Name:Sawera khattak

ID:14915

Subject:Immunology

Submitted to:Sir Zahir

Department:Microbiology

Viva assignment.

Question number :1

Answer:

Blood grouping:

one of the classes (such as those designated A, B, AB, or O) into which individuals or their blood can be separated on the basis of the presence or absence of specific antigens in the blood. — called also blood type.

Principle of Blood grouping:

The ABO and Rh blood grouping system is based on agglutination reaction. When red blood cells carrying one or both the antigens are exposed to the corresponding antibodies they interact with each other to form visible agglutination or clumping.

Materials Required:

Monoclonal Antibodies (Anti-A, B and D)

Blood Lancet

Alcohol swabs

Tooth picks

Sterile cotton balls

Clean glass slide

Ice tray

Biohazard disposal container

Procedure:

- Set the table with all the materials required. Remember to place the Monoclonal Antibody (Mab) kit in an Ice tray.
- Open an Alcohol swab, and rub it at the area from where the blood will be sampled (finger tip). (Discard the swab)
- Open the Lancet cover, put pressure at the tip of the finger from where blood will be sampled (maintain it). Prick the finger tip with the opened Lancet.(Discard the Lancet)
- As blood starts oozing out, make 1 drop fall on the three depressions of the glass slide. (in clinical setup, there will be a fourth well used as a control).

- Place a cotton ball at the site where it was pricked. Using the thumb, put pressure on the area to stop blood flow.
- Take the Anti-A (blue) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 1st spot. Place the bottle back in ice.
- Take the Anti-B (yellow) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 2nd spot. Place the bottle back in ice.
- Take the Anti-D (colorless) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 3rd spot. Place the bottle back in ice.
- Take a tooth pick and mix the content in each well. Discard the tooth pick after using in one well (take a new one for the next well).

After mixing, wait for a while to observe the result.

ABO Blood Grouping

Discovered in 1901 by Karl Land

A person's ABO blood group-A, B, AB, or O—is based on the presence or absence of the A and B antigens on his red blood cells.

The A blood type has only the A antigen and the B blood type has only the B antigen. The AB blood type has both A and B antigens, and the O blood type has neither A nor B antigen.

By the time a person is six months old, he naturally will have developed antibodies against the antigens his red blood cells lack.

That is, a person with A blood type will have anti-B antibodies, and a person with B blood type will have anti-A antibodies.

A person with AB blood type will have neither antibody, but a person with O blood type will have both anti-A and anti-B antibodies.

Although the distribution of each of the four ABO blood types varies between racial groups, O is the most common and AB is the least common.

The ABO grouping is the first test done on blood when it is tested for transfusion.

Principle:

This ABO grouping test is based on the principle of haemagglutination reaction.

Haemagglutination of red cells occur when the red cell antigens (agglutinogens) react with corresponding antibody

(a) positive agglutination is observed by the clumping of red cells as seen on slide or by button formation in the test tube,

(b) negative reaction is observed by uniform distribution of red cells on slide and lack of button formation in test tube.

Procedure:

There are two basic methods to observe the haemagglutination reactions in ABO blood grouping

(i) slide method

(ii) test tube method.

Former is easier to perform and the latter is more sensitive. Many laboratories of developing countries perform the tube test only in case the result of the slide test is doubtful.

However, tube method is recommended for reliable results as per FDA approved guidelines.

Slide Method

Requirement:

Glass slides, Pastuer pipettes, Applicator sticks and centrifuge. Reagents

1. Anti-A sera (blue color):

Human polyclonal or murine monoclonal.

2. Anti-B sera (yellow color):

Human polyclonal or murine monoclonal.

3. Normal saline:

0.9 g/dl sodium chloride in distilled water.

Specimen:

Clotted blood is generally used. Centrifuge the clotted blood at 1500 rpm for few min. to separate serum.

With the help of Pastuer pipette, separate the red cells from the clot and suspend them in saline.

Anti-coagulated blood with proper anticoagulant like EDTA can be used. Store the specimen at 2-8oC if there is any delay in examination.

Blood obtained by finger puncture may be tested directly by the slide method. To avoid clotting of the collected blood (on the slide), it should be mixed quickly with antisera.

Procedure:

1. Prepare a 10% suspension of red blood cells in normal saline as follows:

(i) Mix 0.05 ml (5 drops) of sedimented red cells with 2 ml of normal saline, (ii) Centrifuge at 1,500 rpm for 1 to 2 min.

Discard supernatant, (iii) Add 2 ml of normal saline to the sedimented red cells. Mix well. This gives a 10% suspension of red cells.

2. On one-half of a glass slide, place 1 drop of anti-A sera.

3. On the other-half slide, place 1 drop of anti-B sera.

4. Using Pastuer pipette add one drop of the red cell suspension to each half of the slide.

5. With separate applicator sticks, mix each cell- serum mixture well.

6. Tilt the slide back and forth and observe for agglutination.

Interpretation:

a. Tests that show no agglutination within two minutes are considered negative

b. Do not interpret peripheral drying or fibrin stands as agglutination.

Tube Method:

Requirement:

1. Test tubes (10 x 75 mm or 12 x 75 mm)

2. Microscope

Procedure:

1. Prepare a 5% suspension of red blood cells in normal saline as follows: (i) Mix 0.05 ml (5 drops) of sedimented red cells with 2 ml of normal saline, (ii) centrifuge at 1,500 rpm for 1 to 2 min.

Discard supernatant, (iii) Add 4 ml of normal saline to the sedimented red cells. Mix well. This gives a 5% suspension of red cells.

2. To a small test tube, add one drop of anti-A sera.

3. To a second test tube, add one drop of anti-B sera.

4. Using a Pastuer pipette, add one drop of 5% red cell suspension to each of the two test tubes.

5. Mix well and centrifuge both the tubes at 1,500 rpm for one min. or incubate at room temperature for one min.

6. Examine for agglutination: If the tube is centrifuged red cell sediment will be seen at the bottom of the tube, which is called a button.

Gently tap the button from the tube by a spring action of right index finger and dislodge the cell button.

If, red cells form one or more clumps with clear supernatant fluid, the agglutination is present.

If, red cells re-suspend easily, without any visible clumping, agglutination is absent.

7. In case of any doubt, take a drop of the suspension on a slide and observe under the 10X objective for agglutination.

Forward and Reverse Grouping:

To determine ABO blood group, there are two ways:

1. When the individuals red cells are tested with a known anti – A and anti- B sera, this procedure (as described above) is called forward grouping or front typing or cell typing.

2. When the individuals serum is tested with known group A cells and group B cells, the procedure is called reverse grouping or back typing or serum typing.

Rh Blood Typing:

Rh typing (also called as Rh blood grouping) is next important to ABO blood grouping. It detects only the presence of Rh antigen (or D antigen) out of all Rh factors on the red cells.

Principle:

It is based on the principle of haemagglutination that, the red cells with Rh antigen (D antigen) will clump with anti-D antiserum at room temperature in presence of protein.

The technique is similar to ABO blood grouping and hence, Rh typing is done along with ABO blood grouping.

Procedure:

The Rh typing can also be done by two methods:

1. Slide method and

2. Tube method

Slide Method

Requirements:

Same as that of ABO slide method.

Reagents:

1. Anti-D sera (human polyclonal or human monoclonal).

2. Normal saline

Specimen:

Same as that of ABO method.

Procedure:

1. On a pre-warmed glass slide, place one drop of anti-D serum.

2. By using a Pastuer pipette add one drop of 10% suspension of red blood cells (in case of anaemic patients, use one drop of sedimented red cells)

3. With an applicator stick, mix cell-serum mixture well.

4. Tilt the slide back and forth and observe for agglutination.

5. Tests that show no agglutination within two min. are considered negative.

Tube Method

Requirements:

Same as that of ABO tube method.

Reagents:

1. Anti-D sera (human polyclonal or human monoclonal).

2. Normal saline.

Specimen:

Same as that of ABO method.

Procedure:

1. Prepare a 5% suspension of red blood cells in normal saline.

2. To a test tube add one drop of anti-D serum.

3. Add one drop of cell suspension with the help of Pasture pipette.

4. Mix well and centrifuge both the tubes at 1,500 rpm for one min. or incubate at room temperature for one min.

5. Examine the agglutination reaction in each tube by dislodging the button gently. If necessary, use a magnifying hand lens.

6. Interpretation: Agglutination will be recognised by the formation of small clumps in a clear liquid. As the bottom of the test tube is tapped, the clumps whirl up and then settle down.

This will be marked as positive reaction and the cells are identified as Rh- positive. If the red cells re-suspend homogeneously with no visible clumps, it should be marked as negative reaction and the cells are identified as Rh- negative.

7. All the Rh negative cells must be tested for Du.

Why blood typing is done?

Blood typing is done prior to a blood transfusion or when classifying a person's blood for donation. Blood typing is a fast and easy way to ensure that you receive the right kind of blood during surgery or after an injury. If you're given incompatible blood, it can lead to blood clumping, or agglutination, which can be fatal.

Blood typing is especially important for pregnant women. If the mother is Rh-negative and the father is Rh-positive, the child will likely be Rh-positive. In these cases, the mother needs to receive a drug called RhoGAM. This drug will keep her body from forming antibodies that may

attack the baby's blood cells if their blood becomes mixed, which often happens during pregnancy.

Risks of blood typing:

You will need to have your blood drawn in order to have it typed. Having your blood drawn carries very minimal risks, including:

bleeding under the skin (hematoma)

fainting or feeling lightheaded

infection at the puncture site

excessive bleeding

Blood group chart:

Blood Type	A	B	O	AB
Rh-positive	A+	B+	O+	AB+
Rh-negative	A-	B-	O-	AB-

Question number:2

Answer:

ICT-Immunochromatographic test:

Introduction

Immunochromatography assay (ICA), namely lateral flow test, is a simple device intended to detect the presence or absence of the target analyte. The concept of immune-chromatography is a combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions. The most widespread immunochromatographic system is the test strip**.**

Developing an ICA

Strips used for ICA contain four main components:

1. Sample Application Pad

It is made of cellulose and/or glass fiber and sample is applied on this pad to start the assay. Its function is to transport the sample to other components. Sample pad should be capable of transportation of the sample in a smooth, continuous and homogenous manner. This pretreatment may include separation of sample components, removal of interferences, adjustment of the pH, etc. analyte sample should be added to the sample application pad to start the test.

2. Conjugate Pad

It is the place where labeled biorecognition molecules (labeled antibodies, usually nano colloid gold particle) are dispensed. Material of conjugate pad should immediately release labeled conjugate upon contact with moving liquid sample. Labeled conjugate should stay stable over entire life span of the lateral flow strip. Any variations in dispensing, drying or release of conjugate can change the results of assay significantly. Poor preparation of labeled conjugate can

adversely affect sensitivity of the assay. Glass fiber, cellulose, polyesters and some other materials are used to make conjugate pad.

3. Substrate(Nitrocellulose) Membrane

It is highly critical in determining sensitivity of ICA. Test and control lines are drawn over this piece of membrane. So an ideal membrane should provide support and good binding to capture probes (antibodies, etc.). Nonspecific adsorption over test and control lines may affect results of assay significantly, thus a good membrane will be characterized by lesser non-specific adsorption in the regions of test and control lines. Proper dispensing of bioreagents, drying and blocking play a role in improving sensitivity of the assay.

4. Adsorbent Pad

It works as sink at the end of the strip. It also helps in maintaining flow rate of the liquid over the membrane and stops back flow of the sample. Adsorbent capacity to hold liquid can play an important role in results of assay.

All these components are fixed or mounted over a backing card. Materials for backing card are highly flexible because they have nothing to do with ICA except providing a platform for proper assembling of all the components. Thus, backing card serves as a support and it makes easy to handle the strip.

Major steps in ICA are:

(i) Preparation of labeled antibody and capture antibody against target analyte;

(ii) Immobilizing the labeled antibody onto conjugate pad, and the capture antibody onto the strip membrane to form the Test/Control line.

(iii) Assembling of all components onto a backing card after dispensing of reagents at their proper pads.

(iv) Add samples and buffer onto sample pad.

(v) Wait the sample flow through the test and control line for 5-10min.

(vi) Read the result when the color reveal.

Application:

Immunochromatographic methods are widely used in clinical practice for;

Detection of Toxins

Pregnancy tests- detection of human chorionic gonadotropin (hCG)

Diagnosis of parasitic infections

1. Malaria: Malaria- detect specific antigens (Plasmodium lactate dehydrogenase, Plasmodium aldolase, and P. falciparum histidine-rich protein-2) produced by malaria parasites in the blood of infected individuals.
2. G. lamblia and Cryptosporidium parvum (ImmunoCardSTAT! Cryptosporidium/Giardia)
3. G.lamblia, E. histolytica/E. dispar, and C. parvum on fecal specimen (Triage Micro Parasite Panel)

Diagnosis of bacterial infections

1. Mycoplasma pneumoniae (ImmunoCard Mycoplasma, which detects Mycoplasma pneumoniae specific IgM in serum samples)
2. H.pylori antigens in stool (ImmunoCardSTAT SpSA)

Diagnosis of viral Infections

Antigen detection

1. RSV (Binax NOW RSV, Remel Xpect RSV)
2. Rotavirus (ImmunoCardSTAT! Rotavirus)
3. Influenza A/B (BinaxNOW Influenza A & B)
4. Hepatitis B and Hepatitis C infection

Antibodies detection

Detection of HIV-1 and HIV-2 antibodies (OraQuick Advance Rapid HIV-1/2 antibody test, Reveal G4 Rapid HIV-1 antibody test, Multispot HIV-1/HIV-2 Rapid Test, etc)

Interpretation of test results

- Positive result: A clear line in the control zone and in the test area on the membrane.
- Negative result: A single line in the control zone.
- Invalid: A single line in the test area without a corresponding control line

Advantages

Commercially available and low cost (compared with EIA, Immunofluorescence or RIA)

Comparable or better sensitivity and specificity than other well-established methods

Rapid test

Requirement of small sample volume

Easy to perform (no sample pre-treatment required in most of the cases)

Simple and user-friendly (to perform as well as to interpret test result)

Can be used in the field or rural settings: stability over a wide range of environmental conditions and very long shelf life.

Limitations

Mostly qualitative or semi-quantitative

Most of the devices can detect more than one or two analytes simultaneousl