Lec 3

Introduction to serology:

Serology is the ***in vitro*** study of blood serum (sero = serum). In practice, the term usually refers to the diagnostic identification and measuring of **antibodies** in the serum, and less frequently in **other body fluids** such as semen and saliva. Such antibodies are typically formed in response to an infection against a given microorganism (**bacteria, parasite, fungi, and virus**), or against other foreign proteins in response to a mismatched blood transfusion, or to ones own proteins in instances of autoimmune disease. Serological tests may be performed for diagnostic purposes when an infection is suspected, and in many other situations, such as checking an individual's blood type.

Also, serological blood test help to diagnosis patients with certain immune deficiencies associated with the lack of antibodies.

Seroconversion: is the development of detectable antibodies in the blood that are directed against an infectious agent.

Seroreversion : is the opposite of seroconversion. This is when the tests can no longer detect antibodies in a patient's serum.

In the initial (**primary infection**) phase of the infection, immunoglobulin (IgM) antibodies are produced and as these levels drop and become undetectable, immunoglobin G IgG level rise and remain detectable.

Upon reinfection, IgM antibodies usually do not rise again but IgG level will increase. Thus an elevated IgM titer indicates **recent infection**, while the presence of IgG suggests **past infection** or **immunization.**

Diagnostic accuracy:

♦ Sensitivity and specificity.
♦ Positive and negative predicative values (PPV, NPV).
♦ Positive and negative diagnostic likelihood ratio.

A perfect diagnostic procedure has the potential to completely discriminate subjects with and without disease. The cut-off value divides the population of examined subjects with and without disease in four subgroups considering parameter values of interest:

1-True positive (TP): subjects with the disease with the value of a parameter of interest above the cut-off.

2-False positive (FP): subjects without the disease with the value of a parameter of interest above the cut-off.

3-True negative (TN): subjects without the disease with the value of a parameter of interest below the cut-off.

4-False negative (FN): subjects with the disease with the value of a parameter of interest below the cut-off. As show in table (1).

Test status	**Subject with the disease**	**Subject without the disease**
Positives	TP	FP
Negative	FN	TN

Sensitivity and specificity:

Sensitivity: is expressed in percentage and defines the proportion of true positive subjects with the disease in a total group of subjects with the disease (TP/TP+FN).

Specificity: is a measure of diagnostic test accuracy, complementary to sensitivity. It is defined as a proportion of subjects without the disease with negative test result in total of subjects without disease (TN/TN+FP).

Predictive values:

Positive predictive value (PPV): defines the probability of having the state/disease of interest in a subject with positive result. (TP/TP+FP).

Negative predictive value (NPV): describes the probability of not having a disease in a subject with a negative test result. (TN/TN+FN).

Serological tests: Serological tests are the qualitative and mainly quantitative determination of specific antibodies (or antigens) in the serum through serological procedures which include: ELISA, agglutination, precipitation, complement –fixation, and fluorescent antibodies.

1-Agglutination tests: The interaction between antibody and a particulate antigen results in visible clumping called **agglutination**, particular antigens include; Bacteria, White blood cells, Red blood cells (Hemagglutiation), and Latex particles.

Antibodies that produce such reactions are called agglutinins, all Abs can agglutinate particulate Ags but IgM ,due to its high valence, is particularly good agglutinin and one sometimes infers that an Abs may be of the IgM class if it is a good agglutinating Ab.

Agglutination permits phagocytic cells to engulf invading microorganisms. This is a major role of agglutinin in the immune reaction.

A-Qualitative agglutination test:

Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. (This test can be done on a slide ex: blood group).

B-Quantitative agglutination test;

Agglutination tests can also be used to quantitative the level of antibodies to particulate antigens. In this test one makes serial dilutions of a sample to be tested for antibody and then adds a fixed number of red blood cells or bacteria or other such particulate antigen. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination. (This can be done using a micro titer plate or tubes).

C-Cold Agglutination test:

Cold agglutinins test is performed to detect the presence of antibodies in blood that are sensitive to temperature changes. Cold agglutinins are auto antibodies that cause red blood cells to clump, but only when the blood is cooled below the normal body temperature of 98.6°F (37°C). The clumping is most pronounced at temperatures below 78°F (25.6°C).

The

Purpose:

The cold agglutinins test is used to confirm the diagnosis of certain diseases that stimulate the body to produce cold agglutinins. The disease most commonly diagnosed by this test is mycoplasmal pneumonia, but mononucleosis, mumps, measles, scarlet fever, some types of hemolytic anemia.

Description:

Since cold agglutinins cause red blood cells to clump only at temperatures lower than 98.6°F (37°C), the test consists of chilling a sample of the patient's blood. There is a bedside version of the test in which the doctor collects four or five drops of blood in a small tube, cools

the tube in ice water for 30-60 seconds, and looks for clumping of red blood cells. If the cells clump after chilling and unclump as they rewarm, a cold agglutinin titer (concentration) greater than 1:64 is present. The laboratory test measures the clumping of red blood cells in different dilutions of the patient's blood serum at 39.2°F (4°C).

D-Coombs test (Agglutination test):

Coombs test (also known as **Coombs' test**, **antiglobulin test** or **AGT**). The two Coombs tests are the **direct Coombs test** (**DCT**, also known as **direct antiglobulin test** or **DAT**), and the **indirect Coombs test** (also known as **indirect antiglobulin test** or **IAT**).

In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the RBC surface membrane, and their circulating RBCs can become coated with IgG alloantibodies and/or IgG auto antibodies. Complement proteins may subsequently bind to the bound antibodies and cause RBC destruction.

1-Direct coombs test:

The **direct Coombs test** is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells; a blood sample is taken and the RBCs are washed (removing the patient's own plasma) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct coombs test is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cell.

Examples of disease that give a positive direct coombs test:

1- Alloimmune hemolysis (Hemolytic disease of the newborn HDN, or erythroblastosis fetalis ,and ABO hemolytic disease of the new born).

2- Drug-induced immune-mediated hemolysis. (Penicillin high dose).

2-Indirect coombs test:

The **indirect Coombs test** is used in prenatal testing of pregnant women for IgG Abs that are likely to pass through the placenta into the fetal blood and cause hemolytic disease of the new born. , and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood sample taken from the patient. Then, the serum is incubated with RBCs of known antigenicity, that is, RBCs with known

reference values from other patient blood samples. If agglutination occurs, the indirect Coombs test is positive.

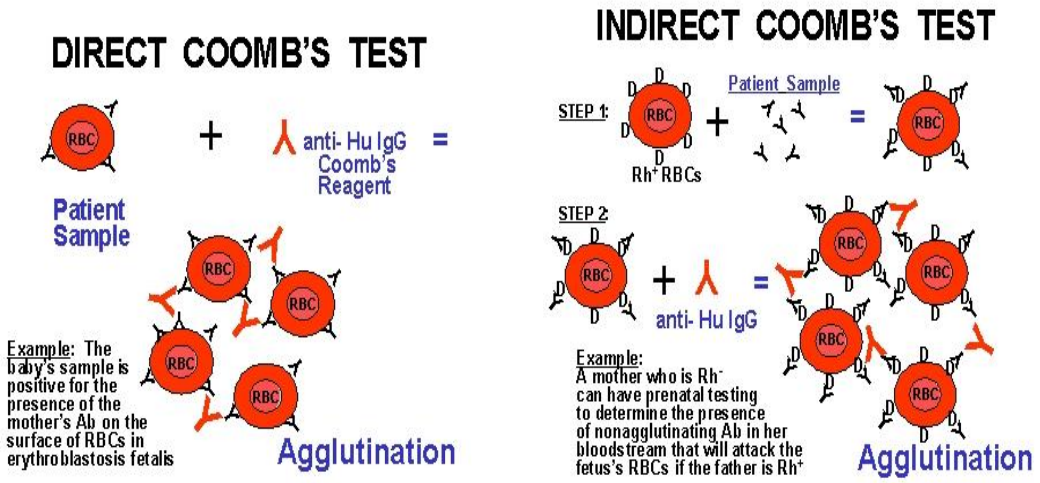

Figure 2: Direct and indirect coombs test.

2-Precipitation test:

Another form of serological test is the precipitation test. In this test, antibodies are called **precipitin**. They react with dissolved antigens and form large complexes that become visible as a fine precipitate. Test such as these can be performed in fluid or gel.

A-Radial immunodiffusion:

Radial immunodiffusion (or Mancini method, Mancini immunodiffusion, single radial immunodiffusion assay SRID) is an immuno diffusion technique used in immunology to determine the quantity of an antigen by measuring the diameters of circles of precipitin complex surrounding samples of the antigen that mark the boundary between the antigen and an antibody suspended in a medium, such as an agar gel. The diameters of the circles increase with time as the antigen diffuses into the medium, reacts with the antibody, and forms insoluble precipitin complexes.

B-Immunoelectrophoresis(IEP):

Immunoelectrophoresis, also called gamma globulin electrophoresis, or immunoglobulin electrophoresis. In immunoelectrophoresis, a complex mixture of antigens is placed in a well punched out of an agar gel and the antigens are electrophoresed so that the Ags are separated according to

their charge. After electrophoresis, a trough is cut in the gel and Abs is added. As the Abs diffuse into the agar, precipitin lines are produced in the equivalence zone when an Ag /Ab reaction occurs.

3-Enzyme-Linked Immunosorbent Assay (ELISA).

The enzyme-linked immunosorbent assay (ELISA) is a common laboratory technique which is used to measure the concentration of an analyte (usually antibodies or antigens) in solution.

Four Typical ELISA Formats:

1-DirectELISA:

In the direct ELISA test, a primary antibody is held on the walls of a microtiter plate. When the sample suspected to contain the antigen is added, there is an antigen-antibody reaction. Next, an enzyme-linked secondary antibody that is capable of reacting with the antigen is added. If antigen is present in the sample, it binds to this enzyme-linked antibody. When a colorless substrate is added, if a color develops, it indicates the presence of antigen.

2-IndirectELISA:

Antigen coated to a polystyrene multiwell plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody. The secondary antibody is usually an anti-species antibody and is often polyclonal.

3-SandwichELISA

Sandwich ELISAs typically require the use of matched antibody pairs, the first antibody, termed **the capture antibody** is coated to the polystyrene plate. Next, the analyte or sample solution is added to the well. A second antibody layer, **the detection antibody**, follows this step in order to measure the concentration of the analyte.

If the detection antibody is conjugated to an enzyme, then the assay is called a **direct sandwich ELISA.** If the detection antibody is unlabeled, then a second detection antibody will be needed resulting in an **indirect sandwich ELISA**.

4-Competition or Inhibition ELISA:

This is the most complex ELISA, and is used to measure the concentration of an antigen (or antibody) in a sample by observing interference in an expected signal output. Hence, it is also referred to as

an **inhibition ELISA**. It is most often used when only one antibody is available to the antigen of interest or when the analyte is small, i.e. a hapten, and cannot be bound by two different antibodies.

ELISA Results

The ELISA assay yields three different types of data output:

1)**Qualitative:** ELISA data provide a simple positive or negative results(yes or no) for a sample.

2) **Quantitative:** the optical density (OD) of the sample is compared to a standard curve. Known concentrations of target molecule are used to produce a standard curve and then this data is used to measure the concentration of unknown samples by comparison to the linear portion of the standard curve. This can be done directly on the graph or with curve fitting software which is typically found on ELISA plate readers.

3-Semi-quantitative:

ELISAs can be used to compare the relative levels of antigen in assay samples, since the intensity of signal will vary directly with antigen concentration.

4-IMMUNOFLUORESCENCE:

Immunofluorescence is an antigen-antibody reaction where the antibodies are tagged (labelled) with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope. Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescence.The fluorochromes commonly used in immunofluorescence are fluorescein isothiocyanate (green) and tetramethyl rhodamine isothiocyanate(red).

Types of immunofluorescence:

1- Direct immunofluorescence.2- Indirect immunofluorescence.3- Microimmunofluorescence.

Direct immunofluorescence: This technique is used to detect antigen in clinical specimens using specific fluorochrome labeled antibody. The steps involved are: Fixation of smear on the slide, treating with labeled antibody, incubation, washing to remove unbound excess labeled antibody and visualization under fluorescent microscope. This technique can be used to detect viral, parasitic, tumor antigens from patient specimens or monolayer of cells.

Another application is identification of anatomic distribution of an antigen within a tissue or within compartments of a cell.

Indirect immunofluorescence:

Indirect immunofluorescence is employed to detect antibodies in patient serum. It is often used to detect auto antibodies. Commonly used in the detection of anti-nuclear antibodies (ANA) found in the serum of patients with SLE.

Microimmunofluorescence:

This is a serological technique employed to detect antibodies in patient serum. It works on the same principle as that of indirect immunofluorescence but is performed on Teflon slides with many wells dotted with antigens. This technique is used in the serodiagnosis of Q fever, Mediterranean spotted fever.

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Immunological marker: C- reactive protein:

C-reactive protein (CRP) is a protein that is produced by the liver in response to factors released by fat cells (adipocyte) .It is a member of pentraxin family of proteins. CRP levels in the blood increases if there is an inflammation anywhere in the body and therefore high levels of CRP is cause for concern and can be evidence of burns, inflammation, trauma, infection, active inflammatory arthritis, some cancers and more recently CRP has been linked to atherosclerosis and heart disease. Because there are a large number of disparate conditions that can increase CRP production, an elevated level does not diagnose a specific disease.

The purpose of CRP is to bind to phosphocholine on surface of microbes, dead cell in order to activate the complement system via the C1q complex and it assists phagocytosis by macrophages which mean that it helps with the destruction and assimilation of bacteria, dead cells and small mineral particles.

CRP (i.e. acute phase protein) is believed to play an important role as an early defense system against infections in the body. Measuring CRP levels is useful in determining how a disease is progressing, and whether or not treatments given for the disease are working.

A high sensitivity test may be used to check your CRP levels called an hs-CRP test. In healthy persons CRP levels are less than 10 mg/L and increases slightly as one ages; higher levels are found in women during

late pregnancy, in women taking oral contraceptives and in cases of mild inflammation and viral infections.

In more recent times elevated levels of CRP have been linked to diabetes, hypertension and as said before heart disease and strokes; high levels of hs-CRP have consistently been used to predict recurrent coronary problems in patients.

Three laboratory methods used to measure serum CRP levels are as follows:

1- **Qualitative**: by latex agglutination
2- 2-**Semiquantitative**: by latex agglutination using serial dilution of sera sample.
3- **Quantitative**: by ELISA most sensitive, immunodiffusion.

Autoimmune disease:

1- Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology that is characterized by chronically inflamed synovial joints and subsequent destruction of cartilage and bone. RA is marked by several key characteristics, including synovitis occurring in a symmetrical fashion, polyarthritis, morning stiffness lasting over an hour, and the development of subcutaneous rheumatoid nodules. RA can present with many symptoms, including: pain, swelling, stiffness, joint deformity, and loss of movement.

Laboratory test used to diagnosis RA:

1-Rheumatoid factor RF:

The first autoantibody detected in patients with RA was RF, RF antibodies may be of the IgM, IgG, or IgA classes, and they target patient (Fc region of IgG.) Following the discovery of RF, the RF test became the primary laboratory test used in the diagnosis of RA and found in 60-80% of patients. **Many methods of RF detection have been developed:**

A- Particle agglutination tests employ latex, charcoal, or human erythrocytes as carrier molecules to which human or rabbit IgG is bound. Latex agglutination tests detecting IgM-RF are the most common methods used in laboratory diagnosis of RA. In rheumatoid arthritis, IgG antibodies produced by lymphocytes in the synovial joint react with the IgM antibodies (RF, rheumatoid factor) to generate immune complexes

that activate the complement and cause the tissue destruction, the lab value for RF of 1:20 or less is considered normal.

B- Nephelometry is another method used for detecting RF. In nephelometry, latex particles are coated with human IgG that captures RF Complexes formed between the IgG and RF are detected by light scattering. The degree of light scatter is dependent upon the concentration of immune complexes formed, making this a quantitative test.

C- A third method of RF detection is an enzyme-linked immunosorbent assay (**ELISA**). It is a solid phase assay that detects IgM- and IgA-RF using human IgG Fc as the substrate, and detects IgM-, IgG-, and IgA-RF if rabbit IgG is used as the substrate.

Rheumatoid factor can be positive in healthy people (in 1% of younger people and up to 5% of those over 70 years old).Rheumatoid factor can be detected in the blood up to 10 years before rheumatoid arthritis symptoms appear. However, rheumatoid factor is detectable after symptoms appear.

2 - Anti cyclic citrulline antibodies (anti-CCP)

It is antibodies that recognized epitopes that contained the amino acid citrulline, which is generated post-translationally from arginine by the enzyme peptidylarginine deiminase .

Anti CCP is a very useful blood test in the diagnosis of Rheumatoid arthritis. It is a protein produced as part of the process that leads to joint inflammation in rheumatoid arthritis. It is a test for early and specific diagnosis of rheumatoid arthritis. At present, anti- CCP antibody is the most specific marker for AR with a specificity of about 98%. Anti CCP antibody is also of prognostic significance in Rheumatoid arthritis. anti CCP antibodies predate the diagnosis of RA and may be found years before symptoms of RA develop.

3-Anti-A2/anti-RA33 antibodies

Anti-A2/anti-RA33 antibodies are directed to the heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2), a nuclear protein that is involved in mRNA splicing and transport antibodies occur in approximately one-third of RA patients.

4-ANA Profile:

The ANA profile is a series of tests that measure the presence of abnormal antinuclear antibodies. When the ANA is positive, it indicates that the patient may have an autoimmune disorder. However, this test alone is not a reliable diagnosis, since ANA may also be present in people who do not have RA.

5-Anti-Sa antibodies

Anti-Sa antibodies (vimentin) are directed to a 50 kDa protein of unknown structure and function that has been isolated from human tissues (spleen, placenta, rheumatoid syn-ovium). Anti-Sa auto antibodies are detected in approximately 40% of patients with established.

6-Anti-BiP antibodies:

It is antibodies to type II collagen (CII), immunoglobulin binding protein (BiP).It is also known as glucose-regulated protein a heat-shock protein family, and is localized in the endoplasmic reticulum. Anti-BiP auto antibodies are found in the sera of more than 60% of RA patients.

7- Erythrocyte Sedimentation Rate (ESR):

This test can help doctors differentiate between rheumatoid arthritis and osteoarthritis. It is also a test that is used to monitor disease activity and how well a patient is responding to treatment.

2-Systemic lupus erythematosus:

Systemic lupus erythematosus; often abbreviated as **SLE** or **lupus**, is a systemic autoimmune disease (autoimmune connective tissue disease) that can affect any part of the body. It is both type II hypersensitivity and a type III hypersensitivity reaction in which bound antibody-antigen (immune complex) pairs precipitate and cause a further immune response.

SLE most often harms the heart, joint, skin, blood vessel, liver, kidney and nervous system, The disease occurs nine times more often in women than in men, especially in women in child-bearing years ages 15 to 35.

Childhood systemic lupus erythematosus generally presents between the ages of 3 and 15, with girls outnumbering boys 4:1, and typical skin manifestations being butterfly eruption on the face and photosensitivity in humans.

Classification:

1- Systemic lupus erythematosus. (SLE is the most common and serious form).

2- Discoid lupus erthrematosus:1-Childhood discoid lupus erythematosus.2-Generalized discoid lupus erythematosus.3-Localized discoid lupus erythematosus.

3-Drug induced lupus erthematosus.

4- Neonatal lupus erthrematosus.

Signs and symptoms:

Symptoms vary from person to person. Almost everyone with lupus has joint pain and swelling. Chest pain when taking a deep breath, Fatigue, Fever with no other cause. General discomfort, (malaise), Hair loss, Mouth sores, Sensitivity to sunlight. Skin rash - a "butterfly" rash in about half people with SLE.

Lab Tests used to diagnose Lupus:

1. General Tests

• Urinalysis.
• Leukocyte Count.
• Hematocrit.
• Platelet Count.
• Creatinine/Blood Chemistry.
• Plasma Proteins.
• Erythrocyte Sedimentation Rate.
• C-Reactive Protein.
• Blood Glucose.
• Cholesterol.

2**. Immune Testing (autoantibodies**).

• ANA
• anti-dsDNA
• anti-Sm
• anti-Ro (SS-A)
• anti-La (SS-B)
• anti-Histone
• anti-RNP
• Lupus Anticoagulant
• Anti-Cardiolipan Antibody • Beta-2 Glycoprotein I

Antinuclear Autoantibody (ANA): is an antibody directed against material in the nucleus of cells and is the initial screening test for lupus. The ANA test is reported as a **titer** and a **pattern**.

Double-stranded DNA auto antibodies (dsDNA): are antibodies directed against genetic material in the cell. They are found in the majority of patients with lupus and elevated levels are characteristic of **lupus affecting the kidneys**. These auto antibodies are highly specific for lupus and are only rarely associated with other autoimmune diseases or infections. These auto antibodies have been found to fluctuate with **disease activity** and are a useful tool for monitoring **disease flares and remissions.**

Histone Auto antibodies: are antibodies directed against nuclear material in the cell. The presence of this antibody is characteristic of **drug-induced lupus**.

Antiphopholipid Antibodies: These auto antibodies include lupus anticoagulants (LA), anti-cardiolipan antibodies, anti- B2-glycoprotein I, Which bind to the surface of blood vessel walls and platelets. This group of auto antibodies is associated with an elevated risk of blood clotting, multiple spontaneous miscarriages and low platelet counts. Patients develop one of these illnesses and are said to have **antiphopholipid syndrome (APS**).

Complement (C3, C4, CH50) : a group of enzymatic proteins (C1-C9) found in normal blood serum that participate and are consumed in the inflammatory process. **Decreased levels of one or all three proteins** can be seen in SLE and may signify kidney disease.

Cryoglobulins : are immune complexes that form in SLE patients as a result of auto antibodies interacting with abnormal cellular components. These immune complexes may then deposit in tissues causing inflammation and damage. Detection of serum cryoglobulins serves as a crude indication of immune complexes circulating in the blood of SLE patients and may be **related to the activity of the disease.**

3-Celiac disease (Gluten- sensitive enteropathy):

Celiac disease is defined as a permanent intolerance to ingested gluten, the structural protein in wheat rye and barley. The ingestion of gluten results in small bowel mucosal inflammation, crypt hyperplasia and villous atrophy. CD is recognized as a chronic autoimmune disorder that

occurs in genetically predisposed individuals, both children and adults and it affects approximately 1% of the world population.

-**Common symptoms include**: Delayed puberty, Growth problems**,** Diarrhea (fatty)**,** Abdominal pain,bloating, Weight loss,Fatigue,Dermatitis herpetiformis, Mouth sores and Iron deficiency

-**Common tests for celiac disease include:**

1-IgA class of Anti-tissue Transglutaminase Antibody (anti-tTG): Tissue transglutaminase is an enzyme that causes the cross linking of certain proteins. Anti-tTG, IgA is the most **sensitive and specificity** blood test for celiac .The IgG class of anti-tTG may be ordered as an alternative in those who have a **deficiency of IgA.**

2-Quantitative immunoglobulin A (IgA): Used to determine if someone is deficient in the IgA class of antibodies and whether the IgG class of autoantibody tests should be performed.

3-Deamidated Gliadin Peptide (DGP) Antibodies, IgA*:* Anti-DGP test is a relatively new test that may be positive in some people with celiac disease who are **anti-tTG negative**, including children less than 2 years old.

4-Anti-Gliadin Antibodies (AGA), IgG and IgA classes*:* Gliadin is part of the gluten protein found in wheat (similar proteins are found in rye, barley, and oats). AGA is an autoantibody directed against the gliadin portion.

5-Anti-Endomysial Antibodies (EMA), IgA class*:* Endomysium is the thin layer that covers individual muscle fibers. Almost 100% of **patients with active celiac disease** and 70% of **patients with dermatitis herpetiformis** (another gluten-sensitive condition that causes an itchy, burning, blistering rash on the skin) will have the IgA class of anti-EMA antibodies.

6-Anti-Reticulin Antibodies (ARA), IgA class*:* Anti-ARA is **not as specific or sensitive** as the other auto antibodies. It is found in about 60% of celiac disease patients.

7-Anti-Actin (F-actin), IgA class*:* The F-Actin antibody test may **indicate increased intestinal damage.**

Lec 5

Serological test of Infectious disease

1-Streptococci

Species of *Streptococcus* are classified based on their hemolysis properties; Alpha-hemolytic species cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar *(S.pneumoniae, S.viridans*). Beta-hemolytic species cause complete rupture of red blood cells on blood agar; this appears as wide areas clear of blood cells surrounding bacterial colonies. Gamma-hemolytic species cause no hemolytic. Beta hemolytic streptococci which include group A, B, C, D, E, F, G, and H**.**

Group A beta-hemolytic streptococci (*S .pyogenes)* produce several enzymes— streptokinase (fibrionlysin), streptodornase (streptococcal decarboxylase) streptolysin, hyaluronidase, and DNase B, erythrogenic toxin (**cause scarlet fever**) that act as antigens in the human body. Serologic tests that measure the presence or level of the enzyme-specific antibodies are valuable diagnostic tools in the identification of conditions or diseases associated with previous streptococcal infections. **The streptococcal antibody tests include:**

1-Anti-streptolysin O (ASO or ASLO): is the antibody made against streptolysin O, an immunogenic, oxygen-labile hemolytic toxin produced by most strains of group A and many strains of groups C and G streptococcus. The O in the name stands for *oxygen-labile*; the other related toxin being oxygen-stable streptolysin-S. The main function of streptolysin O is to cause hemolysis (the breaking open of red blood cells) in particular, beta-hemolytic.

Clinical significance:

When the body is infected with streptococci, it produces antibodies against the various antigens that the streptococci produce. ASO is one such antibody. Raised or rising levels can indicate past or present infection. Historically, it was one of the first bacterial markers used for diagnosis and follow up of **rheumatic fever** or **scarlet fever**.

Acceptable values, where there is no clinical suspicion of rheumatism are as follows:

- **Adults**: less than 200 units, **Children**: less than 400 units

This titer has a significance only if it is greatly elevated (>200), or if a rise in titer can be demonstrated in paired blood samples taken days apart. The antibody levels begin to rise after 1 to 3 weeks of strep infection, peaks in 3 to 5 weeks and falls back to insignificant levels in 6 months. Values need to be correlated with a clinical diagnosis.

Estimation:

It is done by serology methods like **latex agglutination or slide agglutination**, **ELISA** may be performed to detect the exact titer value.To detect the titer value, by a non-ELISA method, one has to perform the above agglutination using a **serial dilution technique**.

2- **Antideoxyribonuclease-B titer (anti-DNase B, or ADB)**

Anti-DNase-B, or ADB, also detects antigens produced by group A strep, and is elevated in most patients with **rheumatic fever** and **post streptococcal glomerulonephritis.** This test is often done concurrently with the ASO titer, and subsequent testing is usually performed to detect differences in the acute and convalescent blood samples. When ASO and ADB are performed concurrently, 95% of previous strep infections are detected. If both are repeatedly negative, the likelihood is that the patient's symptoms are not caused by a **post streptococcal disease**.

What does the test result mean?

Negative anti-DNase B and ASO tests or these antibodies present at very low titers means that **the person tested most likely has not had a recent strep infection.** This is especially true if a sample taken 10 to 14 days later is also negative. An elevated antibody titer of anti-DNase or ASO, or rising titer of these antibodies, means that it is likely that **the person tested has had a recent strep infection**. If symptoms of rheumatic or glomerulonephritis are present, **an elevated anti-DNase B and/or ASO titer may be used to help confirm the diagnosis.**

3-Streptozyme

The streptozyme test is often used as a screening test for antibodies to the streptococcal antigens **NADase, DNase, streptokinase, streptolysin O, and hyaluronidase.** This test is most useful in evaluating suspected post streptococcal disease following *Streptococcus pyogenes* infection, such as rheumatic fever. Streptozyme has certain advantages over ASO and ADB.

4-Rapid detection of pneumococcal antigens in urine may be helpful in selected patients (especially patients with **meningitis** or those **who have received prior antibiotics**). **Latex agglutination tests** are widely used to diagnose pneumococcal meningitis, but the value of these tests for urine or blood is controversial. An **immunochromatographic test** detects the C-polysaccharide wall antigen of *S. pneumoniae*. This urinary antigen test enables a rapid diagnosis (within 15 minutes) of **pneumococcal pneumonia**, but was less sensitive than sputum Gram stains in some studies.

2-Salmonella

There are more than 2,000 different serotypes of salmonella. The bacteria are grouped into different serotypes using their O antigens and H antigens. The O antigen is a molecule that constitutes part of the bacterial cell wall, while the H antigen is part of the flagella or tail. Two of the best-known serotypes *are S. typhi* and *S.typhimurium;* the former is responsible for **typhoid fever and paratyphoid fever**, while the latter causes **food poisoning**. Stereotyping salmonella is a way to tell the medically harmful subtypes of this genus apart.

Serological test of Salmonella infection:

1-Widal test (slid agglutination):

A Widal test slide showing agglutinations in reactions of corresponding O and H antigens.

The **Widal test** is a presumptive serological test for enteric fever whereby bacteria causing typhoid fever are mixed with serum containing specific antibodies obtained from an infected individual. As with all serological tests, the rise in antibody levels needed to perform the diagnosis takes 7–14 days, which limits it applicability in early diagnosis.

The Widal test is positive if TO antigen is more than 1:160 in an **active infection,** or if TH antigen titer is more than 1:160 in **past infection or in immunized persons**.

2-**Tube Agglutination**: Tube agglutination is similar to slide agglutination, except that the procedure is performed in a test tube (or in multiple test tubes) to determine whether the antigen is present. Antibodies that are specific to the bacterial flagella will exhibit a characteristic pattern of agglutination where the bacteria are only loosely clumped together and can be shaken apart.

3-**ELISA test**: Enzyme-linked immunosorbent assays (ELISAs) form a powerful technique to rapidly identify bacteria from an unknown sample by their antigens. The double antibody sandwich assay is the most common technique for salmonella.

3-Brucella:

Brucellosis, also called, **Malta fever**, **Mediterranean fever**.

Serological tests:

1- ***Brucella*** **microagglutination test (BMAT)**: a modified version of the serum (tube) agglutination test (SAT), that can detect antibodies to *Brucella* species - *abortus*, *melitensis* or *suis*. For a diagnosis to be made using serology, two serum samples are required. The first serum sample should be taken when a person is acutely ill (≤7 days after symptom onset); the second serum sample should be drawn 2-4 weeks later to check for a rise in antibodies (a fourfold or greater rise in antibodies would be an individual is positive for brucellosis).

2-Rose Bengal plate test:

The Rose Bengal test (RBT) is a simple, rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum. It is often used as a screening test in **human brucellosis** and would be optimal for small laboratories with limited means. False-negative reactions occur especially in the early stages of acute infection.

Limitation of Rose Bengal Test:

1. Low sensitivity particularly in long evolution (chronic) cases, and relatively low specificity in endemic areas.
2. Moreover, some authors consider that prozones make strongly positive sera appear as negative in RBT.

The positive samples should be checked by the **Complement fixation test (CFT)** or by an IgG specific procedure such as **ELISA**. The RBT can be used in all animal species but positive results should be confirmed by a **quantitative test**.

3- The major antigens that are useful for diagnosis of brucellosis are the smooth (S) lipopolysaccharide (LPS) of the outer membrane and internal proteins. **The serum** (**tube) agglutination test (SAT)** detects antibodies to the S-LPS. Antibodies reacting against S-LPS can also be detected by

other tests, such as **ELISA** and the **Coombs test**. It is important to note that the **Coombs test** remains positive longer than other **agglutination tests.**

4-Ricktettsia

Rickettsia: is a genus of Gram negative bacteria,endospore, highly pleomorohism that can present as cocci ,rods (1–4 μm long) or thread-like (10 μm long). Being obligate intracellular parasites, the *Rickettsia* survival depends on entry, growth, and replication within the cytoplasm of eukaryote, host cell (typically endothelial cells).

Because of this, *Rickettsia* cannot live in artificial nutrient environments and is grown either in biological tissues or embryo cultures (typically, chicken embryos are used). In the past it was positioned somewhere between viruses and true bacteria, However unlike Chalmydia and *Mycolasma*, Rickettsial organisms possess true cell walls similar to other Gram negative bacteria.

Ricktettsia: can be divided into 3 groups:

1- Spottd fever group. (Rocky Mountain spotted fever***) Rickttsia rickettsii***.

2- Typhus group (epidemic typhus). ***Rickettsii prowazakii***.

Murine typhi, endemic typhus. ***(Rickettsia typhi**.)*

3- Scrub typhus group. ***(R. tsutsugamushi***).

Serological diagnosis:

A) Weil-Felix Test: The cheapest and most easily available serological test but is notoriously unreliable. The Weil-Felix test is based on the detection of antibodies to alkali based carbohydrate antigen which are shared by some Rickettsiae and certain strains of *Proteus* species, *P.vulgaris* OX19, and OX2 and *P.mirabilis* OXK. In Weil-Felix test, agglutinating antibodies are detectable after 5 to 10 days following the onset of symptoms, with the antibodies detected being mainly of the immunoglobulin M (IgM) type.

B) Indirect Immunoperoxidase (IIP): IIP used for the diagnosis of scrub typhus. The advantage of the immunoperoxidase assay is that the

results can be read with an ordinary light microscope. In addition, it provides a permanent slide record.

C) Microimmunofluorescence: is the test of choice for the serodiagnosis of rickettsial diseases .The micro-IFA has the advantage that it can simultaneously detect antibodies to a number of Rickettsial antigens (up to nine antigens) with the same drop of serum in a single well containing multiple rickettsial antigen dots.

D) Western Immunoblot: Western immunoblot assay with sodium dodecyl sulfate-gel electrophoresed and electroblotted antigens is a powerful serodiagnostic tool for seroepidemiology and confirmation of serologic diagnoses obtained by conventional tests. It is especially useful **in differentiating true-positive from false-positive results** created by cross-reacting antibodies.

E) Line blot Assay: The line blot assay allows the testing of more than 45 antigens simultaneously. It is a useful test for large-scale screening of sera when quantitative titers are not needed.

5-Infectious mononucleosis (IM):

Infectious mononucleosis (**IM**; also known as **mono**, **glandular fever**, and sometimes colloquially as the **kissing disease** (from its oral transmission) is an infectious, widespread viral disease caused by the Epstein –Barr virus(EBV) one type of Herpesviridae . Typical features of infectious mononucleosis include fever, pharyngitis, adenopathy, malaise, and an atypical lymphocytosis. Splenomegaly, hepatomegaly, jaundice, and splenic rupture can occur in patients with IM, most people are exposed to the virus as children, when the disease produces no noticeable or only flu- like symptoms.

Diagnostic tests.

1-**The monospot rapid latex agglutination test**, a form of the **heterophile antibody test**, is a rapid test for IM due to EBV. It is an improvement on the Paul-Bunnell test. The test is sensitive for heterophiles antibodies produced by the human immune system in response to EBV infection.

2- **Detection of EBN antibodies**:

During the initial three to four weeks of acute IM, EBV early antigen (EA) antibodies will begin to rise but then will quickly decrease over the

next three months or so. Also, early in the course of the illness, EBV viral capsid antigen (VCA) IgM antibody will begin to peak during the first two to six weeks of symptoms. It will then slowly decline over the following two to three months as well. Therefore, if repeat VCA IgM and EA levels remain low during the course of the illness, the diagnosis of acute IM is unlikely.

After two to three months from the onset of symptoms, VCA IgG antibodies begin to appear and will remain elevated for life. Also, around this time EBV nuclear antigen (EBNA) antibodies will begin to be detected. EBNA will also remain elevated for the remainder of one's life, and the presence of these antibodies likely excludes acute IM within the last year.

Antibody to Epstein-Barr nuclear antigen (EBNA), while typically not detectable until six to eight weeks after the onset of symptoms, can help distinguish between **acute and previous infections**. If EBNA is positive in a patient with acute symptoms and suspected infectious mononucleosis, previous infection is suggested. Elevated hepatic transaminase levels are relatively common in patients with infectious mononucleosis, occurring in approximately one half of patients

In summary, VCA IgM and EA antibodies appear in the first few months of symptoms and are indicative of acute IM, while VCA IgG and EBNA antibodies appear much later in the course and during convalescence.

Table 1: **Progression of Epstein Barr Virus Serological Markers**

Infection status	EA	VCA IgM	VCA IgG	EBNA
No prior infection	-	-	-	-
Early infection	+	+	+ / -	-
Late infection	-	- / +	+	- / +
Past/Latent	-	-	+	+

3- Besides specific EBV antibody testing, there is other non-specific laboratory testing that may support the diagnosis of acute IM. During the initial two to six weeks of symptoms the patient will often have

leukocytosis with an elevated number of atypical lymphocytes (greater than 10%) and may also have a mildly decreased platelet count.