Ministry of Higher Education& Scientific Research
Al-Furat Al-Awsat Technical University
Kufa Technical Institute
Medical Lab Tech. Department

Practical Basic Immunology

Second Stage

First Semester

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Important Terms

Immunology:- the branch of sciences dealing with the components of the immune system, immunity against disease, the immune response, and immunological techniques of analysis.

Immunity can be defined as the way in which the body can protect itself from invasion by pathogenic microorganisms, and provide a defense against their harmful effect.

Antibody(Ab):- A protein (immunoglobulin) that recognizes a particular antigen and binds with it, Antibodies are produced by B cells.

Antigen(Ag): A substance that reacts with antibodies or T-cell receptors.

Immune system :- a complex network of cells, tissues, organs, and substances that it produces to help the body fight infections and other diseases.

Specimens and body fluids used in immunology Lab..

1- Serum:- yellow, clear fluid contain protein ,carbohydrate, sugar, lipid, water, but non contain fibrinogen.

Serum is made when we don't add any anti-coagulant then the clotting factors promote clot formation. These clots which contain fibrinogen as a clotting agent efficiently remove red blood cells from the plasma as a solid mass. Serum forms a larger percentage of blood than plasma and is most widely used in research because it removes the unwanted red blood cells more efficiently generating more volume per unit of blood.

2- Plasma:- yellow, clear fluid contain protein, carbohydrate, sugar , lipid , water and fibrinogen. Plasma is made by Starting with whole blood, where an anticoagulant is added immediately after collection, then coagulation is prevented and all components are kept in suspension .If you just leave this blood sample alone without mixing, then all the components will settle out. The heavier cells will sink to the bottom. This results in a clear liquid at the top. This clear upper layer is plasma which is essentially all the components

of blood minus the cells. Under centrifugation, a layer of white cells called the buffy coat forms between the plasma and red blood cells. Plasma retains fibrinogen

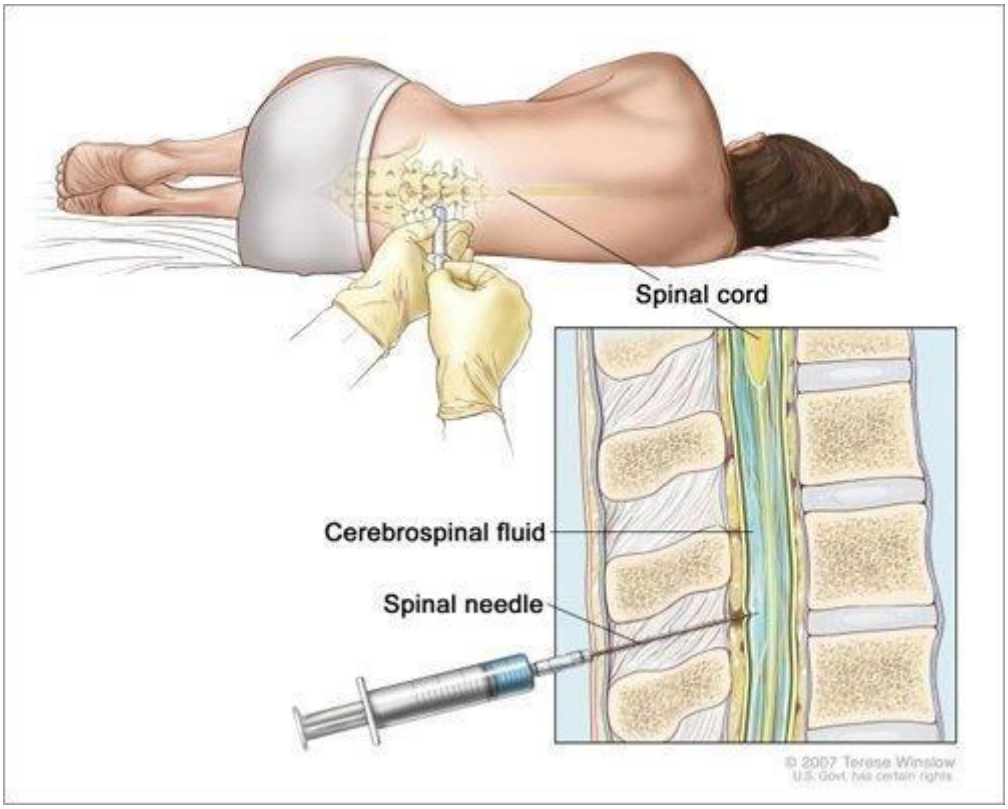
Following are the important difference between plasma and serum:

Plasma	Serum
A transparent, straw-colored, liquid portion of the blood.	An undiluted fluid, the extracellular portion of blood.
It is composed of serum and clotting factor.	It is the part of the blood which lacks clotting factor.
It is acquired after centrifuging blood with the anticoagulant.	It is acquired after centrifuging of coagulated blood.
Anticoagulant is required to obtain plasma from the blood sample.	Anticoagulant is not required to separate the serum from the blood sample.
Consists of 55% of the total volume of blood.	Less volume in comparison to plasma.
Comparatively easier and less time is required to separate the plasma from the blood sample.	Difficult to separate serum from the blood sample. It is a time-consuming process.

Contains fibrinogen.	Lacks fibrinogen.
Consists of 92% water with proteins, salts, lipids, and glucose.	Consists of 90% water with dissolved hormones, proteins, minerals, and carbon dioxide.
Has 1.025 g/ml density	Has 1.024 g/ml density.
Has a long life. It can be preserved for up to ten years.	Has a short life. It can be preserved only for a few months.
Plasma is the main medium for excretory product transportation.	An important source of electrolytes.
Cells are freely suspended in plasma.	Cells are attached together by clot formation

3-Urine:- urine is one of the metabolic wastes. It consists of water, urea (from amino acid metabolism), inorganic salts, creatinine, ammonia, and pigmented products of blood breakdown, one of which (urochrome) gives urine its typically yellowish color. In addition, any unusual substances for which there is no mechanism of reabsorption into the blood remain in the urine . Also possibly found pus cells, Epithelial cells, and RBCs. and crystals when infected by diseases.

4- Cerebrospinal Fluid**(CSF)** : less sample uses in the lab. And similar serum in characterized. Cerebrospinal fluid (CSF) is a clear, colorless, watery fluid that flows in and around your brain and spinal cord. it acts like a cushion that helps protect your brain and spinal cord from sudden impact or injury. The fluid also removes waste products from the brain and helps your central nervous system work properly. A CSF analysis is a group of tests that use a sample of cerebrospinal fluid to help diagnose diseases of the brain and spinal cord and other conditions that affect the central nervous system.



The factors effect on the immunological samples include:-

1- chemical factors -(Base, acid, clean reagent) these material caused change of ph.

2- physical factors-(heat, shaken, light), Heating samples caused denaturation phenomena it mean destroyed the serum protein by heat.

3- Biological factors :- (Bacteria, fungi, insect(fly).

You can keep samples from effect these factors by:-

1- storing in the deep freeze (-40)-(-70)c when need storing for long time increase on 7 days.

2- storing in the refre.(4-8)c when need storing for short time (1 hour- 7 days).

* The urine sample non storing . if you need it for 16 hour can keeping in (4-8) c.

Serum preparation

General objective :-

1-To isolate the serum from other component of blood.

2- prepare the plasma and erythrocyte Ag

3- To learn the difference between plasma and serum .

4- To learn how to get serum without causing hemolysis of RBCs.

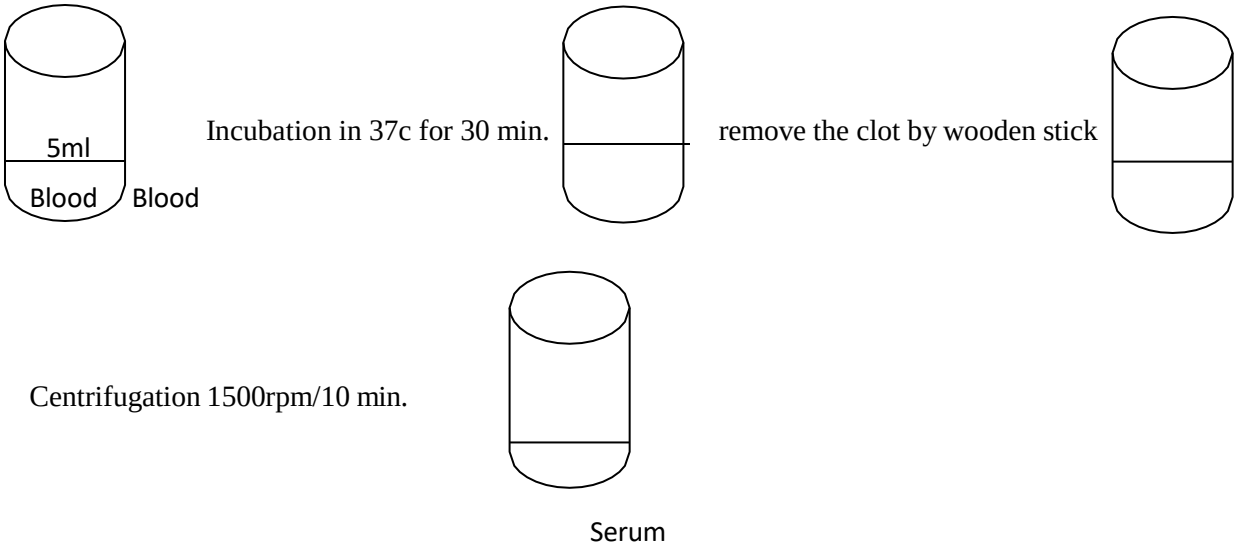
5- To learn how to store the serum.

Materials:-

1- Test tubes. 2-wooden sticks. 3-centerifuge . 4- water bath . 5- incubator.

6-disposable syringe (5 ml).

Method :-



The serum preparing must be yellow ,clear some time was turbid this is mean the serum contain high rate from lipid cannot remove it . and sometime was red this mean occur hemolytic when prepare it , cannot remove it. If the time for centrifugation little the RBCs remain in the super net ,so must be repeat centrifugation .

Plasma and Erythrocyte Ag preparation

Aim:

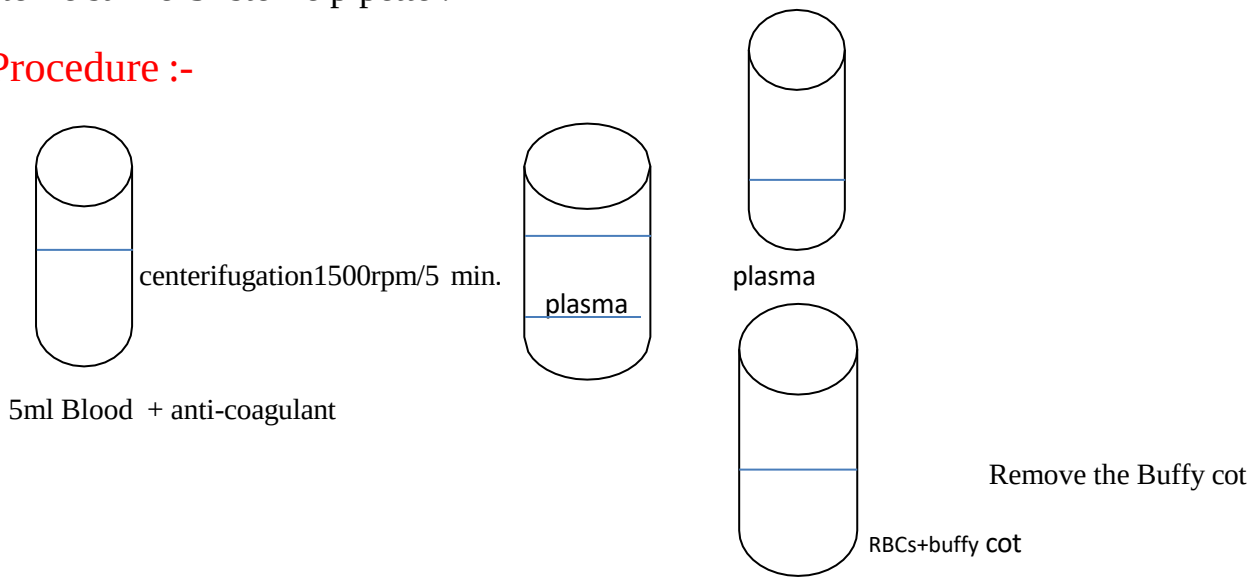
1- prepare the plasma.

2- prepare Erythrocyte Ag.

Materials : -

1- 10 ml of sterile citrated blood . 2- sterile centrifuge tubes . 3- sterile vaccine vials . 4- sterile saline 5- sterile pipette .

Procedure :-



washing RBCs by Normal saline (3 step) to get a good Ag

Blood group	Ag	Ab
A	A	anti-B
B	B	anti-A
AB	A,B	---
O	---	anti A ,B

Dilution and serial dilution (titration)

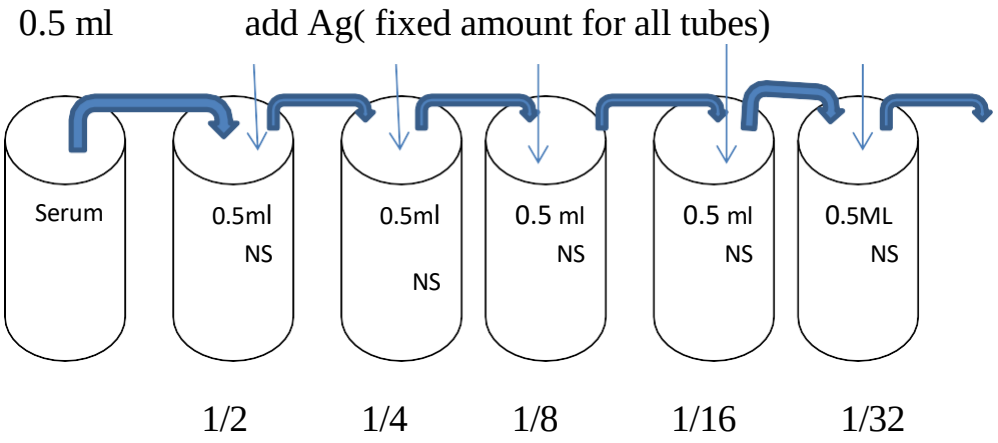
Principle:-

Precipitation reaction :- it is immunological reaction occur between Ag and Ab character by (Ag , Ab soluble and specific for them).

Precipitation reaction uses for identify the Ab when know Ag and identify the Ag when know Ab .

Serial dilution:-

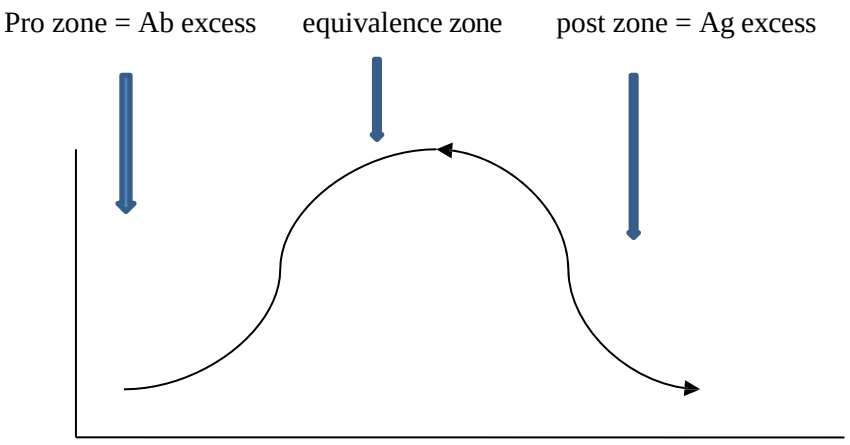
$$\text{Dilution} = \frac{\text{Part}}{\text{All volume}} = \frac{\text{serum}}{\text{serum + N.S}}$$



Micro dilution:-

Micro titer system by sever 1962 . uses metal diluter to transfer and mix the solution .

Titer:- reciprocal of the last dilution give positive result (appearance precipitation).



Precipitation curve

Bactericidal effect of normal serum

Introduction :-

fresh normal serum contain substance capable of killing microorganism (Gve- and Gve+) these substance called humeral factors include :-

1- Antibody (Ig G , IgM , IgA , IgE, ,IgD). 2- Complement [C_1- C_9]

3- proper din . 4- Betalysin . 5- CRP. 6- Bacteriocidin.

These substance can be destroyed if the serum heated at 56c for 30min. .

Principle:-

This study of humeral factors which found in the normal serum and its effect on the common pathogenic bacterial gram ve+ and gram ve – bacteria by mixing both of them with heated and unheated sera .

Materials:-

1- 1.5 ml fresh unheated human serum .

2- 1.5 ml fresh heated human serum .

3- saline (sterile).

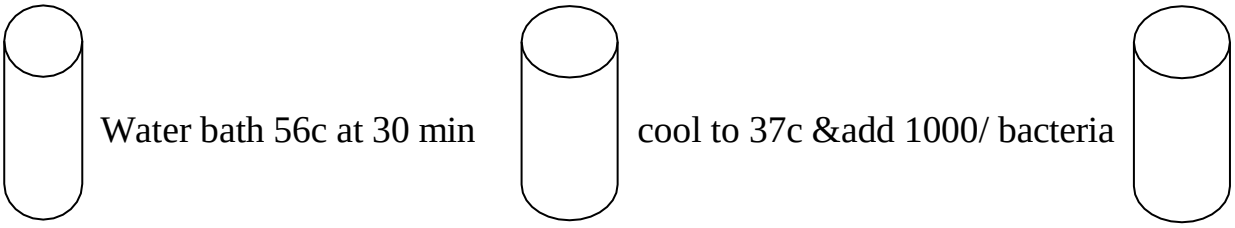
4- pipette volume (1, 2)ml (sterile).

5- Nutrient agar media in plate .

6- 24 hour *staph.* Broth culture and *E. coli* broth culture

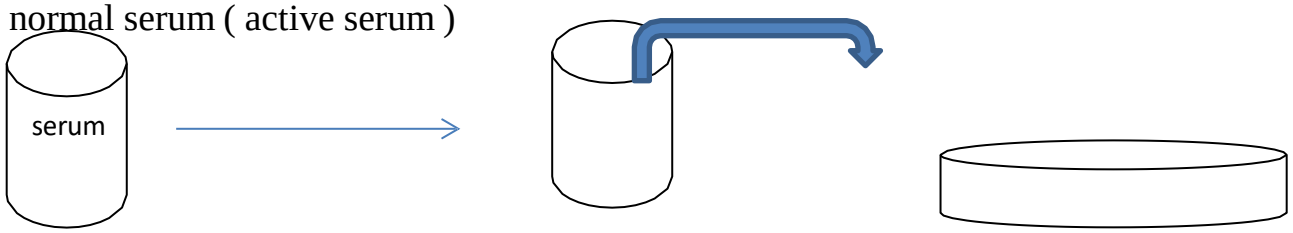
Procedure:-

A- inactivated serum



Incubatin37c for 1 hour → culture 1ml in the plate → incubate 37c for 24 hour → count the colony.

B- normal serum (active serum)



Add 1000bacterial cell incubate in 37c for 1 hour

culture 1ml in the plate → incubate 37c for 24 hour → count the colony

* Why you incubate the serum and Bacteria in 37c for 1 hour ?

* why culture 1ml after incubation 1 hour ?

* why you cool the serum for 37c ? .

* why you use water bath 56c at 30 min. ?

Bacterial Antigen

Bacterial antigen of the genus of *salmonella* have been widely used in general they consist of the somatic (o) antigen and protein flagellin as flagella (H) antigen .

Aim:-

Vaccine preparation

Principle :-

To prepare vaccine (Ag stimulate immune response and safe to use). *Salmonella typhi*. is pathogenic treated with chemicals formalin 0.6% absolute Al-cohol to kill it .

Material :-

1- scrow cap test tubes.

2- sterile formalin – saline .

3- absolute al – cohol.

4- nephlometer tubes system .

5- salmonella N. agar .

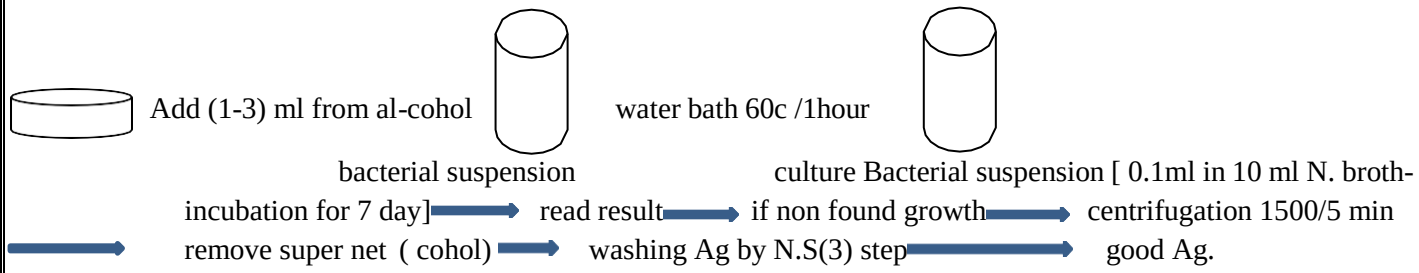
6- glass rod (L-shape).

7- sterile cotton – stoppered pipettes.

8- sterile vaccine vials.

Procedure : -

A- O-Ag



B- H-Ag

culture0.1ml in 10 ml N. broth → incubation for 7 day → read result → if non found growth → centrifugation 1500/5 min. → remove super net (formalin) → washing Ag by N.S(3) step → good Ag.

*O-Ag :- it is polysaccharide Ag . it is stand by heat but destroyed by chemical factor .

* H-Ag :- it is flagellin Antigen . it is with stand by chemical factor but destroyed by heat.

Q- why you used al-cohol and heat in O-Ag preparation ?

Q- why you use formalin in H-Ag preparation ?

Q- why you washing bacterial Ag before uses it ?

Antibodies production in Experiment animal

Introduction : -

The purpose of this exercise is to stimulate the production of antibodies in rabbit so as to study the properties of antigen-antibody reaction in vitro by using serological method. The rabbit will be assigned to each of group of student by used picric acid because this reagent can react with fur of rabbit .they are to be handled very gently .

Aim :-

Antibody production.

Material:-

1- Antigen preparation .

2- tuberculin syringe for injection .

3- cotton ball .

4- centrifuge tubes and screw cap tubes.

Procedure :-

Each group of students give prepare Ag to rabbit in marginal vein by tuberculin syringe (25) gag by dependent these tables.

bacterial suspension		Erythrocyte Ag 10%	
Day	mount	day	mount
First day	0.01 ml	First day	1 ml
3rd day	0.25 ml	Second day	1 ml
5th day	0.25 ml	3rd day	1 ml
8th day	0.5 ml	4th day	1 ml
10 day	0.75 ml	6th day	1 ml
12 day	1 ml	8th day	1 ml

Sven to 10 day after the last injection the animal may be trial bleed .Post immunization bleeding by : -1- intracardialy. 2- via the marginal ear vein

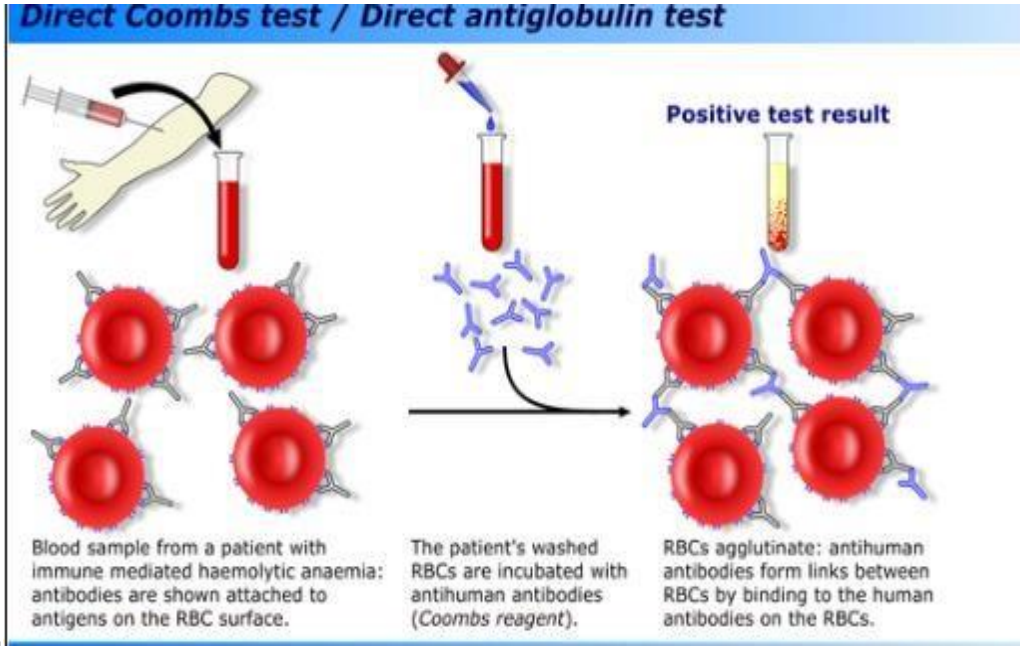
After bleeding preparation the serum . the serum will be frozen until testing .

Agglutination

Agglutination:- mean immune reaction occur between Ag and Ab by found large molecule (RBCs or latex).

Types of agglutination:-

1- direct agglutination:- coombs test



2- indirect agglutination:-

Ag on the latex or RBCs + Abs from serum = agglutination

This aggl. Used for diagnosis bacterial infection.

3- Reversed agglutination :-

Abs on the RBCs or Latex + Ag from serum = agglutination This aggl. Used for diagnosis viral infection.

Phases of agglutination :-

1-Primary phase :-

Ag + Abs = Immune complex

2- Secondary phase :-

IC + IC = Lattice formation

Agglutination test may be performed on a slide if agglutination is rapid , but tube tests more readily adapt to semi- quantization , or tittering of the agglutination .

Pregnancy test

Pregnancy is the term used to describe the period in which a fetus develops inside uterus. Pregnancy usually lasts about 40 weeks, or just over 9 months, as measured from the last menstrual period to delivery.

pregnancy tests detect the hormone human chorionic gonadotrophin (HCG), which starts to be produced around 6 days after fertilization.

Human chorionic gonadotropin (HCG) **is a glycoprotein hormone secreted by the developing placenta beginning shortly after fertilization.** At the time of the first missed menstrual period, HCG concentrations in serum and urine are about 100mIU/ml and double in concentration every 1.2 to 2 days. Peak levels of over 100,000 mIU/ml HCG are seen late in the first trimester of pregnancy.

The direct pregnancy assay is based upon the latex agglutination reaction between latex particles coated with anti-HCG antibodies and HCG present in the test specimen. The presence of HCG in a urine specimen results in the formation of an agglutination matrix which is visually differentiated from the non-agglutinating negative control. The direct pregnancy latex test is calibrated to detect HCG levels at or greater than 200mIU/ml. The Pregnancy latex test kit comprises; latex test reagent, positive/ negative controls, pipette-stirrers and an agglutination slide

advised **women Don't drink too much water**, or any liquid, before taking a pregnancy test because **Excess fluids can impact the accuracy of test**, also **dilute of hCG levels which can change the test result**

Getting a negative result doesn't mean not pregnant, it may just mean hCG levels are not high enough for the test to detect the hormone in urine.

Procedure:-

+ cont.	Test / urine	- cont.
+ cont. with latex	Filter urine with latex	- cont. with latex

After 3 min. from mixing the material read the result.

Interpretation:-

Positive : visible agglutination .

Negative :- smooth suspension with no visible agglutination at the end 3 min.

Doubtful (+) :- No visible agglutination but rather rough suspension at the end 3 min. with hold the interpretation test again at a later date .

***HCG found in human body in many cases include :-

1- pregnant women.

2- chronic inflammation .

3- uterus cancer .

4- prostate cancer

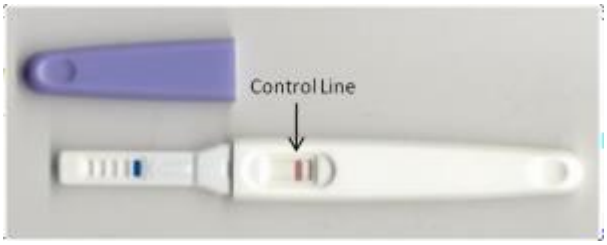
*** Notes :-**

Must be filtration the urine before used it in the test To remove any large molecule example RBCs , pus cell , Epithelial cell , crystal it is effect on the reaction between Ag (HCG) and Ab (anti HCG) .

Also can diagnose the pregnancy by using specific strips.

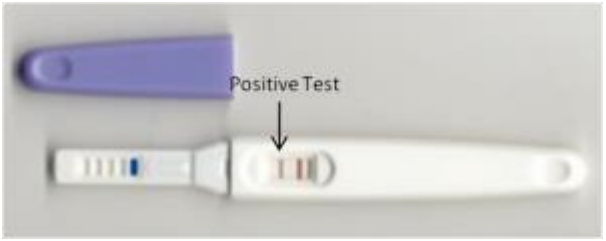
RESULT INTERPRETATION

Negative Result: NOT PREGNANT



Only one color band appears on the top Control (C) region. There should be no apparent band on the Test (T) region .

Positive Result:



color bands appear on the Control (C) and Test (T) regions. The color intensity of the bands may vary according to concentration and level of HCG development. The test line is usually slightly weaker in intensity in comparison to the control line. The pattern of increasing intensity of the test line is a much better predictor of pregnancy .

C-Reactive Protein (CRP) Test

C-Reactive Protein (CRP), also known as **Pentraxin 1**, CRP is an acute phase reactant, a protein made by the liver and released into the blood within a few hours after tissue injury, the start of an infection, or other cause of inflammation. It is named CRP because it is only react with c- substance found in *streptococcus* cell wall.

Normally, the body has low levels of C-reactive protein in blood. High levels may be sign of a serious infection or other disorder. it can indicate a host of other health concerns, including infection, arthritis, kidney failure, and pancreatitis.

In general, the normal CRP level is less than 0.9 milligrams per deciliter (mg/dL). if increased over that mean is a sign of acute inflammation .so a diet which includes fish, olive oil, walnuts, flaxseeds and chia seeds will bring down inflammation and CRP levels.

A CRP test may be used to find conditions that cause inflammation. These include:

1-Bacterial infections.

2-A fungal infection

3-Inflammatory bowel disease

4-An autoimmune disorder

CRP test uses

1- if found symptoms of a serious bacterial infection, include: [Fever ,Chills ,Rapid breathing, Rapid heart rate ,Nausea and vomiting].

2-used to monitor for success of treatment.

3- It is done to check for infection after surgery. CRP levels normally rise within 2 to 6 hours of surgery and then go down by the third day after surgery. If CRP levels stay elevated 3 days after surgery, an infection may be present .

Reagent:-

1-Ag.

2- Ab (serum) .

3- positive and negative control .

4-Buffer solution PH= 8.8 .

Principle of CRP Test

The C-Reactive Protein test is based on the principle of the latex agglutination. When latex particles complexed human anti-CRP are mixed with a patient's serum containing C reactive proteins, an visible agglutination reaction will take place within 2 minutes.

Procedure of CRP Test

Qualitative Test

1-Bring all reagents and serum sample to Room Temperature and mix latex reagent gently prior to use. Do not dilute the controls and serum.

2-Place 1 drop of Serum, Positive control and Negative control on separate reaction circle on glass slide.

3-Then add 1 drop of CRP latex reagent to each of the circles.

4-Mix with separate mixing sticks and spread the fluid over the entire area of the cell.

5-Tilt the slide back and forth slowly for 2 minutes observing preferably under artificial light, Observe for visible agglutination .

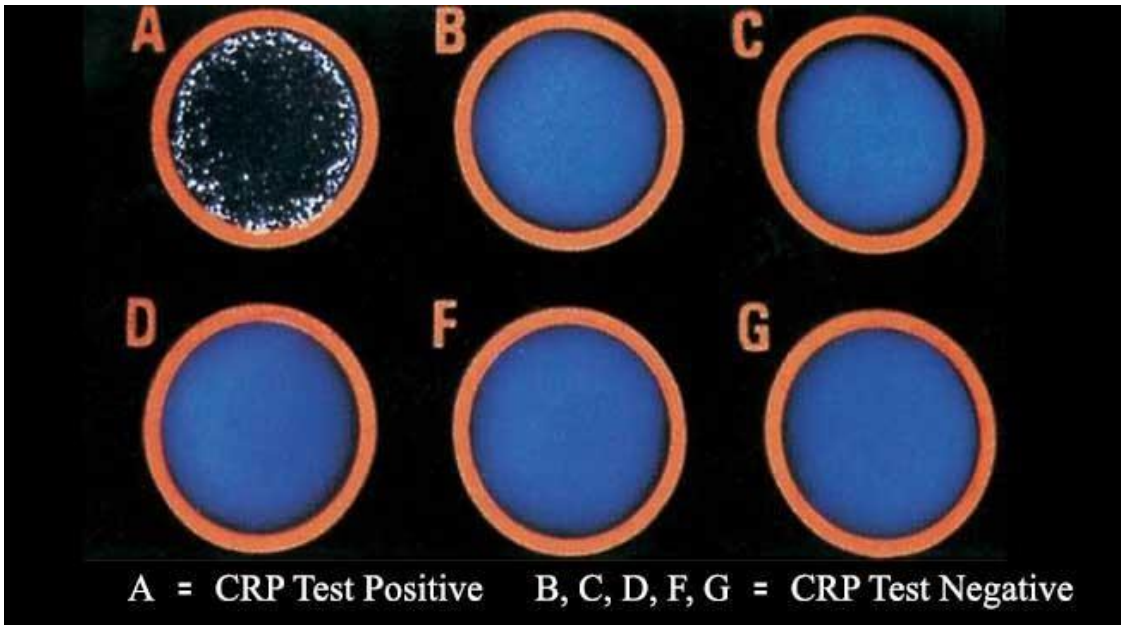
Semi-Quantitative Test

1. Prepare dilution of the specimen with physiological saline 0.9%, as indicated in the following table

Dilution	CRP (ug/ml) in undiluted sample
1:2	14
1:4	28
1:8	56
1:16	112
1:32	224
1:64	448

2. Then proceed for each dilution as in qualitative test.

Result Interpretation of CRP Test



Positive: Agglutination of latex particles, indicating the presence of C – reactive protein at a significant and detectable level.
Negative: No Agglutination.
For Semi-Quantitative Test Results, the last dilution of serum with visible agglutination is the CRP titer of the serum.

CALCULATION OF TITRE:
CRP ug/ml = 7 x D, where D is the highest dilution of serum showing agglutination and 7 is the sensitivity in ug/ml

Widal Test

Widal Test is an agglutination test which detects the presence of serum agglutinins (H and O) in patients serum with typhoid and paratyphoid fever. the Widal test is reliable and can be of value in the diagnosis of typhoid fevers in endemic areas. The patient's serum is tested for O and H antibodies against the following antigen suspensions :

S. *Typhi* O antigen suspension

S. *Typhi* H antigen suspension

S. *Paratyphi* A O antigen suspension

S. *Paratyphi* A H antigen suspension

S. *Paratyphi* B O antigen suspension

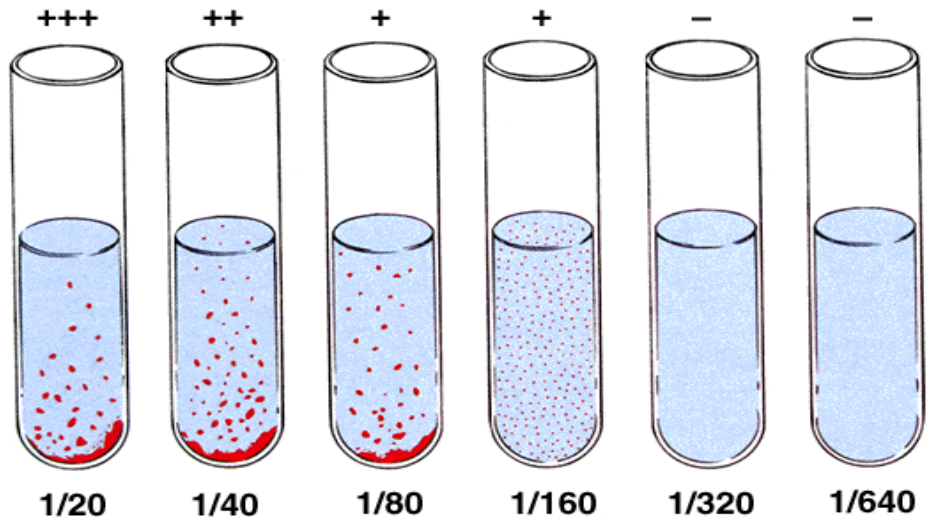
S. *Paratyphi* B H antigen suspension

S. *Paratyphi* C O antigen suspension

***S.* Paratyphi C H antigen suspension**

- *Salmonella* antibody starts appearing in serum at the end of the first week and rises sharply during the 3rd week of endemic fever. In acute typhoid fever, O agglutinins can usually be detected 6–8 days after the onset of fever and H agglutinins after 10–12 days.
- *Salmonella* antigen suspensions can be used as slide and tube techniques.

The main principle of the Widal test is that if the antibody is present in the patient's serum, it will react with the antigen and gives visible agglutination in the tube. The antigens used in the test are "H" and "O" antigens of Salmonella types.



Agglutination of tube" Titer = 160

Slide test

-Place one drop of positive control on one reaction circle of the slide.

-Pipette one drop of Isotonic saline on the next reaction circle. (-ve Control).

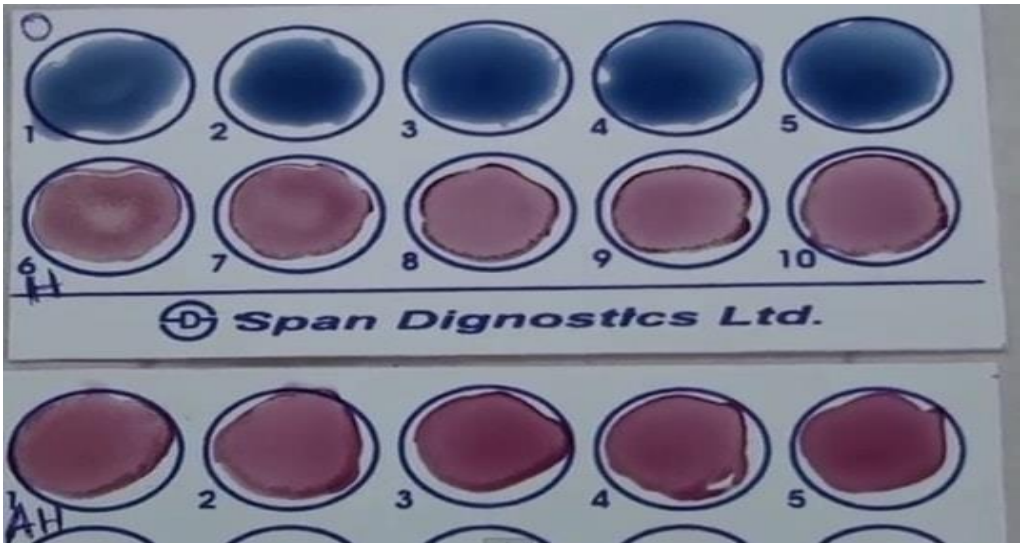
-Pipette one drop of the patient serum to be tested on the remaining four reaction circles.

-Add one drop of Widal test antigen suspension ‘H’ to the first two reaction circles.

-Add one drop each of ‘O’, ‘H’, ‘AH’, and ‘BH’ antigens to the remaining four reaction circles.

-Mix the contents of each circle uniformly over the entire circle with separate mixing sticks.

-Rock the slide, gently back and forth, and observe for agglutination macroscopically within one minute.



interpretation

- Agglutination is a positive test result and if the positive reaction is observed with 20 µl of the test sample, it indicates the presence of clinically significant levels of the corresponding antibody in the patient serum.
- No agglutination is a negative test result and indicates the absence of clinically significant levels of the corresponding antibody in the patient serum.

Limitations of Widal Test

-The Widal test may be falsely positive in patients who have had previous vaccination or infection with *S. Typhi*.

- the test cannot distinguish between current infection and previous infection or vaccination against typhoid.

-False positive Widal test results occur in typhus, acute falciparum malaria (particularly in children), chronic liver disease, and nephrotic syndrome.

-False-negative results may be associated with early treatment, with "hidden organisms" in bone and joints, or due to antibody responses being blocked by early antimicrobial treatment.

Weil – Felix Test

First described in 1916, the Weil-Felix reaction is a test used in the diagnosis of rickettsial infections .The known pathogenic rickettsia species are gram-negative, obligate intracellular , no motile , spherical or rod organism bacteria. but cannot reproduction outside living cell .They are separated into 3 groups: the epidemic and endemic typhus group, the scrub typhus group, and the spotted fever group .Rickettsia can infect arthropods(Lice , mites , ticks) and transmitted to human.

The test was developed upon the observation that certain serotypes of Proteus bacteria display antigenic cross-reactivity with Rickettsia species. The isolation of these antigens

helped identify antibodies against the Rickettsia disease groups. *P. Vulgaris* OX19 antigen reacts with antibodies to the typhus group, *P. mirabilis* OXK antigen reacts with antibodies to the scrub typhus group, and *both P. Vulgaris* OX2 and OX19 antigens react with antibodies to the spotted fever group .Due to its low sensitivity and specificity, the Weil-Felix test has its use and is no longer recommended in routine practice. The current standard in the diagnosis of rickettsial infections is indirect immunofluorescence.

Principle:-

weil-felix test to base on cross – reaction of Ab to Rickettsia with Ag to the polysaccharide somatic Ag present on certain strain of proteus .

Procedure:-

Weil-Felix test can be performed in slide or in test tube.

Slide method:

- Place 50-100µl of patient serum on the slide
- Add a drop of desired antigen (Proteus OX19 or OX2 or OXK)
- Mix the suspension by rotating the slide for 1 minutes
- Visible agglutination indicates the positive test

Application:

Very useful for diagnosis in case of epidemic typhus

Limitations:Weil-Felix test is a nonspecific test and cannot be relied on for diagnosis of rickettsial infection. Orientia tsutsugamushi gives a false negative test while Proteus spp gives a false positive test.

Sensitivity= less than 33% Specificity= 46%

Syphilis disease

Syphilis is a sexually transmitted infection (STI) caused by a type of bacteria known as *Treponema pallidum*. The first sign of syphilis is a small, painless sore. It can appear on the sexual organs, rectum, or inside the mouth. This sore is called a chancre

Syphilis is only spread through direct contact with syphilitic chancres. It can't be transmitted by wearing another person's clothing, sharing a toilet with another person or using another person's eating utensils. It is treatable, but If it's left untreated, it can spread through the body and cause complications in organs.

Stages of syphilis infection:- The four stages of syphilis are:

- **Primary stage:-** The primary stage of syphilis occurs about (3-4)after contracting the bacteria. It begins with a small, painless sore called a chancre. it's highly infectious. The sore remains for anywhere between 2-6 weeks. Syphilis is transmitted by direct contact with a sore. This usually occurs during sexual contact.
- **secondary stage**:- **Skin rashes** may develop during the second stage of syphilis. The rash won't itch . Other symptoms of secondary syphilis may include: (Headaches, swollen lymph nodes ,fatigue , fever , weight loss ,hair loss aching joints)
- Latent stage :- The third stage of syphilis is the latent. The primary and secondary symptoms disappear, and there won't be any noticeable symptoms at this stage. This stage could last for years before progressing to tertiary syphilis.
- Tertiary stage:- The last stage of infection is tertiary syphilis. Tertiary syphilis can occur years after the initial infection. it can be life-threatening. Some other potential outcomes of tertiary syphilis include: (Blindness , deafness , mental illness , memory loss , destruction of soft tissue and bone , neurological disorders, such as stroke or meningitis ,heart disease , neurosyphilis, which is an infection of the brain or spinal cord)

*Syphilis is most infectious in the first, two stages

*When syphilis is in the latent stage, the disease remains active but often with no symptoms. Tertiary syphilis is the most destructive to health.

Treating syphilis

Primary and secondary syphilis are easy to treat with a penicillin injection. if the patient is severely allergic to penicillin can use (doxycycline).

Mothers infected with syphilis are at risk for premature births or maybe will pass the disease on to the fetus.

VDRL Test

Venereal Disease Research Laboratory (VDRL) Test is a slide flocculation test used in the diagnosis of syphilis. it is not a specific test because the antigen used in this test is cardiolipin, which is extracted from beef heart. This test is also classified as a non-treponemal. The VDRL test isn't perfect, but it's a trusted test that can be a first step in helping to determine the infection.

In cases, may screen for syphilis even without symptoms . For example, in case pregnant as a routine part of care, when treated for another STI such as HIV, and to sure treatment worked.

The VDRL test needs a blood sample, that doesn't require to fast or stop taking any medications

Principle: Patients suffering from syphilis produce antibodies(Wassermann) that react with cardiolipin antigen in a slide flocculation test, which are read using a microscope.

Requirements: Patient's serum, water bath, cardiolipin, VDRL slide, mechanical rotator, syringe, pipettes, and microscope. positive and negative serum controls.

Procedure: Patients' serum is inactivated by heating for 30 minutes in a water bath (56 c) to remove non-specific inhibitors (example complement). The test can be worked both

qualitatively and quantitatively to diagnose the disease and determine the antibody titers . Depending on the size the results are classified as weakly reactive (W) or reactive (R).

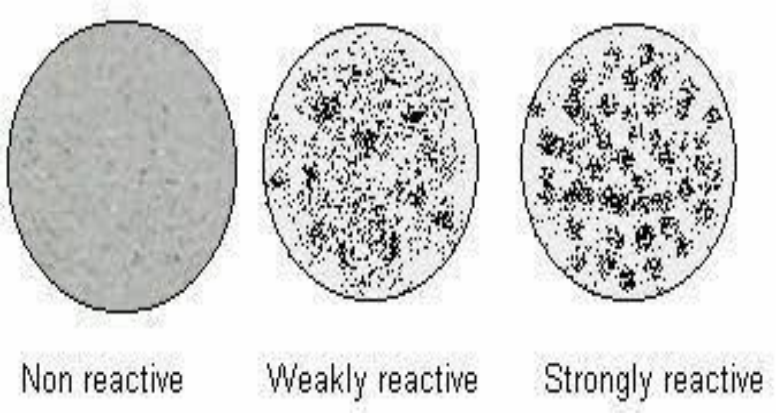
Result

If the test comes back **negative** for syphilis antibodies, the result don't have syphilis. but If the test comes back **positive** for syphilis antibodies, that probably (but not definitely) have syphilis, will use a specific test to confirm the results (TPHA test).

false-negative results-when infected with syphilis before less than three months.

Or in late-stage syphilis

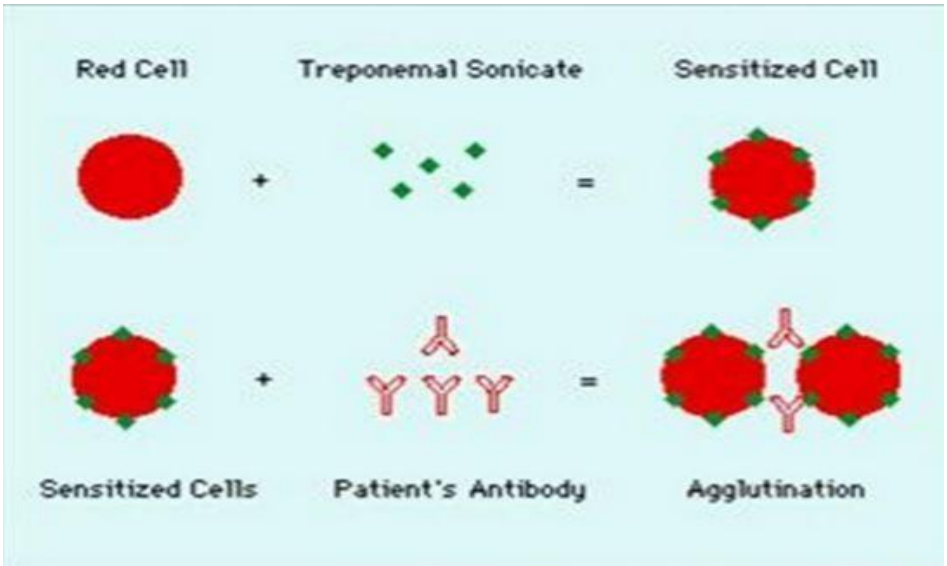
false-positive results because infection by HIV, malaria , pneumonia (certain types only) , systemic lupus erythematosus



Treponema pallidum Haemagglutination Test (TPHA)

test used specific antigens for the detection of antibodies to *Treponema pallidum* in the diagnosis of Syphilis.

TPHA Test Principle



The Red cell used is took from avian or chicken

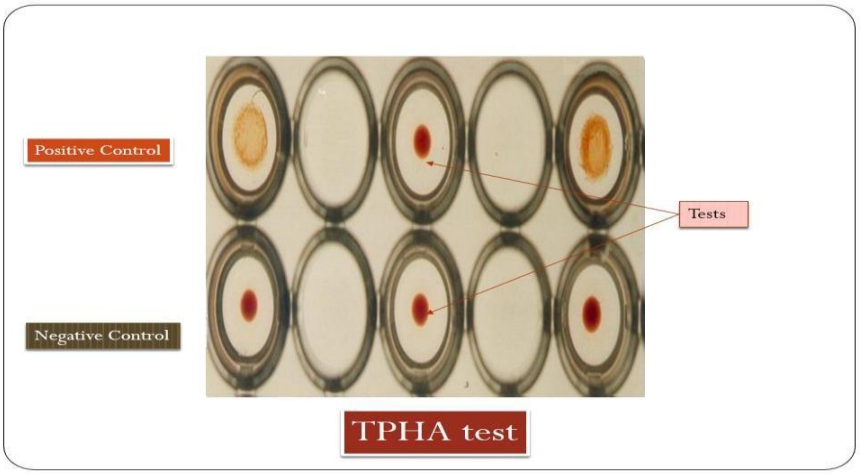
Reagents

1. **Test cell and Control cell suspensions.** 3-**Buffer**
2. **Inactivated serum (56** ºC for 30 mint.).

Procedure

Before performing the test procedure, bring the sample, diluent, control, and test cells at room temperature (25 – 30ºC). The TPHA test blood is a routine blood and does not require any specific preparations. A sample for the test is drawn from vein of arm. the TPHA test procedure is carried out by using a test plate with three wells.

Results and Interpretation



Results	Test Cells	Control Cells
Strongly Reactive	Full cell pattern covering the bottom of the well.	No agglutination tight button
Weakly Reactive	Cell pattern covers approx. 1/3 of well bottom	No agglutination tight button
Nonreactive	Cells settled to a compact bottom, typically with a small clear center.	No agglutination tight button

Hydatid cyst

•Human echinococcosis is a parasitic disease caused by tapeworms of the genus Echinococcus.

•The two most important forms in humans are cystic echinococcosis and alveolar echinococcosis.

•Humans are infected through ingestion of parasite eggs in contaminated food, water or soil, or after direct contact with animal hosts.

•Echinococcosis is often expensive and complicated to treat and may require extensive surgery and/or prolonged drug therapy.

•Prevention programs focus on deworming of dogs, which are the definitive hosts.

Echinococcosis occurs in 4 forms

1-hydatid disease - caused by infection with a species complex centered on *Echinococcus granulosus;*

2-alveolar echinococcosis, caused by infection with *E. multilocularis*.

3-polycystic caused by infection with *E. vogeli*.

4-Unicystic caused by *E. oligarthrus.*

symptoms

hydatid disease the infection is located most often in the liver and lungs, and less in the bones, kidneys, spleen, muscles, and central nervous system. The asymptomatic incubation period of the disease can last many years until hydatid cysts grow to an extent that triggers clinical signs. **Abdominal pain, nausea, and vomiting** when hydatids occur in the **liver**. If the **lung** is affected, clinical signs include **chronic cough, chest pain, and shortness of breath** and Non-specific signs include anorexia, weight loss, and weakness Alveolar echinococcosis is characterized by an asymptomatic incubation period of 5–15

years and the slow development of a primary tumor-like lesion which is usually located in the liver. Clinical signs include weight loss, abdominal pain, general malaise, and signs of hepatic failure.

diagnosis :- Imaging techniques, such as CT scans, ultrasonography, and MRIs, are used to detect cysts. After a cyst has been detected, serologic tests may be used to confirm the diagnosis, by detecting IgG antibodies to hydatid cyst fluid.

The Casoni skin test is a hypersensitivity-based skin test used to detect hydatid disease. Although once a major test in diagnosing hydatid disease it has largely been superseded by newer more sensitive, specific, and safer serological tests. The test is only 63.8% sensitive and 47% specific to hydatid disease .

Echinococcus agglutination Test

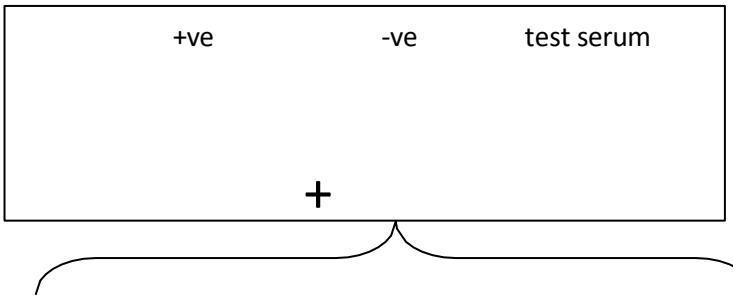
Reagent :-

1- Ag (hydatid fluid).

2- control Ag (sheep serum) for confirm the positive result).

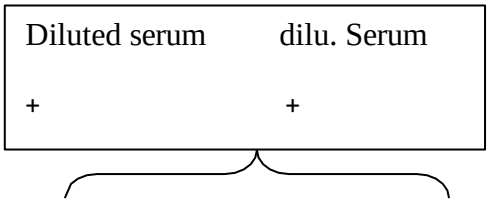
3- positive and negative control serum .

4- Buffer solution PH=8.2 for quantitation determination of Abs titers.



Negative (no agglutination) positive (agglutination)

Non infection dilution serum (1/2,1/4,1/8)



+ aggl. +aggl.

Confirmative test

Dilution the serum 1/16,1/32, 1/64

And repeat the work if the result was positive this mean infection.

Treatment

albendazole and Mebendazole are the two most commonly used drugs to treat.

albendazole- fixed doses of 400 mg twice a day in adults for four weeks, If drug treatment fails, the cyst is surgically removed.

Toxoplasmosis

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*. Infections with toxoplasmosis usually cause no obvious symptoms in adults because their **immune system usually keeps the parasite from causing illness**. Occasionally, people may have a few weeks or months of mild, flu-like illness such as muscle aches and tender lymph nodes. the parasite remains in the person's body in an inactive state. It can become reactivated if the person becomes immunosuppressed.

Toxoplasmosis is not passed from person to person, except in instances of mother-to-child (congenital) transmission and blood transfusion or organ transplantation. People typically become infected by three principal routes of transmission:-

1-Foodborne:- as eating undercooked, contaminated meat ,eating food that had contact with raw contaminated meat and drinking unpasteurized milk

2-Animal-to-human (zoonotic).

3-Mother-to-child (congenital).

An organ transplant and a blood transfusion from a Toxoplasma-positive donor can be caused the infection.

Diagnosis

serologic testing test that measures immunoglobulin G (IgG) and immunoglobulin M (IgM) .also be made by direct observation of the parasite in stained tissue sections, cerebrospinal fluid (CSF), or other biopsy material.

Toxoplasma IFA(Indirect Fluorescent Antibody) test is used to detect specific antibodies to *Toxoplasma gondii.*

Interpretation:-

1- any serum antibody titer is > 16 should be considered evidence of infection at some time with *Toxoplasma gondii*.

2- Antibodies appear within 2 wk of the onset of infection and peak within 2 months.

3- Antibodies may be detected in normal adults.

Infection mononucleosis Test(glandular fever)

I.M: is an acute self-limited disease of the reticuloendothelial system diagnosed on the bases of 3 characteristic:

1-clinical symptoms.

2-heamatological manifestation(granulocyte and monocyte)

3-serological result (agglutination test)

Patient suffering from I.M. disease in general character by :-

1- depressed cell-mediated immunity

2-form variety of humeral antibody(heterophil Ab)

3-Abs to human erythrocyte and lymphocyte

4-Abs to virus especially the EBV

5-serodiagnosis of patient with I.M. based on detection of paul-bunnel antibody in the patient sera

Principle:- Dependent on the reaction between the Ag (EBV)binding with the Horse RBCs and Abs (anti – EBV) found in the serum .read result dependent on appearance of agglutination .

Material:

1-positive and negative control. 2-4% horse erythrocyte suspension

3-slide and wooden stick.

Procedure:-

1- Allow reagent and serum sample to reach 18-25c one drop of positive control serum , one drop of patient s serum .

2- add one drop of mixed (well shacked) erythrocyte suspension to each drop of serum.

3- use the wooden stick applicator to mix the erythrocyte- serum mixture .

4- using a bright light to check for hem agglutination .

Result:

EBV antibody test

Normal (negative):	No IgM antibody against EBV is present. If the antibody IgG is present, it may mean that you have been exposed to EBV in the past.
Abnormal (positive):	The antibody IgM against EBV is present.

Interpretation:-

1-the test is usually positive when symptoms appear but it may remain negative up to 3wk after clinical onset

2-usually antibody appear (7-14) day after onset of disease.

Viral Hepatitis

Hepatitis may be caused by viral infection. Hepatitis A, B, C, D, and E viruses infect hepatocytes and cause acute or chronic hepatitis. HBV and HCV can progress to chronic disease, but HDV and HEV may also present chronically in some circumstances. The symptoms of viral hepatitis are nonspecific and include jaundice, fever, and a lack of appetite. Diagnosis cannot be made by clinical evaluation alone. Chronic and sometimes

acute viral infections can be asymptomatic or mildly symptomatic. However, even in the absence of symptoms, chronic viral hepatitis infections can result in cirrhosis, liver failure, or hepatocellular carcinoma if undiagnosed and untreated .

HAV is a vaccine-preventable disease. HAV causes an acute disease that may be asymptomatic or present with enteric symptoms. Populations at an increased risk for hepatitis A include travelers and persons who use illegal drugs .For diagnosis of HAV, use immunoglobin M (IgM) HAV antibody testing .

HBV is a vaccine-preventable disease that may occur acutely or chronically. Chronic infection is often asymptomatic until the onset of cirrhosis or end-stage liver disease. Transmission of HBV generally occurs through contact with the blood or body fluids of an infected person. may be transmitted from mother to fetus .Several laboratory tests should be used for the diagnosis of HBV.

The table below details the expected HBV test results for HBV markers in various clinical situations.

Interpretation	HBsAg	IgM Anti-HBc	IgG Anti-HBc	Total Anti-HBc	Anti-HBs
No past or current infection or immunization	Negative	Negative	Negative	Negative	Negative
Immune due to vaccination	Negative	Negative	Negative	Negative	Positive
Acute HBV	Positive	Positive	Negative	Positive	Negative
Chronic HBV	Positive	Negative	Positive	Positive	Negative

HCV may occur acutely, but the majority of cases (75-80%) become chronic. Acute and chronic diseases are commonly asymptomatic. Transmission of HCV is parenteral .

Good Luck