ASSIGMENT IMMUNOLOGY;-

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SUBMITTED TO ;-SIR ZAHIR

PROCRDURE OF BLOOD GROUPING TEST;-

The test to determine your blood group is called ABO typing. Your blood sample is mixed with antibodies against type A and B blood. Then, the sample is checked to see whether or not the blood cells stick together. If blood cells stick together, it means the blood reacted with one of the antibodies.

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.

PRINCIPAL OF BLOOD GROUPING;-

Principle behind blood tests: Blood clumping or Agglutination observation.

Compatibility between the blood groups of donor and recipient determines the success of a blood transfusion. The AB0 and Rh blood groups are looked at while conducting the test. In a diagnostic lab, Monoclonal antibodies are available for A, B and Rh antigen. Monoclonal antibody against Antigen A (also called Anti-A), comes in a small bottles with droppers; the monoclonal suspension being BLUE in colour. Anti-B comes in YELLOW colour. Anti-D (monoclonal antibody against Rh) is colourless. All the colour codes are universal standards. When the monoclonal antibodies are added one by one to wells that contain the test sample (blood from patient), if the RBCs in that particular sample carry the corresponding Antigen, clumps can be observed in the corresponding wells. A drop of blood is left without adding any of the antibodies; it is used as a control in the experiment. The monoclonal antibody bottles should be stored in a refrigerator. It is recommended to tilt the bottle a couple of times before use in order to resuspend the antibodies that have settled at the bottom of the bottle.

QUESTION NO 2;-

IMMUNO CHROMATOGRAPHIC TEST;-

Immunochromatographic tests rely principally on the capture of the target antigen (or sometimes antibodies) from various specimens. The assay utilizes antibodies mounted on a paper strip or a nitrocellulose membrane as the immobile capture antibody (test area).

Lateral flow tests, also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence of a target substance in a liquid sample without the need for specialized and costly equipment. ... The sample pad acts as a sponge and holds an excess of sample fluid.

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 Immunochromatographic assays

1. Practical Immunology and Serology Hawler Medical University College of Health Sciences Medical Microbiology Dept. Ass. Lec. Amer Ali Khaleel (M.Sc. Medical Immunology) Lab.3 Immunochromatographic assays 3rd stage

2. Introduction: Immunochromatographic assays, also called lateral flow dipstick immunoassay or simply strip tests,They are a logical extension of the technology used in latex agglutination tests, the first of which was developed in 1956 by Singer and Plotz.

3. Principal of Immunochromatography kit: Principal of immunochromatography is the same as ELISA sandwich method, only difference is in that immunological reaction is carried out on the chromatographic paper by capillary action. For this system, two kinds of specific antibodies against antigen are used.One of the antibodies is immobilized on the chromatographic paper, and the other is labeled with colloidal gold and infiltrated into sample pad. An immunochromatographic unit is completed by attaching the sample pad at the end of the membrane.

4. Principal of Immunochromatography kit: When the liquid sample is dropped on the sample pad, the antigen in the sample forms an immunocomplex with the antibody labeled with colloidal gold. Its complex moves along with the liquid sample, and makes a contact with the antibody immobilized on the membrane, followed by forming an immunocomplex with the immobilized antibody, resulting in generating a colored red purple line. Appearance of red purple line on the membrane indicates the presence of antigen of interest in the sample. Since the liquid of the sample migrates through the membrane very fast, it makes it possible to detect the presence or absence of antigen within 15 minutes.

5. Basic Components of Lateral flow test: 1. Sample pad. 2. Conjugate (detector) pad. 3. Detection conjugate. 4. Nitro-cellulose membrane. 5. Test and control reagent lines. 6. Absorbent (sink) pad. 7. Plastic-adhesive backing card. The following components are “optional” and are not necessary or included in many lateral flow platforms: 8. Laminate Tape. 9. A Strip housing / Cassette. Typical lateral flow test strip configuration (Card, Cassette, Dipstick, Strip)

6. Lateral flow tests: how they work: Step 1: Sample placement To perform the test, a sample is placed on the sample pad at one end of the strip. The sample may be used alone as is commonly done with urine or serum or whole blood or plasma compatible tests, or it may be mixed with a buffer specific to the test.

7. Lateral flow tests: how they work: Step 2: Molecules solubilized With the addition of the sample, the detector molecules are solubilized. When solubilized the detector molecules mix with and bind to the analyte in the sample (if analyte is present).

8. Lateral flow tests: how they work: Step 3: Capillary action Then capillary action draws the fluid mixture up the sample pad and into the membrane. The sample/detector molecule mix continues to move up the membrane until it reaches the analyte capture molecule. In these lines a second (and third) antibody or antigen, immobilized as a thin stripe in the nitrocellulose will then capture the complex if it is positive for the target analyte. The control line should always show as a visible line, otherwise the test is invalid and must be repeated. If the test is positive, a colored (typically pink or purple) line develops along with the control line.

9. Lateral flow tests: how they work: Step 4: Excess absorbed Excess buffer along with any reagents not captured at the test of control line will then move into the absorbent wicking pad.

10. The benefits of immunochromatographic tests includes: 1.Can detect antigen or antibody. 2.Commercially available. 3.Easy to perform. 4.Limited/no instrumentation. 5.Single use, rapid test. 6.User friendly format. 7.Very short time to get test result. 8.Long-term stability over a wide range of climates. 9.Relatively inexpensive to make.

11. Limitations: 1-Results are qualitative. 2-Rapid tests can be less sensitive or less accurate compared to existing tests. Includes Strip or Cassette or Multi-devices or Midstream (hCG & LH only). Test format: Multi-devices

12. Applications of Immunochromatographic assays: It can be applied for multiple test platforms for liver, sexually transmitted diseases, cardiac markers, as well as women’s and men’s health (hCG, VDRL, CMV, PSA , H. Pylori, Troponin I, TORCH, HIV, HBsAg, HBV, HCV, FOB, RA, CRP, ASO, SLE, IM, Salmone.