**INTENDED USE**

The Aria HIV 1+2 Ag-Ab ELISA Kits is a solid phase enzyme linked immunoabsorbent assay for the qualitative detection of HIV-1 P24 antigen, anti-HIV-1 including subtype O and anti-HIV-2 antibodies (including isotype IgG, IgM and IgA in human serum or plasma. It is intended for professional use only as an aid in the early identification of infection with HIV-1 and HIV-2 viruses.

**TEST PRINCIPLE**

The Aria HIV 1+2 Ag-Ab ELISA Kits is a solid phase enzyme linked immunoabsorbent assay based on the principle of the double antibody/sandwich technique for the detection of the various antibodies against HIV-1 and/or HIV-2 and HIV antigen in human serum or plasma.

**INTRODUCTION**

Human immunodeficiency virus type I and type II (HIV-1+2) are enveloped single strand RNA positive viruses. The causative relationship between HIV-1 and HIV-2 viruses 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 (1). HIV-2 has been isolated from West African AIDS patients and from seropositive asymptomatic individuals (2).

Infection with HIV induces the immune system to produce antibodies against viral proteins from different parts of the HIV genome. ENV, GAG and POL. Diagnosis of anti-HIV seropositivity is based on the detection of these specific antibodies. HIV antigen is produced during the viral replication phase and generally appears some days after exposure then decreases quickly as antibodies are being produced. Years later, antigenemia may again increase, and is indicative of intense viral replication. Kits to detect markers of HIV infection have been available since 1985.

The Aria HIV 1+2 Ag-Ab ELISA Kits is a latest generation of HIV kits for the simultaneous detection of the presence of anti-HIV antibodies and HIV antigens.

**REAGENTS AND MATERIALS PROVIDED**

1. Solid microwells pre-coated with:
   a. Recombinant HIV-1 and HIV-2 antigens;
   b. Anti P-24 antibody coated microwells;
2. Liquid conjugates composed of:
   a. Recombinant HIV-1 and HIV-2 antigens conjugated with horse reddish peroxidase (HRP-HIV-1+2 conjugates);
   b. Anti-P24 antibody conjugated with biotin (Biotinylated P24 Ab) and avidin conjugated with HRP (HRP-avidin conjugates).

**ASSAY PROCEDURE**

1. Bring all reagents, controls to room temperature (18°C-28°C).
2. Dilute concentrated Wash Buffer 30 fold with water as following:
   - Full plate: 580 mL
   - Half plate: 290 mL
   - A quarter plate: 145 mL
   - 30 mL wash buffer: 10 mL
   - Final volume: 660 mL
   - 300 mL

3. Place the reagents and controls into the designated reagent wells, respectively. The reaction is stopped with Stop Solution and absorbance is read using a spectrophotometer at 450/620-690 nm.

1. 2.3 Test wells:
   - TMB substrate A 6 mL
   - TMB substrate B 6 mL
   - Stop solution 6 mL

**WARRANTS 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 use expired devices.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolized blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucous membrane protective clothing and disposable gloves while handling the kit reagents.
7. 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. All the reagents are stable through the expiration date printed on the label if not opened. Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
10. The enzyme reaction is very sensitive to metal ion. Thus, do not allow any metal element to come into contact with the enzyme reaction solution.
11. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
12. Use a new distribution pipet for each specimen. Never use the specimen container to distribute conjugates and standards.
13. The wash procedure must be avoided completely before adding the Washing Solution. Insufficient washing will result in poor precision and falsely elevated absorbance.

**SPECIMEN COLLECTION AND PREPARATION**

- Specimen should be prepared from a whole blood specimen obtained by accepted venipuncture technique. This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerated at 2°C-8°C. If storage period longer than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of toxicologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

**PREPARATION OF THE REAGENTS**

1. Warm up the concentrated Washing Buffer at 37°C to dissolve the precipitant if it appears.
2. Mix each reagent before adding to the test wells.
3. Determine the number of microwells needed and mark on the ELISA Working Sheet the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

**ASSAY PROCEDURE**

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
2. Add specimens according to the designation on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

**STORAGE AND STABILITY**

All reagents except the concentrated wash buffer are ready to use as supplied. Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8°C.
B. Add 50 μL (or 1 drop) of TMB substrate A and 50 μL (or 1 drop) of TMB substrate B into each well including the blank well.

9. Incubate at 37°C in dark for 10 minutes.

10. Stop the reaction by adding 50 μL (1 drop) of stop buffer to each well. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

11. Set the microplate reader wavelength at 450nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620-690nm can be used as a reference wavelength to optimize the assay result.

INTERPRETATION OF RESULTS

A. Set up the cut-off value
The cutoff value = 0.10 + N
N: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if less than 0.05.

B. Calculation of specimen OD ratio
Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

<table>
<thead>
<tr>
<th>Specimen OD</th>
<th>Cutoff Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen OD ratio = Specimen OD / Cutoff Value</td>
<td></td>
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</tbody>
</table>

C. Assay validation
The mean OD value of the HIV Ab positive controls = > 0.80. The mean OD value of the HIV negative controls = < 0.05. The mean OD value of the P24 positive control = > 0.80.

Check the procedure and repeat assay if above conditions are not met.

D. Interpretation of the results

<table>
<thead>
<tr>
<th>Specimen OD ratio</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 1.00</td>
<td>≥ 1.00</td>
</tr>
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</table>

1. The negative result indicates that there is no detectable HIV antibody and antigen in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the AntiHIV 1+2 Ab-Ag Test. They should be retested in duplicate before final interpretation.

Non repeatable reactions are often caused by:
- Inadequate microwell washing.
- Contamination of negative specimens by serum or plasma with a high antibody titer.
- Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
- Contamination of the stopping solution

If after retesting a specimen, the absorbance value of the 2 duplicates is less than the cut-off value, the initial result is non repeatable and the specimen considered to be negative with the AntiHIV 1+2 Ab-Ag Test.

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HIV antigen and anti-HIV antibodies in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.

2. The AntiHIV 1+2 Ab-Ag ELISA Kits limited to the qualitative detection of HIV antibody and antigen in human serum or plasma. The intensity of the color does not have linear correlation with antibody and antigen titer in the specimen.

3. A negative result for an individual subject indicates absence of detectable HIV P24 antigen, anti-HIV-1 and 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 P24 antigen, anti-HIV-1 and HIV-2 antibodies present in the specimen are below the detection limits of the assay, or the antigen and antibodies that are detected are not present during the stage of disease in which a specimen is collected.

REFERENCES