INTENDED USE

The **OnSite HIV-1/2 Ab Rapid Test** is an indirect lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of IgG anti-HIV-1 and anti-HIV-2 antibodies in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with HIV. Any reactive specimen with the OnSite HIV-1/2 Ab Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

Human immunodeficiency virus type I and type II (HIV-1 and HIV-2) are enveloped single-strain RNA positive virus. The causative relationship between HIV-1 and HIV-2 virus and acquired immunodeficiency syndrome (AIDS) has been established over decades. HIV-1 has been isolated from patients with AIDS and AIDS-related complex, and from healthy individuals with a high risk for developing AIDS. HIV-2 has been isolated from West African AIDS patients and from zero-positive asymptomatic individuals.

The two types of HIV have significant variation in sequences. HIV-1 has been divided into three groups: group M (for major), including at least ten subtypes (A through J), group O (for outlier); and group N (for non-M, non-O). Similarly, the HIV-2 has been classified into at least five subtypes (A through E). Some HIV-1 variants show up to 50% homology in their envelope genes with the sequences of more common prototype strains.

Both HIV-1 and HIV-2 virus can elicit strong immune responses, including the production of anti-virus antibodies. Presence of specific anti-HIV-1 and/or anti-HIV-2 virus antibody in blood, serum, and plasma indicates the exposure of an individual to the HIV-1 and/or HIV-2 virus, being of great value for clinical diagnosis.

The **OnSite HIV-1/2 Ab Rapid Test** utilizes the conserved envelope antigen domains, which allows IgG antibodies to the HIV-1 including O subtype or HIV-2 to be detected.

TEST PRINCIPLE

The **OnSite HIV-1/2 Ab Rapid Test** is an indirect lateral flow chromatographic immunoassay. The test strip consists of: 1) a burgundy colored conjugate pad containing mouse monoclonal anti-human IgG antibody conjugated with colloidal gold (Human IgG Conjugates), 2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands) and a control band (C band). The T1 band is pre-coated with recombinant HIV-1 antigen gp120 / gp41 for the detection of antibodies to HIV-1. T2 band is pre-coated with HIV-2 antigen gp36 for the detection of antibodies to HIV-2, and the C band is pre-coated with goat anti-mouse IgG antibody.

When an adequate volume of test specimen is dispensed into the sample pad of the test strip, the specimen migrates by capillary action across the strip. IgG anti-HIV-1 antibodies if present in the sample migrate through the conjugate pad where they bind to the Human IgG Conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV-1 antigen, forming a burgundy colored band on the T1 region, indicating a positive test result. Absence of this band in the test region suggests a HIV-1 antibody negative result.

IgG anti-HIV-2 antibodies if present in the sample migrate through the conjugate pad where they bind to the Human IgG Conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV-2 antigen, forming a burgundy colored band on the T2 region, indicating a positive test result. Absence of this band in the test region suggests a HIV-2 antibody negative result.

The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-mouse IgG mouse anti-human IgG conjugates regardless of the presence of any colored T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 50 test devices, each sealed in a foil pouch with two items inside:
   a. One strip device
   b. One desiccant.
2. Sample diluent (2 bottles, 5 mL/bottle)
3. 50 mini plastic droppers.
4. One package insert (instruction for use)

MATERIALS REQUIRED AND AVAILABLE FOR PURCHASE

1. Positive Control (1 vial, red cap, 1 mL, Cat # R0016-P)
2. Negative Control (1 vial, green cap, 1 mL, Cat # R0016-N)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood for the testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 20 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 20 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e., an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C-30°C. The positive and negative controls should be kept at 2°C-8°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

**Plasma**

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

**Serum**

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C-8°C if not tested immediately. Store specimens at 2°C-8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen’s ID number.

Step 4: Fill in the mini plastic dropper with the specimen not to exceed the specimen line as shown in the following image. The volume of the specimen is around 5 µL.

Note: Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 5µL volume.

Holding the dropper vertically, dispense all of the specimen into the sample pad making sure that there are no air bubbles.

Then add 2 drops (about 70-100 µL) of Sample Diluent immediately.
Step 5: Set up timer.

Step 6: Read the test result in 20 minutes. Positive result could be visible as short as 1 minute.

Don’t read results after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

### QUALITY CONTROL

Using individual OnSite HIV-1/2 Ab Rapid Test strips as described in the Assay Procedure above, run 1 positive control and 1 Negative Control (both provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

Expected results are as follows:

**Negative Control**

Only the C band shows color development, the two T bands (T1 and T2) show no color development.

**Positive Control**

The C band and two T bands (T1 and T2) show color development.

The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

### INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no HIV antibodies are detected in the specimen. The result is negative.

2. **POSITIVE RESULT**:  
   
   2.1 In addition to the presence of C band, if T1 band is developed, the test indicates for the presence of antibodies to HIV-1 in the specimen. The result is HIV-1 positive.
   
   2.2 In addition to the presence of C band, the test indicates for the presence of antibodies to HIV-2 in the specimen. The result is HIV-2 positive.
   
   2.3 In addition to the presence of C band, if both T1 and T2 bands are developed, the test indicates for the present antibodies to HIV 1 and or HIV 2. The test result is HIV positive.

   To differentiate the type of virus infection, dilute the specimen at 1:100 dilution with the Sample Diluent provided, re-run the test. Interpret the result as illustrated above (also See Limitations of Test §).

   Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID**: If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands as indicated below. Repeat the assay with a new device.

### PERFORMANCE CHARACTERISTICS

1. **Clinical Performance For HIV-1 Ab Test**

A total of 2,000 samples from susceptible subjects were tested by the OnSite HIV-1/2 Ab Rapid Test and by a Chinese State Drug Administration (SDA) licensed EIA. Comparison for all subjects is showed in the following table:

<table>
<thead>
<tr>
<th>EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>31</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>1958</td>
<td>1969</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>42</strong></td>
<td><strong>1958</strong></td>
<td><strong>2000</strong></td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100%, Relative Specificity: 99.4%, Overall Agreement: 99.5%

2. **Clinical Performance For HIV-2 Ab Test**

A total of 300 samples from susceptible subjects were tested by the OnSite HIV-1/2 Ab Rapid Test and by a Chinese State Drug Administration (SDA) licensed EIA. Comparison for all subjects is showed in the following table:

<table>
<thead>
<tr>
<th>EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>274</td>
<td>275</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>26</strong></td>
<td><strong>274</strong></td>
<td><strong>300</strong></td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100%, Relative Specificity: 99.6%, Overall Agreement: 99.8%

### LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to HIV in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The OnSite HIV-1/2 Ab Rapid Test is limited to the qualitative detection of antibodies to HIV-1 or HIV-2 in human serum or plasma. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HIV-1 or HIV-2 antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with HIV-1 or HIV-2.
4. A negative result can occur if the quantity of the HIV-1 or HIV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. As illustrated in INTERPRETATION OF ASSAY RESULT-2.3, all the three positive bands (T1, T2 and C) may develop when tested with samples containing high titer of HIV-1 antibodies. To differentiate the cross reactivity: dilute the test specimen with Sample Diluent at 1:100 dilution, then re-test the diluted specimen with a new test device. Only T1 band and C will appear if it is a HIV-1 Ab response. If T1, T2 and C band all appear, the test indicates for the exposure of both HIV-1 and HIV-2.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

### REFERENCES