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INFECTIOUS MARKER TESTING IN BLOOD BANK

Pathology

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ABSTRACT

As we all know that blood transfusion is the greatest need of all mankind. Every patient who comes to the hospital needs blood transfusion, there could be various reasons behind it. Blood or blood components are required during operation procedures, gunshot patient, accidental patient, gynae patient ,dengue patients etc. It's the duty of all the blood bank to ensure safe transfusion. All the recommended tests need to be conducted diligently using proper protocol and according to user manuals. Tests to be conducted are HIV, HBSAG, HCV, Core antibodies, syphilis, Malaria. Non-Reactive results of all the above-mentioned investigation ensures safe transfusion of blood. While performing all the test we should always follow kit manuals and protocol. To achieve best results all the test kits should be kept at 2-to-6-degree temperature. And should be brought to room temperature prior to conducting every test.

KEYWORDS

HIV, MALARIA, SYPHILIS, ELISA, HCV, HBSAG, WASHING, SPAN, INTERPRETATION

TEST FOR MALARIAL PARASITE: (OPTIMAL)

The purpose of identification of malarial parasite in donor's blood sample is to provide safe blood to the recipient for the prevention of transfusion related transmission of malaria [8]

Principle:

The test detects the presence of Plasmodium Lactate Dehydrogenate, an enzyme produced both in the sexual and asexual form of the parasite**.** the DiaMed OptiMAL conjugate well contains an indicator-tagged monoclonal (mouse hybridoma) antibody to plasmodium lactate dehydrogenate, dried on to this surface. This monoclonal antibody reacts with all isoenzyme LDH of the genus plasmodium.

Sample / material

- Fresh anticoagulated blood collected from donor
- DiaMed OptiMAL Dipstics
- DiaMed OptiMAL Conjugate wells
- DiaMed OptiMAL Wash wells
- DiaMed OptiMAL Wash holder buffer
- Pipettes

Test procedure

- Take a dipstick from the container; write the donors identification on the label.
- Place 1 conjugates well and 1 wash well on the holder
- Dispense one drop of buffer (approx-20µl) into the conjugate well and 4 drops (approx-80µl) into the wash well. Allow to stand for one minute.
- Draw donor's blood from the collection tube into the pipette.
- Add one drop of blood (10µl) into the conjugate well. Mix gently with the stirrer of the same pipette. Allow to stand for 1 minute. (Discard the tip into the suitable container)
- Place the appropriate dipstick vertically into the conjugate well and leave it into the well for 10 minutes (the blood migrates towards the filter pad and the control band will appear progressively).
- Transfer the dipstick from the conjugate well to the wash well and leave it until the reaction field until the reaction field of dipstick is cleared of the blood and the procedure control band becomes clearly visible (within 5-10 minutes)
- Remove the dipstick from wash well and read the reaction.

Interpretation of results:

- Positive reaction: the pLDH present in the sample reacts with the anti-pLDH conjugate and rise up the dipstick where it is captured by one or both of the specific pLDH antibodies, causing the appearance of the coloured band.
- Negative reaction: only the control band will be the visible

Validation of the test:

The test can be considered valid when the reaction field of the dipstick is cleared of blood and the control band is clearly visible, presenting a dense line.

The test is invalid in two cases:

- When the control band does not appear.
- When the dipstick is not sufficiently cleared

Note: In both the condition repeat the test

Precaution

- Remove the required number of dipsticks and conjugate wells and then to close immediately the containers because the presence of humidity may decrease the stability of reagents.
- Always hold the dipstick by the label, do not touch the reaction field.
- The reagent, dipsticks and conjugate wells of different lots must not be mixed and used [7]

REFERENCES: Manufacturer guidelines (Dia Med OptiMAL)

TEST FOR SYPHILLIS: (RAPID PLASMA REGAIN CARD TEST)

The purpose is detection of antilipodial antibodies in donors blood samples.

Principle:

When a specimen contains regain antibody, flocculation's occurs due to coagglutination, which appear as pink-red clumps against white background of the card. This coagglutination is read macroscopically. Non- Reactive specimens show an even light pink colour.[9]

Materials required:

- Disposable plastic cards.
- Disposable plastic droppers.
- Disposable applicator sticks.
- Antigen delivering dropper.

Procedure:

- Place one drop serum or plasma on the card with the help of disposable serum dropper or disposable micro tip using a pipette.
- After gently mixing the TRUST (Toludine Red Unheated Serum Test) antigen suspension, place one drop (15-20µl) using the antigen delivery dropper or disposable micro tip using a pipette.
- Mix the drops well and spread out the pool of liquid uniformly with the entire area of the circle by using the disposable applicator stick supplied in the kit.
- Rock the card gently to and fro for 4 minutes and observe under good light source for the appearance of agglutination.

Reporting of results:

Positive result – Pink aggregates which may be deposited at the periphery of the liquid appearing before the 4th minute of rotation.

Negative result – Complete absence of pink aggregates with a uniform light pink background at the end of the 4th minute rotation.[6] [1]

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA):

ELISA is the most common used assay for the detection of Antibody or Antigen for the viruses. The tests that are performed are for:

- Hepatitis B Surface Antigen (HBsAg).
- Hepatitis C Virus (HCV).
- Human Immunodeficiency Virus (HIV).
- Hepatitis B Core (HBc) Total.

General Precautions to be taken for ELISA tests:

- Centrifuge the clotted donor's samples and transfer the serum into fresh pre labeled test tubes. Do not take serum directly from clotted sample. If there is delay in testing store the sample at 4ºC.
- Reagent should be stored properly according to manufacturer guidelines and should bring at room temperature prior to start the procedure.
- Check all packaging before using the kits, if the packaging is damaged the technician must check that component of kit is intact before using them.
- Store unused strips according to manufacturer guidelines.
- Verify that conjugate sphere is on the bottom of the wells before removing the strip sealers. Before testing begins, the technician should inspect the micro-Elisa strip holder and ensure that all strips are secure.
- All reagents must be mixed well before use.
- Routinely maintain aspiration / wash system to prevent carry over from highly reactive specimen to non reactive specimens.
- To avoid contamination, do not touch the top of the bottom of the strips, the edge of the wells, or the conjugate sphere with finger or pipette tips.
- Do not allow the micro-Elisa wells to dry once the assay has begun.
- Remove any bubble in the well by gentle tapping.

ORTHO Antibody to HBsAg ELISA:

Principle:

This is a two-stage test carried out in the microwell coated with antibody to HBsAg. There are two stages of the test.

1st Stage: - Working conjugate comprised of antibody conjugate to HRP and diluted in conjugate diluent that is blue in color is added to the test well. The test specimen is then added to the test well. The test specimen is then added to the test well and a SOM (Sample omission monitoring) read is performed. The plate is incubated for a specified length of time. If HBsAg is present in the specimen, it will bind to the antibody coated on the well and simultaneously bind to the conjugate to form immobilized antibody-HBsAg-conjugate complexes. If HBsAg is not present, these complexes will not be formed. Unbound serum or plasma proteins will be removed in the subsequent washing steps.

2nd stage: - An enzyme detection system composed of o-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If the bound conjugate is present, the OPD will be oxidized, resulting in a colored end-product. In this reaction, peroxidase is divalently oxidized by the hydrogen peroxide to form an intermediate compound, which in turn is reduced to its initial state by subsequent interaction with the hydrogen ion donating OPD. The resulting oxidized form of OPD has an Orange Color. Sulfuric acid is then added to stop the reaction.

The color intensity depends on the amount of bound conjugate in the well. Therefore, Color intensity is a function of the concentration of the HBsAg present in the specimen. The color intensity is measured with a microwell reader at 490 or 492nm.

Material

- Specific kit for the test.
- Distilled or deionized water
- Adjustable multichannel or single channel capable of delivering 50µL and 200µL.
- Fixed or adjustable single channel micropipettes capable of delivering 20µL to 200µL.
- 5µL to 300µL disposable pipette tips.
- Incubator
- Micro shaker
- Elisa reader
- ELISA washer
- 1 mol / l sulfuric acid
- Disposable gloves
- Sodium hypochlorite solution (5%)
- Absorbent tissue
- Biohazard waste container

Procedure:

Add 50µL working conjugate in all wells except A-1.

↓

Add 150µL N.C (in 3 wells), P.C(in 2 wells) and specimen in the appropriate wells.

↓

Incubate at 37°C for 90 minutes.

↓

Wash 6 times.

↓

Add 200µL substrate solution in all wells.

↓

Incubate at room temperature in the dark for 30 minutes.

↓

Add 50µL stop solution in all wells.

↓

Read the absorbance at 492 and 620 nm within 15 minutes.

Validity of the results:

- The value of the substrate blank should be greater than or equal to -0.001 and less than or equal to 0.050.
- At least two negative calibrator wells absorbance must be greater than or equal to -0.006 and less than or equal to 0.012.
- The positive control absorbance value should be greater than or equal to 0.225 and is less than 2.500 and both the positive control values should not differ by more than 0.230.

Calculation of the cutoff value:

The cutoff is calculated by the following method: - N.C (mean) + 0.030 [5]

ORTHO HCV 3.0 ELISA TEST SYSTEM:

Principle:

The assay procedure is a three-stage test carried out in a microwell coated with a recombinant hepatitis C virus (rHCV) antigen (c22-3, c200 and Ns5)

1st Stage: - A diluted test specimen is incubated in the test well for a specified length of time. If the antibody reactive to any of the three antigens is present in the specimen, antigen-antibody complexes will be formed on the microwell surface. If the anti-HCV is not present, complexes will not be formed. In the subsequent washing step, unbound serum or plasma proteins will be formed.

2nd Stage: - Murine monoclonal antibody conjugate to horseradish peroxidase is added to the microwell. The conjugate binds specifically to the human IgG portion of the antigen-antibody complexes are not present, the unbound conjugate will be removed by the subsequent washing.

3rd Stage: - An enzyme detection system composed of o-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If the bound conjugate is present, the OPD will be oxidized, resulting in a yellow-colored end-product. In this reaction, peroxidase is divalently oxidized by the hydrogen peroxide to form an intermediate compound, which in turn is reduced to its initial state by subsequent interaction with the hydrogen ion donating OPD. The resulting oxidized form of OPD has an Orange Color. Sulfuric acid is then added to stop the reaction.

The color intensity depends on the amount of bound conjugate in the well. Therefore, Color intensity is a function of the concentration of anti-HCV present in the specimen. The color intensity is measured with a microwell reader at 490 or 492nm.

Material:

- Specific kit for the test.
- Distilled or deionized water
- Adjustable multichannel or single channel capable of delivering 50µL and 200µL.
- Fixed or adjustable single channel micropipettes capable of delivering 20µL to 200µL.
- 5µL to 300µL disposable pipette tips.

- Incubator
- Micro shaker
- Elisa reader
- ELISA washer
- 1 mol / l sulfuric acid
- Disposable gloves
- Sodium hypochlorite solution (5%)
- Absorbent tissue
- Biohazard waste container

Procedure:

Blank well A-1
↓
Add 200 L sample diluent in all wells.
↓
Add 20µL N.C (in 3 wells) and P.C. (in 2wells)
↓
Incubate at 37°C for 60 minutes.
↓
Wash 5 times.
↓
200µL substrate solution in all wells.
↓
Incubate at room temperature for 30 minutes.
↓
50µL stop solution in all wells.
↓
Read the absorbance at 492 and 620 nm within 15 minutes.

Validity of the results:

- The reagent blank absorbance value of the reagent blank well should be greater than equal to 0.050.
- Individual negative calibrator values must be less than or equal to 0.120 and greater than or equal to 0.120 and greater than or equal to -0.005.
- Both the positive control values should be greater than or equal to 0.800 and should not differ by more than 0.600.

Calculation of cutoff value:

The cutoff value is calculated with the following formula: - NC (mean) + 0.600 [2]

ORTHO HIV-1/HIV-2 Ab-CAPTURE ELISA:

Principle:

The assay procedure is a three-stage test carried out in a microwell coated with a combination of four recombinant human immunodeficiency virus type 1 and type 2 (rHIV-1 and rHIV-2) antigens. The recombinant DNA yeast- derived antigens correspond to four viral proteins sequences: two HIV-1 envelopes. One HIV-1 core and one HIV-2 envelope.

1st stage: - The test specimen or assay control or calibrator is diluted into specimen diluent, which is green in color. The addition of the specimen or assay control or calibrator results in a distinctive color change which can be monitored both visually and photometrically at 610nm. The diluted specimen or assay control or calibrator is then incubated in the test well for a specified length of time. If the antibody reactive to any of the four antigen is present in the specimen, antigen-antibody complexes will be formed in the subsequent washing step, unbound serum or plasma proteins will be removed.

2nd stage: - A mixture of four HIV-1 and HIV-2 recombinant conjugate to horseradish peroxidase is added to the microwell. The conjugate binds specifically to the human HIV-1 and /or HIV-2 immunoglobulin (IgG and IgM) portion of the antigen-antibody complexes. If the antigen-antibody complexes are not present, the unbound conjugate will be removed by the subsequent washing.

3rd Stage: - An enzyme detection system composed of o-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If the bound conjugate is present, the OPD will be oxidized, resulting in a yellow-colored end-product. In this reaction, peroxidase is divalently oxidized by the hydrogen peroxide to form an intermediate compound, which in turn is reduced to its initial state by subsequent interaction with the hydrogen ion donating OPD. The resulting oxidized form of OPD has an Orange Color. Sulfuric acid is then added to stop the reaction.

The color intensity depends on the amount of bound conjugate in the well. Therefore, Color intensity is a function of the concentration of anti-HCV present in the specimen. The color intensity is measured with a microwell reader at 490 or 492nm.

Material:

- Specific kit for the test.
- Distilled or deionized water
- Adjustable multichannel or single channel capable of delivering 50µL and 200µL.
- Fixed or adjustable single channel micropipettes capable of delivering 20µL to 200µL.
- 5µL to 300µL disposable pipette tips.
- Incubator
- Micro shaker
- Elisa reader
- ELISA washer
- 1 mol / l sulfuric acid
- Disposable gloves
- Sodium hypochlorite solution (5%)
- Absorbent tissue
- Biohazard waste container.

Procedure:

Well A-1 Blank.
↓
50µL specimen diluent in all wells except A-1.
↓
150µL N.C(3 wells), P.C(2 wells) & specimen in the appropriate wells.
↓
Incubate at 37°C for 60 minutes.
↓
Wash 5 times
↓
Add 200µL Substrate solution in all wells.
↓
Incubate at room temperature in the dark for 30 minutes.
↓
Add 50µL stop solution in all wells.
↓
Read the absorbance at 492 and 620 nm within 15 minutes.

Validity of results:

- Substrate blank absorbance value should be greater than or equal to -0.020 and less than or equal to 0.050.
- Individual negative calibrator values must be less than or equal to 0.085 or greater than equal to -0.005.
- HIV-1 positive control absorbance value should be greater than or equal to 0.500 and HIV-2 positive control absorbance value should be greater than or equal to 0.500.

Calculation of cutoff value:

The cutoff value is calculated with the following formula: -- N.C (mean) + 0.250 [4]

HEPATITIS B VIRUS CORE ANTIGEN (recombinant) ORTHO HBc ELISA:

Principle:

The assay procedure is a three-stage test carried out in a microwell coated with recombinant derived hepatitis B core antigen (rHBcAg).

1st Stage: - A test specimen is placed directly in the test well containing specimen diluent and incubated for a specified length of time. If anti-HBc is present in the specimen, antigen-antibody complexes will form on the microwell surface. If anti-HBc is not present, complexes will form on the microwell surface. If anti-HBc is not present, complexes will not form and the unbound serum or plasma proteins will be removed in the washing step.

2nd Stage: - Antibody conjugate is added to the test well. The antibody conjugate is a mixture of murine monoclonal antibodies specific for human IgG and IgM. The conjugate will bind specifically to the antibody portion of the antigen-antibody complexes. If antigen-antibody complexes are not present, the unbound conjugate will be removed by washing.

3rd Stage: - An enzyme detection system composed of o-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If bound conjugate is present, the OPD will be oxidized, resulting in a colored end-product. Sulfuric acid is then added to stop the reaction.

The color intensity depends on the amount of bound conjugate and therefore is a function of the concentration of anti-HBc present in the specimen. The color intensity is measured with a microwell reader.

Material:

- Specific kit for the test.
- Distilled or deionized water
- Adjustable multichannel or single channel capable of delivering 50µL and 200µL.
- Fixed or adjustable single channel micropipettes capable of delivering 20µL to 200µL.
- 5µL to 300µL disposable pipette tips.
- Incubator
- Micro shaker
- Elisa reader
- ELISA washer
- 1 mol / l sulfuric acid
- Disposable gloves
- Sodium hypochlorite solution (5%)
- Absorbent tissue
- Biohazard waste container.

Procedure:

Well A-1 Blank.
↓
200µL specimen diluent in all wells except A-1.
↓
10µL N.C(3 wells), P.C(2 wells) & specimen in the appropriate wells.
↓
Incubate at 37°C for 60 minutes.
↓
Wash 5 times
↓
200µL conjugate in all wells except A-1
↓
Incubate at 37°C for 60 minutes
↓
Wash 5 times
↓
Add 200µL Substrate solution in all wells.
↓
Incubate at room temperature in the dark for 30 minutes.
↓
Add 50µL stop solution in all wells.
↓
Read the absorbance at 492 and 620 nm within 15 minutes [3]

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