PART A - CONFIRMATION OF HIV

EXPERIMENT 1

ELISA - HIV

REQUIREMENTS

Sample - Sera extracted from the blood of patients attending Integrated Counselling for Testing Centre (ICTC).

Components

1. **Microlisa-HIV Strip Plates** - 12 Strips (12x8 wells). Breakway microwells coated with HIV-1 & HIV-2 recombinant proteins packed in a pouch with dessicant.

2. **Sample Diluent** - 1 bottle (20 ml). Buffer containing protein stabilizers and antimicrobial agents as preservative.

3. **Enzyme Conjugate Concentrate (100x)** - 1 vial (0.25 ml). Anti-human IgG conjugated with horseradish peroxidase with protein stabilizers.

4. **Conjugate Diluent** - 1 bottle (15 ml). Buffer containing protein stabilizers.

5. **Wash Buffer Concentrate (25x)** - 1 bottle (50 ml). PBS with surfactant. Dilute 1:25 with distilled water before use.

6. **Tetra Methyl Benzidine (TMB) Concentrate (100x)** - 1 vial (0.25 ml). To be diluted in TMB substrate diluents before use.
7. **Substrate (TMB Diluent)** - 1 bottle (20 ml). Buffer containing substrate.

8. **Negative Control** - 1 vial (2.0 ml). Ready to use, normal human serum negative for HIV, HCV and HBsAg.

9. **Positive Control** - 1 vial (2.0 ml). Ready to use, inactivated and diluted human serum, positive for HIV antibodies and non-reactive for HBsAg and HCV, contains sodium azide as preservative.

10. **Stop Solution** - 1 vial (6 ml). Ready to use, 2N sulphuric acid.

**Accessories**

1. Micropipettes and microtips
2. Elisa reader
3. Elisa washer
4. Timer
5. Distilled water
6. Incubator 37°C
7. Graduated Cylinders for reagent dilution
8. Vortex mixer
9. Sodium hypochlorite solution
10. Disposable gloves
11. Absorbent tissue paper
12. Glassware
TEST PROCEDURE

1. The strip holder is fitted with the required number of Microlisa-HIV strips.

2. 100 µl sample diluents was added to A-1 well as blank.

3. 100 µl Negative Control was added in each well no. B-1 & C-1 respectively. Negative Control was ready to use and hence no dilution was required.

4. 100 µl Positive Control was added in D-1, E-1 & F-1 wells. Positive Control was ready to use and hence no dilution was required.

5. 100 µl sample diluents was added in each well starting from G-1 followed by addition of 10 samples.

6. Cover seal was applied.

7. Incubation was given at 37°C for 30 min.

8. While the samples were incubating, Working Wash Solution and Working Conjugate were prepared.

   Preparation of Wash Buffer : For 50 ml; 2 ml concentrated buffer with 48 ml of distilled water.

   Preparation of Working Conjugate : dilute conjugate concentrate 1:100 in conjugate diluents. For 56 wells, 70 µl of enzyme conjugate concentrate in 7 ml of conjugate diluent.

9. The plate was took out from the incubator after the incubation time was over and the wells were washed 5 times with working wash solution in Elisa washer.
10. 100 µl of working conjugate solution was added in each well including A-1.

11. Cover slip was applied.

12. Incubation was given at 37°C for 30 min.

13. Step 9 was again repeated for washing.

14. 100 µl of working substrate solution was added in each well including A-1.

15. Then, plate was incubated at room temperature for 30 min in dark.

16. Finally, 50 µl of stop solution was added.

17. Absorbance was noted at 450 nm within 30 min in ELISA READER after blanking A-1 well.

**INTERPRETATION OF RESULT**

Samples with absorbance value less than the cutoff value are considered non reactive by QUALISA – HIV ELISA kit & are considered negative for HIV.

Samples with absorbance value equal to or greater than cutoff value are considered reactive by QUALISA – HIV ELISA kit. The original sample should be retested in duplicate. Initially reactive sample that do not reactive in either of duplicate are considered negative for HIV. Initially reactive sample that react in either or both duplicate are considered repeatedly reactive.
EXPERIMENT 2

COMBAIDS – RS Advantage

PRINCIPLE

Dot immunoassay employs the same principle as Enzyme Immuno Assay (EIA) were by the immobilised antigen-antibody complex is visualized by means of colour producing (chromogenic) reaction. In EIA the colour is developed by a coupled reaction between enzyme, substrate and chromogen whereas in combaids-RS Advantage the coloured end-point is developed by a Colloidal Gold-Protein-A Signal Reagent. Each tooth of the comb is spotted with a circular spot, one near the tip with an optimally standardized blend of HIV 1 and HIV 2 recombinant antigens and/or synthetic peptides (Test spot), and the other spot, a little above the first spot is spotted with “Control Reagent” (Control spot). When incubated with a specimen containing HIV 1 and/or 2 antibodies, these antibodies bind directly to the HIV antigens present in the “Test area” on the tooth of the Comb. The immune complex is directly visualized after incubation with the Colloidal Gold-Protein-A Signal Reagent. A positive result is indicated by the presence of pink coloured spot/dot in the “Test area” near the tip of the tooth of the Comb where antigens are spotted. Built in Control is visualised separately in the upper part of the tooth (“Control area”), where Control Reagent has been spotted, serving as the procedural control. A pink coloured spot/dot will always appear at the “Control area” with
recommended test specimen irrespective of the presence or absence of HIV antibodies in the specimen.

REQUIREMENTS

Sample - Sera extracted from the blood of patients attending Integrated Counselling for Testing Centre (ICTC).

REAGENTS

Reagent 1: Washing Buffer (5x)
Reagent 2: Colloidal Gold Signal Reagent
Reagent 3: Sample Diluent
Reagent 4: Negative Control
Reagent 5: Positive Control
Reagent 6: Antigen & Control Reagent Coated Combs

Accessories

1. Wash tray
2. 8 Microwell strips with four-row frame
3. Seal bag
4. Disposable plastic droppers
5. Clamp
6. Reference colour index for SPIA (Solid Phase Immunosorbent Assay)
7. Timer
8. Dust cover
9. 5% Sodium or Calcium hypochlorite
10. Disposable gloves  
11. Measuring cylinder - 100ml  
12. Micropipettes (100µL) and pipette tips

**TEST PROCEDURE**

1. First of all, all the reagents and samples were brought to room temperature before starting the test.  
2. Sample numbers were marked on the micro test wells.  
3. Concentrated washing buffer solution was diluted by adding 15 ml of concentrate washing buffer (5x) to 60 ml distilled water, taking care to avoid foaming.  
4. 2 drops (0.1mL) of Sample Diluent in microtest wells were added.  
5. 2 drops (0.1mL) of Samples and controls were added to each of the above wells containing Sample Diluent.  
6. The combs were placed into respective wells. (the combs were rocked gently back and forth 2-3 times in the well.)  
7. Then it was incubated for 10 minutes at room temperature.  
8. In the meantime, 4 drops (0.2mL) of Colloidal Gold Signal Reagent were added into each of another set of unused microtest wells.  
9. The comb was removed from the sample containing wells and the tips of the teeth were blotted on absorbent material. Then, the comb was held vertically with tips pointing down and was rocked forward and backward in the wash solution for a total of ten times.
Materials and Methods

10. Combs were placed into microtest wells containing Colloidal Gold Signal Reagent (gently rock the Comb back and forth 2-3 times in the well).

11. Then it was incubated for 10 minutes at room temperature. After incubation, washing procedure was repeated again.

12. The combs were placed on a clean surface with reactive (labelled) side up. The Combs were allowed to air-dry completely.

13. Finally, the colour development was noted on the spotted area on the tip of the teeth of the comb for reactivity as well as for control dot appearance.

INTERPRETATION

Positive Results

A positive result is indicated by presence of pink coloured spot/dot, both in the “test” and “control” area as shown in the picture. Furthermore, the intensity of pink coloured spot/dot developed in the “test” area should be equal to or more than 1.0 colour index when compared with the Reference colour index chart for SPIA (supplied in the kit) for the test to be positive.

Negative Results

A negative result is indicated by the presence of only one pink coloured spot/dot in the control area as shown in the picture. If no pink spot/dot or faint spot/dot is seen in test area having colour index equal to or less than 0.00
compared with the reference index chart for SPIA then that test is considered as negative.

**Indeterminate Result**

The test should be considered as “indeterminate” in case of faint colouration of dot/spot in test area having colour intensity between 0.00 and 1.0 colour index when compared with Reference colour index chart for SPIA. In such cases it is recommended to repeat the test to confirm the results, if the results is still “indeterminant”, fresh sample should be drawn often after 4-8 weeks and retested again.

**Invalid Results**

The test is to be considered as “Invalid” if no pink coloured dot/spot is visible in “control” area irrespective of presence or absence of pink coloured dot/spot in the test area. In such cases the test should be repeated using a new comb and fresh specimen.
EXPERIMENT 3

SD BIOLINE HIV 1/2

PRINCIPLE

The SD BIOLINE HIV -1/2 test is an immunochromatographic (rapid) test for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to HIV-1 and HIV-2 simultaneously in human serum, plasma or whole blood.

The SD BIOLINE HIV -1/2 test contains a membrane strip, which is precoated with recombinant HIV-1 capture antigen (gp41,p24) on test bend 1 region and with recombinant HIV-2 capture antigen (gp 36) on test band 2 region, respectively. The recombinant HIV-1/2 antigen (gp41,p24 and gp36) — colloidal gold conjugate and the specimen sample move along the membrane chromatographically to the test region (T) and form a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has letter of 1, 2 and C as test Line 1 (HIV-1), Test line 2 (HIV-2) and Control Line on the surface of the device. Both the Test Lines and Control Line in result window are not visible before applying any sample. The Control Line is used for procedural control. Control Line should always appear if the test procedure is performed properly and the test reagent of Control Line are working.
REQUIREMENTS

Sample: Sera extracted from the blood of patients attending Integrated Counselling for Testing Centre (ICTC).

Reagent and Components
1. Test cards individually foil pouched with a dessicant
2. Sample dispensing plastic dropper with each test pouch.
3. Micropipette 10 Microlitre
4. Assay diluents
5. Lancets
6. Negative control
7. Positive control

PROCEDURE
1. The test cards were removed from the foil pouch and placed on a clean dry surface.
2. Add 10 µl of serum or plasma specimen into the sample well.
3. Add 4 drops (120 µl) of assay diluent dispensed into the sample well on the card.
4. Test results interpreted in the 15 minutes.
INTERPRETATION

Positive Result

The presence of two bands at a control line (c) and test band 1 within the test result window indicates a positive result for HIV-1.

The presence of two bands as a control line (c) and test band 2 within the test result window indicates a positive result for HIV-2.

The presence of three lines as a control line (c), test line 1 and test line 2 within the test result window indicates a positive result for HIV-1 and/or HIV-2.

Negative result

The presence of only band at control line within the result window indicates a negative result.
EXPERIMENT 4

HIV TRI-DOT

PRINCIPLE

The HIV TRI-DOT test is a visual, Rapid, Sensitive and accurate immunoassay for the detection of HIV-1 and HIV-2 antibodies (IgG) in human serum or plasma using HIV-1 and HIV-2 antigens immobilized on a Porous immunofiltration membrane. Sample and reagent pass through the membrane and are absorbed into underlying absorbent.

As the patient's sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens.

Conjugate binds to the Fc portion of the antibodies to give distinct pinkish purple DOT against a white background.

REQUIREMENTS

Sample : Sera extracted from the blood of patients attending Integrated Counselling for Testing Centre (ICTC).

Reagents and Components

1. Buffer solution
2. Liquid Conjugate
3. Test device
4. Dropper
PROCEDURE

1. 3 drops of buffer solution were added to the centre of the device.
2. The dropper was held vertically and 1 drop of sample was added using the sample dropper.
3. 5 drops of buffer solution was added.
4. 2 drops of liquid conjugate were added directly from the conjugate vial.
5. 5 drops of buffer solution were added.

NOTE: Read results immediately and discard the device considering it to be potentially infectious.

It is important to allow each solution to soak in the test device before adding the next solution.

RESULTS

Positive

If two dots, one for the control and the other for HIV-1 appear the sample is reactive for antibodies to HIV-1.
If two dots, one for the control and the other for HIV-2 appear the sample is reactive for antibodies to HIV-2.
If all the three dots, one each for control, HIV-1 & HIV-2 appear sample is reactive for antibodies to HIV-1 & HIV-2.

Negative

If only control dot appears the specimen is negative for antibodies to HIV-1 and HIV-2 and interpret sample as non-reactive.
PART B-I TEST FOR ASSOCIATION OF HIV POSITIVE PATIENT WITH HEPATITIS-B

EXPERIMENT 1

DETECTION OF HBV BY ELISA

HBsAg is a solid phase Enzyme Linked Immunosorbent Assay that employs highly purified, high affinity monoclonal & polyclonal antibodies to HBsAg having reactivity for both ad & ay subtypes.

PRINCIPLE OF THE ASSAY

Microwell strips are coated with monoclonal anti HBsAg antibody. A polyclonal antibody to HBsAg is conjugated to horseradish peroxidase (HRPO). The sample & the conjugate are added in the coated wells & incubated simultaneously. The wells are washed to remove unbound components. Bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid & absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula & absorbance of all the wells is compared with the cutoff value. Any sample having absorbance more than the cutoff value is considered reactive.

Components - QUALISA - HBsAg has following components

1. Coated microwells- Microwells coated with monoclonal anti HBsAg antibody. Ready to use.
2. Positive control- HBsAg positive & HIV/HCV non reactive serum diluted in stabilizer solution with preservative.
3. Negative control- Inactivated & stabilized human serum non reactive for HIV1, HIV2, HBsAg & HCV.
4. Conjugate- Polyclonal anti HBsAg HRPO conjugate.
5. Conjugate activator- Buffered solution containing activator & preservatives.
7. Wash buffer- Buffer contains surfactant (20x). To be diluted 20 times with distilled or deionized water.
10. Instruction for use
11. ELISA protocol sheet.
12. Plate sealer.

TEST PROCEDURE

1. Bring all the reagent & specimen to room temperature before use.
2. Take out required number of strips & immediately close the pouch.
3. Prepare ELISA protocol sheet indicating the location of controls & specimen.
4. Use controls in duplicate.
5. Add 50µl activated conjugate to all the wells.
6. Add 100 µl undiluted control or specimen in separate wells. Mix by gentle tapping.

7. Apply plate sealer & incubate according to the procedure chosen.

8. Wash each well by filling 350 µl diluted wash buffer & aspirating/flicking off six times with 1 minute soak time between each wash. Blot dry.

<table>
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<tr>
<th>Procedure</th>
<th>Incubation period</th>
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<td>Short procedure</td>
<td>60 min. at 37°C</td>
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<tr>
<td>Standard procedure</td>
<td>120 min. at 37°C</td>
</tr>
<tr>
<td>Overnight procedure</td>
<td>Overnight at 22-28°C</td>
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</tbody>
</table>

9. Add 100µl substrate & incubate at 22–28°C away light for 30 minutes.

10. Stop reaction by adding 100 µl stop reaction. The stop solution should be added in the same sequence as substrate addition.

11. Read the absorbance at 450 nm with 600–700 nm as reference within 30 minutes of stopping the reaction.

**Run Criteria**

The individual absorbance value of negative controls should be less than 0.1.

The individual absorbance value of positive controls should be more than 1.0.
INTERPRETATION

The cutoff value is calculated by adding 0.1 to average absorbance value of negative control.

Cut Off Value = Av. NC + 0.1

Samples with absorbance value less than the cutoff value are considered non reactive by QUALISA – HBsAg ELISA kit & are considered negative for HBsAg.

Samples with absorbance value equal to or greater than cutoff value are considered reactive by QUALISA – HBsAg ELISA kit. The original sample should be retested in duplicate. Initially reactive sample that do not reactive in either of duplicate are considered negative for HBsAg. Initially reactive sample that react in either or both duplicate are considered repeatedly reactive.
EXPERIMENT 2

RAPID TEST FOR HEPATITIS B - SD BIOLINE HBsAg

PRINCIPLE

The SD BIOLINE HBs Ag Test is an in-vitro immunochromatographic, one step assay designed for qualitative of HBsAg in human serum or plasma. This test cassette contains a membrane strip, which is pre-coated with mouse monoclonal anti-HBs capture antibody on test band region. The mouse monoclonal anti-HBs-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms.

The SD BIOLINE HBsAg test cassette has a letter of T and C as “Test Line” and “Control Line” on the surface of the cassette. Both the Test Line and Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control. The control line should always appear if the test procedure is performed properly and the reagents of control line are working. The SD BIOLINE HBsAg can identify HBsAg in plasma or serum specimens with a high degree of sensitivity.

REQUIREMENTS

Sample: Sera extracted from the blood of patients attending Integrated Counselling For Testing Centre (ICTC).
**Components**

1. Test Strips/Devices
2. Dropper

**Accessories**

1. Timer
2. Absorbent tissue paper

**TEST PROCEDURE**

1. Test Strips were removed from foil pouch, placed on a flat and dry surface.
2. All the strips were marked with sample number.
3. Three drops (using dropper) or 100 ul (using pipette) of serum/plasma was added into sample well provided for the test on the strips.
4. Wait for 20 minutes for the indication of colored line in the test region.

**INTERPRETATION**

1. A colour band will appear at left section of the result window to show that the test is working properly. This band is the “Control Band”.
2. The right section of the result window indicates the test results. If another colour band appears at the right section of the result window, this band is the “Test Band”.
Positive Result

The presence of two colour bands ("T" band and "C" band) within the result window, a no matter which band appears first, indicates a positive result.

Negative Result

The presence of only one purple colour band within the result window indicates a negative result.

Invalid Result

If the purple colour band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.
PART B-II TEST OF ASSOCIATION OF HIV POSITIVE PATIENT WITH HEPATITIS C

EXPERIMENT 1

DETECTION OF HCV BY ELISA

QUALISA-HCV is an Enzyme Linked Immunosorbent Assay that employs highly purified synthetic peptides representing most conserved antigenic segments of Core, NS3, NS4 & NS5 antigens from multiple genotypes. These antigens are so selected that they recognize all six major genotypes of HCV prevalent worldwide. Microwell strips are coated with synthetic peptides representing Core, NS3, NS4 & NS5 antigens. Samples along with positive & negative controls are added in the coated wells & incubated. The wells are washed to remove unbound components & goat antihuman IgG conjugated to horseradish peroxidase (HRPO) is added. After a short incubation the wells are washed again & bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid & absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value (COV) is calculated by the given formula & absorbance of all the wells are compared with the cutoff value. Any sample having absorbance more than the COV is considered reactive.

Components - QUALISA-HCV has following components:

1. Coated microwells- Microwells coated with synthetic peptides representing HCV derived from multiple genotypes of HCV. Ready to use.
Materials and Methods

2. **Positive control** - Inactivated & stabilized human serum reactive for HCV & non reactive for HIV & HBsAg with preservatives.

3. **Negative control** - Inactivated & stabilized human serum non reactive for HIV 1 & HIV 2, HBsAg & HCV.

4. **Conjugate** - Goat anti human IgG HRPO conjugate (50x). To be diluted 50 times with conjugate diluents.

5. **Conjugate diluents** - Buffered solution containing stabilizing proteins & preservatives. Ready to use.

6. **Same diluents** - Buffer solution containing stabilizing proteins & preservatives. Ready to use.

7. **Substrate** - Solution containing tetramethyl benzidine (TMB) & hydrogen peroxide. Ready to use.

8. **Wash buffer** - Buffer contains surfactant (20x). To be diluted 20 times with distilled or deionized water.

9. **Stop solution** - Diluted acid. Ready to use.

10. **Microwell holder**.

11. **Instruction for use**.

12. **ELISA protocol sheet**.

13. **Plate sealer**.

**PROCEDURE**

1. Bring all the reagent & specimen to room temperature before use.

2. Take out required number of strips & immediately close the pouch.

3. Prepare ELISA protocol sheet indicating the location of controls & specimen.
4. Use controls in duplicate.
5. Add 200 µl sample diluents to separate wells.
6. Add 10 µl of controls or specimen in the wells.
7. Gently shake the plate to mix thoroughly. Care should be taken to avoid spillage.
8. Apply plate sealer & incubate for 30 minutes at 22-28°C.
9. Wash each well by filling approximately 350 µl diluted wash buffer & aspirating/flicking off six times. Blot dry.
10. Add 100 µl substrate & incubate at 22-28°C away from light for 30 minutes.
11. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
12. Read the reaction at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

INTERPRETATION

The individual absorbance value of negative control should be less than 0.1

The individual absorbance value of positive control should be more than 1.0

Calculation

The cutoff value (COV) is calculated by adding 0.3 to average absorbance value of negative control.

Cutoff value = Av. NC+0.
Samples with absorbance value equal to or greater than cutoff value are considered reactive. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of the duplicates are considered negative for antibodies to HCV. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
EXPERIMENT 2

RAPID TEST FOR HCV

PRINCIPLE

The Hepatitis C virus (HCV) is now recognized as a major agent of chronic hepatitis, transfusion acquired non-A, non-B hepatitis and liver disease throughout the world. HCV is an enveloped positive-sense, single-stranded RNA virus. HCV diagnostic kits detects the presence of HCV antibodies in human serum by immunoassay. We have constructed HCV genes for the expression of recombinant antigens in bacterium systems such as E. coli and focused on structural and non structural regions of HCV-encoded poly-protein, which are definitely immunogenic. The major immunoreactive antigens of these proteins have been reported as Core, NS3, NS4 and NS5 regions of HCV genome, which are known to be highly immunodominant regions. For diagnosis of HCV infection, these recombinant proteins were used as Capture materials and coated on the membrane of an immunochromatographic (rapid) test. Compared to the first generation HCV test using single recombinant antigens, multiple antigens using recombinant proteins have been added in the new generation of tests to avoid non-specific cross-activity and to increase the sensitivity of the HCV antibody test. The SD BIOLINE HCV test is an immunochromatographic (rapid) test for the qualitative detection of antibodies specific to HCV, in human serum, plasma and whole blood.
The SD BIOLINE HCV test contains a membrane strip, which is pre-coated with recombinant HCV capture antigen (core NS3, NS4 and NS5) on test band region. The protein A - colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antigen-antibody-protein A gold particle complex forms with high degree of sensitivity and specificity. The SD BIOLINE HCV test window has been clearly labelled. “T” for “Test Line” T and “C” for “Control Line”. Both the Test Line and Control Line in the result window are not visible before applying any samples. The Control Line is used for procedural control and should always appear if the test procedure is performed correctly.

REQUIREMENTS

Sample : Sera extracted from the blood of patients attending Integrated Counselling For Testing Centre (ICTC).

Components

1. Test strips
2. Capillary pipette or micropipette
3. Assay diluents

TEST PROCEDURE

1. The test device was removed from foil pouch, placed it on a flat, dry surface.
2. 10 µl of serum/plasma was added into the sample well using a micropipette.
3. 4 drops (120 µl) of assay diluents was added into the sample well.

4. Interpretation of test results in 5-20 min.

**INTERPRETATION**

1. A color band will appear in the left section of the result window to show that the test is working properly. This band is control line (C).

2. Color band will appear in the right section of result windows. This band is test line.

**Positive Result**

The presence of two colour bands ("T" and "C" bands) in the result window, no matter which band appears first, indicates a positive result.

**Negative Result**

The presence of only one colour band (Control Band “C”) in the Result Window indicates a negative result.

**Invalid Result**

If the Control band “C” is not visible in the Result Window after performing the test, the result is considered invalid. This specimen must be retested using a new test device.
PART B-III  DETECTION OF SYPHILIS IN HIV POSITIVE PATIENTS

EXPERIMENT

RAPID PLASMA REAGIN (RPR) TEST

PRINCIPLE

Syphilis is a sexually transmitted (venereal) disease caused by the spirochaete *Treponema pallidum*. After infection the host forms Treponemal antibodies to *Treponema pallidum*, in addition, the host also forms Non Treponemal antilipoidal antibodies in response to the lipoidal material released from the damaged host cell. These antibodies are traditionally referred as ‘Reagins’. The Rapid Plasma Reagin (RPR) / Carbon Antigen test is a macroscopic non Treponemal flocculation test for the detection and quantitation of antilipoidal.

Non-Treponemal tests like Carbogen are of great value when used for screening and follow up of therapy. During the testing procedure, the specimen, serum or plasma is mixed with the Carbogen reagent and allowed to react for eight minutes. If antilipoidal antibodies are present in the specimen, they will react with the Carbogen reagent forming visible black floccules. If antilipoidal are not present in the specimen, there will be no flocculation.
REQUIREMENTS

Sample - Sera extracted from the blood of patients attending ICTC.

Reagents

Reagent 1: RPR Antigen Suspension
Reagent 2: Positive Control Serum
Reagent 3: Negative Control Serum

Accessories

1. Disposable plastic cards
2. Disposable plastic droppers
3. Disposable applicator sticks
4. Antigen delivery dropper (for delivering a drop of approx. 15-20 µl)
5. Rubber teats

TEST PROCEDURE

1. Pipette one drop (50 µl) of the test specimen, positive control negative control onto separate reaction circles of disposable slide using a sample dispensing pipette.

2. Add one drop of well mixed Carbogen reagent next to the test specimen, positive control and negative control by using the reagent dropper provided with the kit. Do not let the dropper tip touch the liquid on the slide.
3. These drops were mixed well and spread out the pool of liquid uniformly within the entire area of the circle by using the disposable applicator stick supplied in the kit.

4. The card was rocked gently to and fro 9 min and observed under good light source for appearance of agglutination.

**INTERPRETATION**

- Large and medium black floccules against white background - Reactive.
- Small black floccules against white background - Weakly Reactive.
- No floccules, even grey backgrounds - Non Reactive.

Flocculation is a positive test result and indicates the presence of antilipoidal in the test specimen. No Flocculation is a negative test result and indicates the absence of antilipoidal antibodies in the test specimen.