The Performance of First Response HIV-1/2 O Card Test was evaluated at various external laboratories & institutes. The summary of reports are as follows:

<table>
<thead>
<tr>
<th>Name of the Institute</th>
<th>Year of Testing</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Institute of Biologicals, India</td>
<td>2003</td>
<td>100 %</td>
<td>99.87 %</td>
</tr>
<tr>
<td>Ghana Health Service, Ghana</td>
<td>2004</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>National Institute for Communicable Diseases, South Africa</td>
<td>2004</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Virology Laboratory, University Teaching Hospital, Zambia</td>
<td>2006</td>
<td>100 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

**Precision**

a) Within run precision was determined by using 10 replicates of four different specimens containing different concentrations of antibodies. Within run precision was observed 100%.

b) Between run precision was determined by using the four different specimens containing different concentrations of antibody in 3 different replicates with 3 different lots of test devices. Between run precision was observed 100%.

**Reference**

2) Eve M. Lackritz, M.D., Glen A. Satten, Ph. D, etc.: Estimated risk of transmission of the Human Immunodeficiency Virus by Sexually Intervened Patients. Nature Medicine, Volume 2, Number 6, June 1996.
4) V.S. Ivanov, Z.K. Suvorova, L.D. Tchikin, A.T. Kozhich and V.T. Ivanov : Effective method for purification of human Immunodeficiency virus-1 gag epitope derived from a recombinant antigen based on only synthetic peptides. Researchers have constructed HIV-1 & 2 genes for the expression of recombinant antigens in bacterium systems such as E. coli and focused on HIV-1 & 2 proteins, which are definately immunogenic. The major immunoreactive antigens of these proteins have been reported to have HIV-1 gp120, p24, and HIV-2 gp36 based on western blot analysis. The First Response HIV-1/2 O Card Test is an immunochromatographic (rapid) qualitative test for the detection of antibodies of all classes specific to HIV-1 including Group O and HIV-2 in human serum, plasma or whole blood.

**Assay Principle**

First Response HIV-1/2 O Card Test is based on the principle of immunochromatography in which nitrocellulose membrane is precoated with recombinant HIV-1 capture antigens (gp41) including Group O and p24 on test band “1” region and with recombinant HIV-2 capture antigen (gp36) on test band “2” region. The Test Device has markings “C,” “T” and “1” & “2” on it at the corresponding positions of “Control Line”, “HIV-1” & “HIV-2” on the membrane. No line will be visible in the Result Window before applying any sample. When the test sample along with Assay Diluent flows through the nitrocellulose membrane, the recombinant HIV-1 & 2 antigens (gp41, p24 and gp36) conjugated with colloidal gold particles bind to HIV-1 & 2 antibodies present in test sample. This Conjugated antigen-antibody complex moves through the nitrocellulose membrane and bind to the corresponding immobilised HIV 1 antigens and HIV 2 antigens (Test Lines) leading to the formation of colour visible line as the capture antigen-antibody conjugated antigen complex, indicating reactive results. Control band will appear irrespective of reactive or non reactive sample. The control band will serve to validate test performance.

**Materials Provided**

First Response HIV-1/2 O Card Test kit contains the following items to perform the assay.

**Reagents**

- Test Device with sample pipette and desiccant (Test device enclosed nitrocellulose test strip on which test and control lines are coated).
- Assay Diluent (Carbonate buffer saline containing proteins and preservative).

**Materials Required but Not Provided:**

- Sterile lancet

**Storage & Stability**

First Response HIV-1/2 O Card Test should be stored at 4 - 30°C. Do not freeze the kit or components. Assay Diluent (opened & unopened) & the unopened Test Device are stable until the expiry date printed on the label, when stored at room temperature 4 - 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the Test Device from the foil pouch. The shelf life of the kit is as indicated on the outer package. Do not use Test Device and Assay Diluent beyond the date of expiry.
Precautions
1) For in vitro diagnostic use only.
2) Test Devices and Assay diluent of different lot must not be used.
3) Do not use Test Device if the pouch is not intact.
4) Do not use the Lancets if the seal is broken.
5) Check the desiccant for saturation, immediately after opening the pouch.
6) Do not smoke, eat or drink while handling specimens and performing a test.
7) The test device, alcohol swab, lancet and sample pipette are intended for single use only.
8) Follow the assay procedure strictly, deviation will invalidate the results.
9) Perform the test by using kit assay diluent, any other diluent or fluid will invalidate the results.
10) Do not touch the tip of Assay Dilluent bottle, it might contaminate Assay Diluent.
11) Wear protective gloves while handling specimens. Dispose of used gloves as biohazard waste. Wash hands thoroughly afterwards.
12) Avoid splashing or aerosol formation.
13) Clean up spills thoroughly using an appropriate disinfectant.
14) Decontaminate and dispose off all used specimens, test devices, alcohol swabs, lancets and sample pipettes as a biohazard waste.

Specimen Collection and Storage
1) Capillary blood collection: Clean the area to be lanced with an alcohol swab and allow it to air dry. Squeeze the end of the fingertip and pierce with a sterile lancet provided. Take a 20 μl sample pipette provided, immerse the open end in the blood drop and then release the pressure to draw blood into the sample pipette.
2) Venous Blood collection: Collect the Whole Blood in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture.
3) Plasma collection: Collect the Whole Blood in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture. Centrifuge the tube to obtain Plasma.
4) Serum: Collect Whole Blood in collection tubes without having any anticoagulants by venipuncture. Keep it in standing position for 30 min and centrifuge it at 3000 rpm for 10-15 minutes to obtain serum.
5) Whole Blood specimen may be used for testing immediately or may be stored at 2-8°C for upto 3 days.
6) If Serum or Plasma specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than three days, freezing at -20° C is recommended. They should be brought to room temperature prior to use.
7) Serum or Plasma specimens containing precipitate may yield inconsistent test results. Such specimens must ALWAYS be centrifuged prior to assay.

Test Procedure
1) Bring the First Response® HIV 1-2 O Card Test kit components to room temperature prior to testing.
2) Remove the Test Device and the sample pipette from the foil pouch and place it on a flat, dry surface.
3) Slowly add 20 μl (two drops) of Whole Blood or 10 μl (One Drop) of serum / plasma to the sample well (S) using the Sample Pipette. Dispose off used sample pipette as biohazard waste.
4) Add 15 μl (One Drop) of the Assay Diluent to the sample well (S).
5) Observe for development of colored bands in the Results Window.
6) Interpret test results at 5-15 minutes. (After recording the results, dispose off test device as a biohazard waste).
7) Do not interpret after 15 minutes.

Sample Pipette

Test Procedure

Result Window

Sample Well

Add 1 drop of Assay Diluent to the Sample Well (S).

Add 2 drops of Whole Blood or 1 drop of Serum / Plasma to the Sample well (S).

Gently release to draw blood

Gently squeeze the bulb

Invert open end in blood

If the room temperature is significantly lower than 15°C, then interpret the result at 20 minutes. Do not interpret after 20 minutes.

Interpretation of the Test

Negative Result
If only one color band appear, at control line 'C' as in the figure, the specimen is negative.

Positive Results
If two color bands appears, one at control line ‘C’ and other at test line HIV-1 ‘1’ as in the figure, the specimen is reactive for antibodies to HIV-1.

HIV - 1 Positive

HIV - 2 Positive
If two color bands appears, one at control line ‘C’ and other at test line HIV-2 ‘2’ as in the figure, the specimen is reactive for antibodies to HIV-2.

HIV 1 & 2 Positive
If all three color bands appears, one at control line ‘C’ and other two at test lines HIV-1 ‘1’ & HIV-2 ‘2’ as in the figure, the specimen is reactive for antibodies to HIV-1 & HIV-2.

Invalid Result
If no color band appears at the control line ‘C’ within the stipulated time then the result is invalid.

Caution
1) The anti-coagulants such as heparin, EDTA and citrate do not affect the test result.
2) Hemolytic sample may give reddish background even after end of test time.
3) Repeat the test in case of very faint test line or if have any doubt for test line.
4) False negative results may arise because of hook effect due to very high titer of antibody in sample. Repeat the test by using different dilution of same sample.
5) Although a positive result may indicate infection with HIV-1 or HIV-2 virus, a diagnosis of AIDS can only be made on clinical grounds. If an individual meets the case definition for AIDS established by the centers for Disease Control. For samples repeatedly tested positive, more specific supplemental tests must be performed.
6) Immunochromographic testing alone cannot be used to diagnose AIDS even if the antibodies against HIV-1 and HIV-2 are present in a patient specimen. A negative result at any time does not preclude the possibility of HIV-1 or HIV-2 infection. Due to similarity in the genetic structures of HIV-1 and HIV-2, appearance of both test bands for HIV-1 and HIV-2 in the reactive sample may indicate mixed infection.

Performance Characteristics

<table>
<thead>
<tr>
<th>Method</th>
<th>Reference</th>
<th>First Response® HIV 1-2 O Card Test</th>
<th>Total Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial ELISA</td>
<td>Positive</td>
<td>121</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>121</td>
</tr>
<tr>
<td>TOTAL RESULTS</td>
<td>121</td>
<td>121</td>
<td>242</td>
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