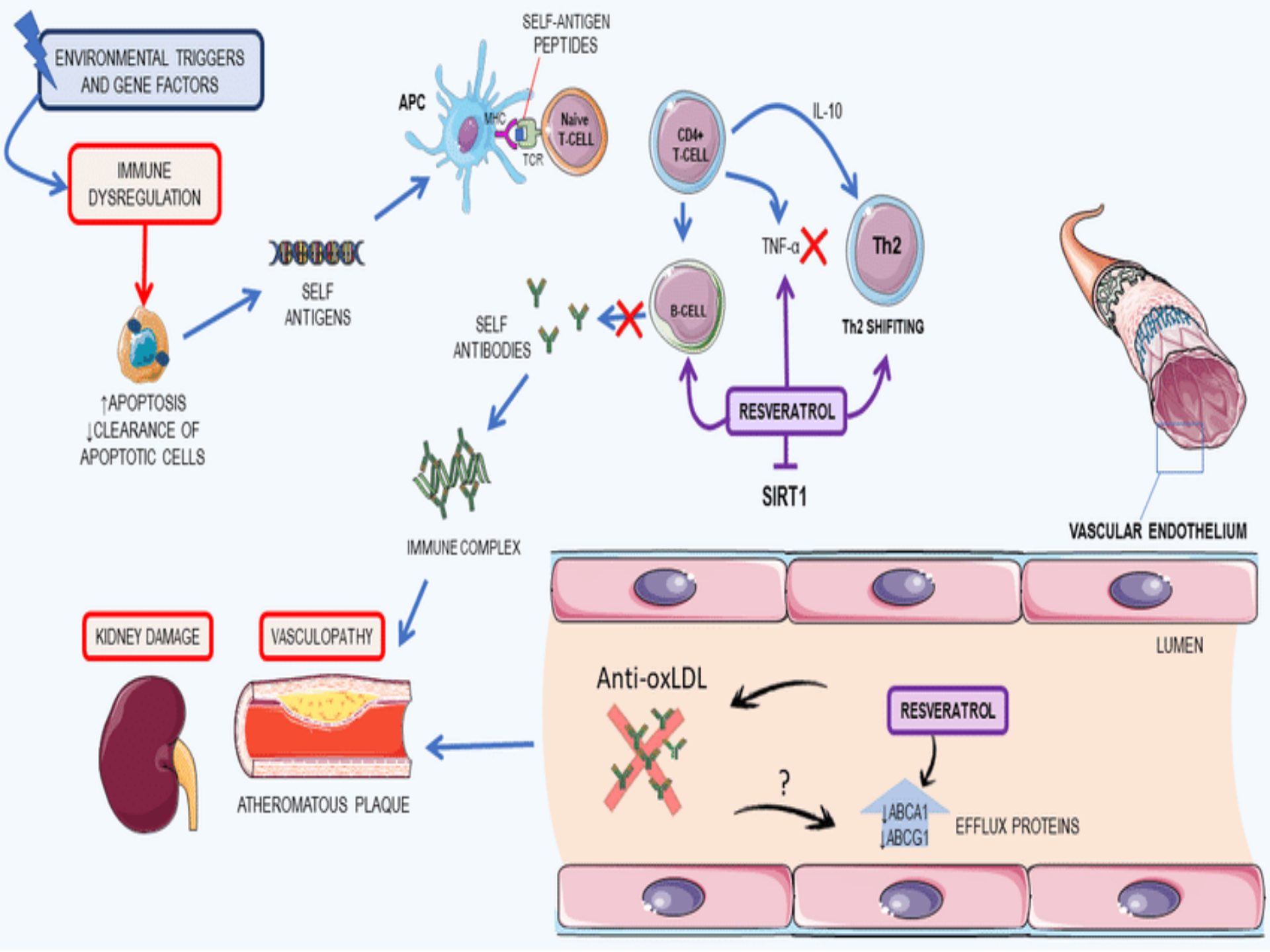
Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of autoantibodies and a diversity of clinical manifestations. It most commonly presents in women during their child-bearing years, in which the immune system targets intracellular particles that contain both nucleic acids and nucleic acid binding proteins.

Etiology

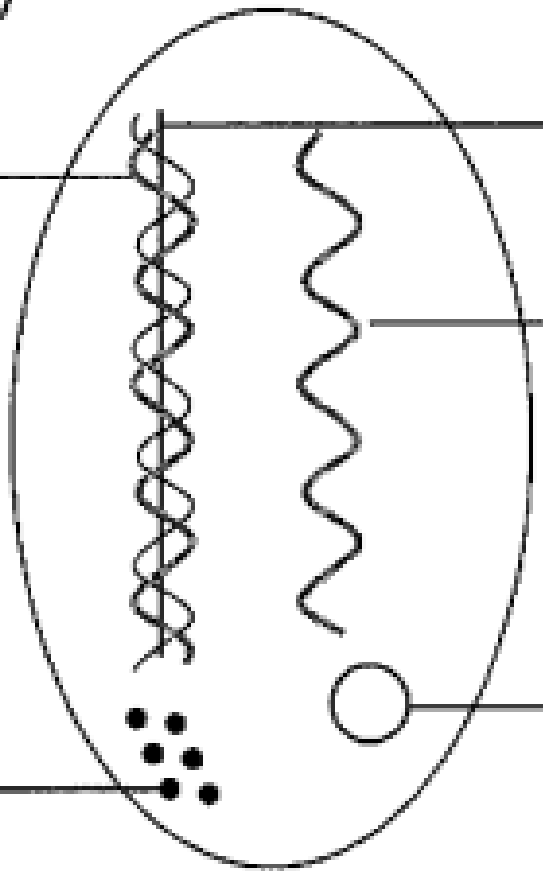
Although the etiology of SLE is unknown, multiple factors are associated with the development of SLE, including genetic (HLA- DR2/DR3), racial, hormonal, immune abnormalities and environmental factors (ultraviolet light, viral infection involving molecular mimicry between organism and self for example anti-Sm autoantibody react with p24 gag protein of retroviruses and that anti- Ro recognizes a nucleocapsid protein on vesicular stomatitis virus)

Etiology


INATE SUSCEPTIBILITY
•HLA type (DR3/2)
•Immunoregulatory genes (multiple)
•Complement levels
•Hormonal levels
ENVIRONMENTAL STIMULI
•UV exposure
•Microbial response
•Drugs
AUTOIMMUNE PROLIFERATION
•Hyperactive B-cell/T-cell activation
•High ratio of CD4:CD8 T-cells
•Defective immune complex clearance
•Impaired tolerance
AUTOANTIBODY PRODUCTION
•Apoptosis & self exposure
•Self-recognition
•Foreign-Ab cross reaction


ENVIRONMENTAL TRIGGERS AND GENE FACTORS
IMMUNE DYSREGULATION
↑APOPTOSIS
↓CLEARANCE OF APOPTOTIC CELLS
SELF ANTIGENS
SELF-ANTIGEN PEPTIDES
APC
MHC
TCR
Naive T-CELL
CD4+ T-CELL
IL-10
TNF-α
Th2
Th2 SHIFITING
B-CELL
SELF ANTIBODIES
RESVERATROL
SIRT1
IMMUNE COMPLEX
VASCULAR ENDOTHELIUM
LUMEN
KIDNEY DAMAGE
VASCULOPATHY
ATHEROMATOUS PLAQUE
Anti-oxLDL
RESVERATROL
?
↓ABCA1
↓ABCG1
EFFLUX PROTEINS

Double stranded DNA
Systematic lupus erythematosus
(chronic active hepatitis)



Histones

Single-stranded DNA
Non-specific
i.e. elderly and many
rheumatic conditions

Nucleolus
Scleroderma

Non-histone nuclear proteins

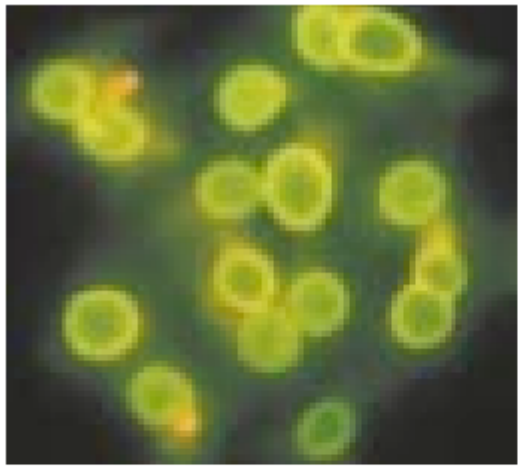
e.g. Sm — systemic lupus erythematosus
RNP — mixed connective tissue disease
Ro — vasculitis/SLE/Sjögren's syndrome
La — Sjögren's syndrome

Table 10.13 Laboratory findings in untreated systemic lupus erythematosus (SLE)*.

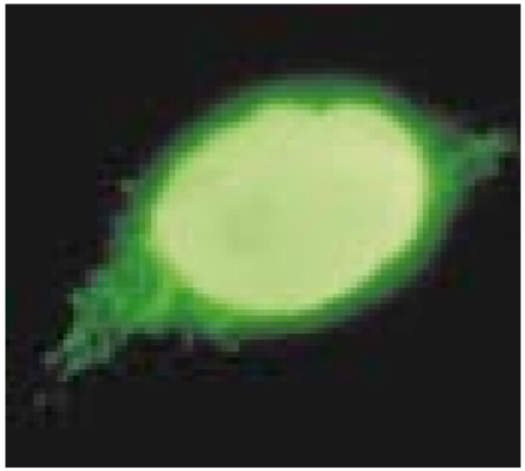
Immunological test	%	Haematological	%	Others	%
dsDNA binding	70–85	Raised ESR	60	C-reactive protein–normal unless infection present	
Antinuclear bodies (high titre; IgG class)	95	Leucopenia	45	Proteinuria	30
Raised serum IgG level	65	Direct Coombs' test positive	40		
Low serum complement C3/C4 levels	60	Lupus anticoagulant	10–20		
Platelet antibodies	60				
Cryoglobulinaemia	60				
Antibodies to ENA:					
Sm	30				
RNP	35				
Ro	30				
La	15				
Antibodies to phospholipids	30–40				
Rheumatoid factor (low titre)	30				
Skin biopsy IgG, C3 and C4 deposits in normal skin	75				

* Figures show percentage of patients with positive tests.

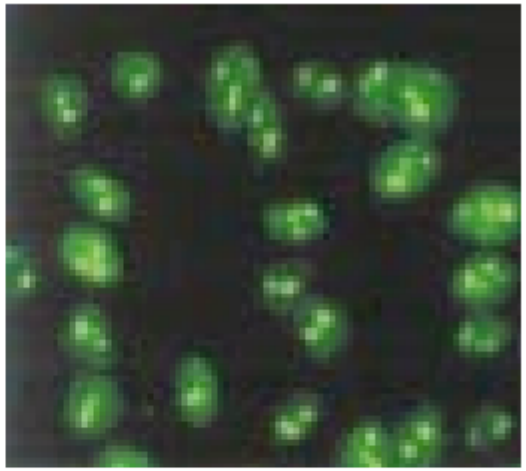
ESR, erythrocyte sedimentation rate; ENA, extractable nuclear antigens; RNP, ribonucleoprotein.



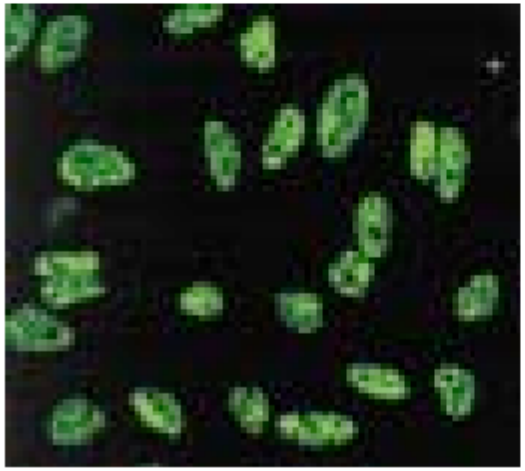
1. Rim pattern
(150x magnif; anti-DNA)



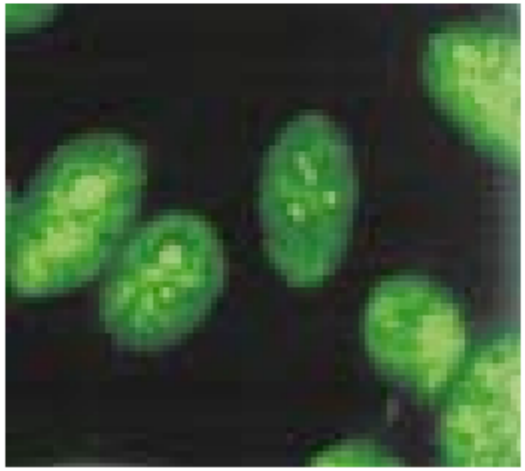
2. Homogenous pattern
(435x enlarged; anti-DNA)



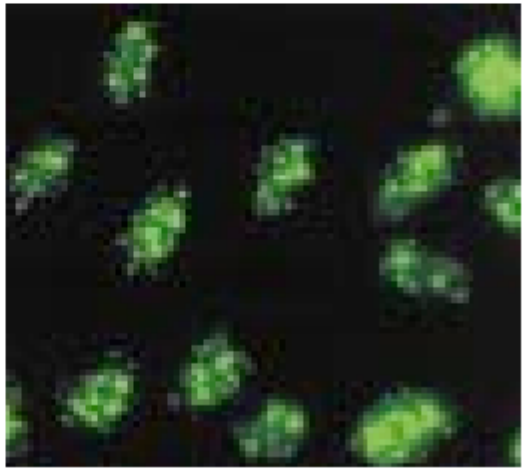
3. Nucleolar pattern
(e.g. fibrillarin)



4. Coarse-speckled pattern
(U1RNP/Sm)



5. Fine-speckled pattern
(Ro/La)



6. Anti-centromere antibody
pattern

C-Reactive Protein (CRP)

C-Reactive Protein (CRP), was the first acute phase protein in inflammatory reactions. CRP is required for the activation of complement system. After an inflammatory stimulus, a significant rise in CRP may be detected in the serum within 6 hours. As such, CRP was for many years thought of as a sensitive, CRP also has an anti-inflammatory role in autoimmune diseases such as systemic lupus erythematosus (SLE

Clinical Indication

CRP production is part of the nonspecific acute-phase response to inflammation, infection, and tissue damage. CRP values are non specific and can never be diagnostic on their own, but can contribute to the evaluation of the inflammatory response, Furthermore, since trace element levels in the serum may change during inflammation, CRP levels used for evaluation of the an on-going inflammatory response. The acute phase response develops in a wide range of acute and chronic inflammatory conditions like bacterial, viral, or fungal infections; rheumatic and other inflammatory diseases; malignancy; and tissue injury or necrosis. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver

PRINCIPLE

The CRP-latex particles are coated with antibodies to human CRP. The CRP-latex Reagent has been standardised to detect serum CRP levels at or above 6 mg/L which is considered the lowest concentration of clinical significance. When the latex suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination is seen within 2 minutes.The presence or absence of a visible agglutination indicates the presence or absence of CRP in the specimen .

PROCEDURE (QUALITATIVE METHOD)

1-Allow each component to reach room temperature before use.

2.Place one drop of the Negative CRP Control onto a circle of the agglutination slide.

3- Place one drop of the Positive CRP Control onto an adjacent circle of the agglutination slide.

4- Using the pipette-stirrers provided, place one drop of serum specimen(s) onto the remaining circle(s) of the agglutination slide.

5- Shake gently and re-suspend the CRP latex reagent.

6- Add one drop next to each drop of controls and serum on the agglutination slide.

7- Stir with individual pipette-stirrers and spread mixture over entire area of the test circle.

8- Gently rock the agglutination test slide for two minutes and observe the test circles for agglutination. Interpret results at two minutes.

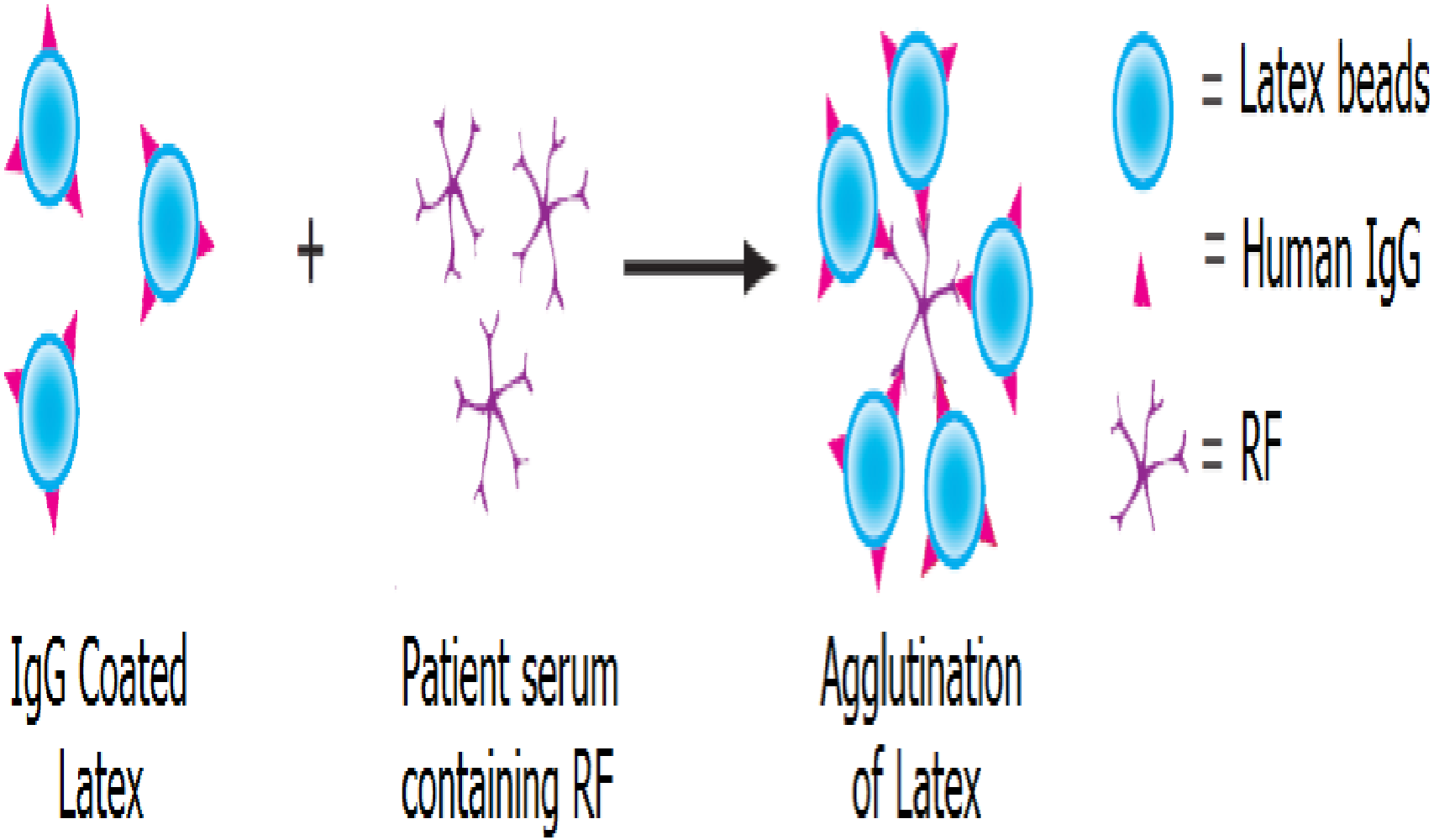
9- At the end of the test rinse the slide with distilled water and dry on air.

SEMI-QUANTITATIVE DETERMINATION

Tube No.	1	2	3	4	5	6	-
Serum (ml)	0.5	0.5	-	-	-	-	-
Saline (ml)	-	0.5	0.5	0.5	0.5	0.5	-
	0.5 →	0.5 →	0.5 →	0.5 →	0.5 →	0.5 →	0.5 Discarded
Dilutions	1/1	1/2	1/4	1/8	1/16	1/32	-
Concentration IU/ml	0.3	0.6	1.2	2.4	4.8	9.6	-

Rheumatoid factor (RF)

Rheumatoid factor (RF) is an anti-antibody, which in-vitro, is detected by its ability to agglutinate latex particles coated with human IgG. RF in patient sample, if present, will attach to the IgG coating the latex particles. Agglutination of the latex particles is a positive result indicating the presence of RF.


= Latex beads
= Human IgG
= RF
IgG Coated Latex
Patient serum containing RF
Agglutination of Latex

Why did doctor order this test?

- Doctors may order a blood test to check for the presence of RF if they suspect you have an autoimmune condition, such as rheumatoid arthritis or Sjögren syndrome. Other health problems that can cause higher-than-normal levels of RF include:

• Chronic infection

• Cirrhosis, which is scarring of the liver

• cryoglobulinemia, which means there are or abnormal proteins in the blood

• dermatomyositis, which is an inflammatory muscle disease

• inflammatory lung disease

mixed connective tissue disease

• lupus

• cancer

QUALITATIVE METHOD Procedure;

1. Bring all reagents and specimens to room temperature.

2. Place one drop of the positive control and 40ul of the patient serum into separate circles on the slide.

3. Gently and add one drop of RF latex reagent on each circle of sample to be tested and control.

4. Use separate Applicator sticks/stir sticks to spread reaction mixture over entire area of the particular field.

5. Tilt the slide back and forth for 2 minutes in a rotary shaker so that the mixture rotates slowly.

6. Observe for agglutination after 2 minutes under bright artificial light. Interpretation Agglutination of latex particles is considered a positive reaction, indicating the presence of rheumatoid factor at a significant and detectable level.

Positive result: An agglutination of the latex particles suspension will occur within two minutes, indicating a RF level of more than 18 IU/ml.

Negative result: No agglutination of the latex particles suspension within two minutes.