Lab .2/ Immunology

Rheumatoid factor (RF) Test:

Principle, Procedure and Interpretation.

+ **The five subclasses of antibodies**

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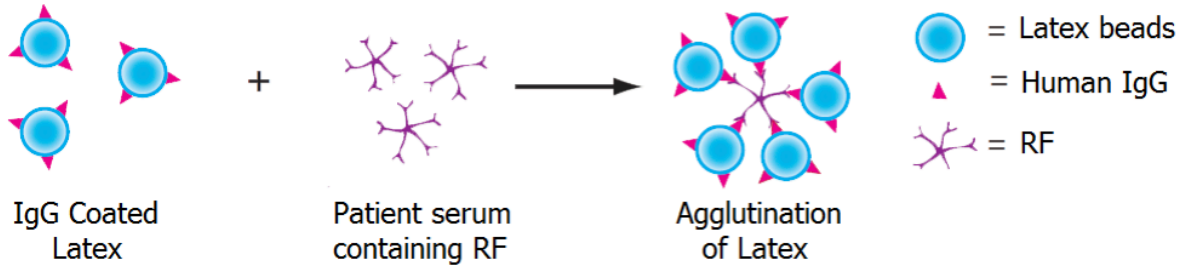
Rheumatoid factor (RF)

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting primarily the joints and per-articular tissues. For many years it has been known that several abnormal proteins circulate in the blood of patients with RA. These proteins, because of their obvious correlation with the disease, became known as rheumatoid factor (RF). Research of these proteins has characterized them as usually being a group of IgM class immunoglobulin's that interacts with antigenic determinants on human IgG molecules (i.e., they are anti-antibodies). Rheumatoid factor is detected in 60-80% of cases of diagnosed rheumatoid arthritis.

Principle:

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.



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

Some health problems may cause elevated RF levels, but the presence of this protein alone is not used to diagnose these conditions. These illnesses include:

- HIV/AIDS
- hepatitis
- influenza
- viral and parasitic infections
- chronic lung and liver diseases
- leukemia

Materials: Materials provided with the test set;

1. RF Latex Direct Reagent

2. Positive Control Serum

3. Negative Control Serum

4. 6- Well Test Slide

5. Disposable Pipettes

Materials required but not provided;

1.Test Tubes (for quantitative method). 2. Serological Pipettes. 3. Laboratory Timer. 4.Laboratory Rotator (optional). 5. Isotonic Saline (0.85% sodium chloride, for quantitative method

Specimens:

This test should be performed on fresh serum. The samples maybe stored refrigerated (2-8^{o}C) for maximum of 7days. If longer storage is required, store at -20^{o}C. Heavy bacterial contamination may cause positive agglutination.

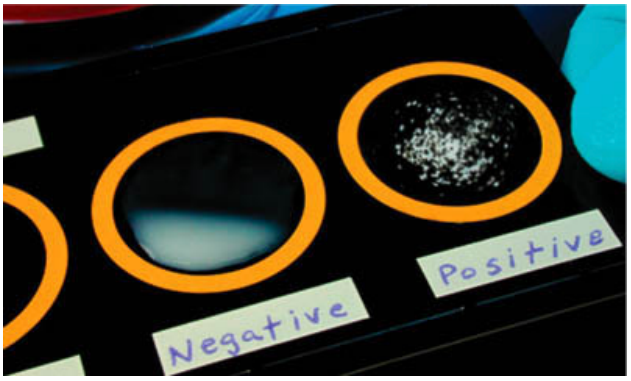
QUALITATIVE METHOD Procedure;

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.



SEMI-QUANTITATIVE METHOD

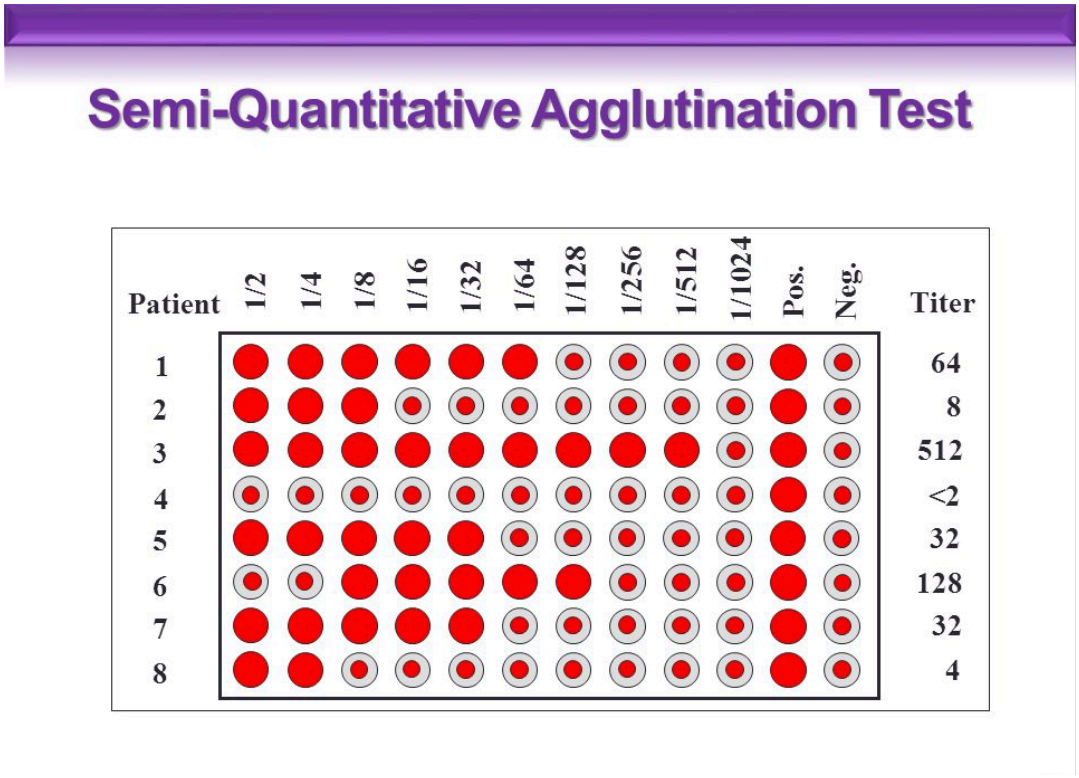
1. Using isotonic saline prepare serial dilutions of the test sample positive in the qualitative method 1:2, 1:4, 1:8, 1:16,1:32, 1:64, 1:128 and so on as follows :
   a. For each specimen to be tested, add 100 µL of 0.9% saline into test tubes numbered 1 to 5.
   b. Add 100 µL of specimen onto test tube 1.
   c. Mix the mixture. Avoid formation of bubbles.
   d. Transfer 100 µL of mixed sample from tube 1 to 2.
   e. Repeat this serial dilution procedure in tube 3 to 4, and then 5. Dispose 100 µL from test tube 5 after mixing
   f. Tubes 1 to 5 now represent a dilution series as follows:

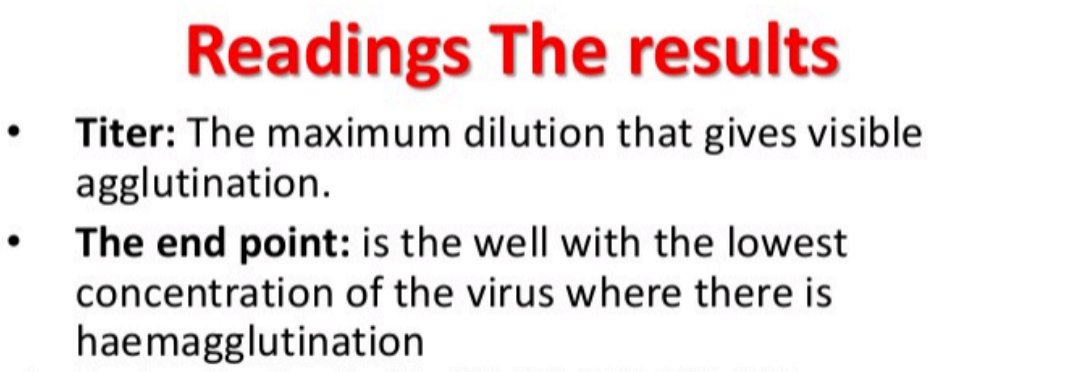
Tube Number	1	2	3	4	5
Dilution	1:2	1:4	1:8	1:16	1:32

2. Perform the qualitative test procedure using each dilution as test specimen.

Results of the dilution:

The results of your test are reported as a titer, which is a measurement of how much your blood can be diluted before RF antibodies are undetectable. In the titer method, a ratio of less than 1:80 is considered normal, or less than 60 units of RF per milliliter of blood.





Limitations:

1. RF is not detected in all patients diagnosed with RA.
2. RF may be detected in increased amounts in patients with infectious mononucleosis, sarcoidosis, lupus, syndrome, TB or leprosy, and other conditions of acute or chronic immune response. The significance of a positive result should be interpreted with caution. Testing should be done to confirm diagnosis of RA.
3. Procedure must be followed carefully and results read at the appropriate time. Reading

after the specified time may result in mis-interpretation due to drying of specimen/reagents.

4. Some products may produce questionable results from hemolyzed, lipemic or contaminated specimens.
5. Avoid contamination of reagent or reagent dispensing dropper.

The five subclasses of antibodies are:

1. **Immunoglobulin A (IgA),** which is found in high concentrations in the mucous membranes, particularly those lining the respiratory passages and gastrointestinal tract, as well as in saliva and tears.

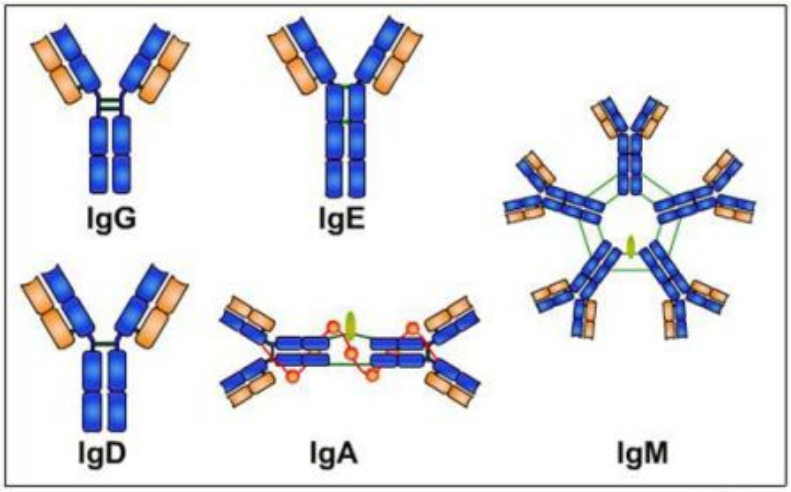
2. **Immunoglobulin G (IgG),** the most abundant type of antibody, is found in all body fluids and protects against bacterial and viral infections.

3. **Immunoglobulin M (IgM),** which is found mainly in the blood and lymph fluid, is the first antibody to be made by the body to fight a new infection.

4. **Immunoglobulin E (IgE),** which is associated mainly with allergic reactions (when the immune system overreacts to environmental antigens such as pollen or pet dander). It is found in the lungs, skin, and mucous membranes.

5. **Immunoglobulin D (IgD),** which exists in small amounts in the blood, is the least understood antibody.

IgA, IgG, and IgM are often measured together. That way, they can give doctors important information about immune system functioning, especially relating to infection or autoimmune disease.



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