



DoubleCheck™

HIV 1&2



Code: 60332000 Version: 332/E11 Format: 40 tests

The **HIV 1 & 2 DoubleCheck™** is a rapid test for the qualitative detection of antibodies to human immunodeficiency virus types 1 and 2 (HIV-1 & HIV-2), in human serum or plasma. Forty individual tests may be performed with one kit.

Introduction

The Human Immunodeficiency Virus (HIV) is a retrovirus, identified in 1983 as the etiologic agent of the Acquired Immunodeficiency Syndrome (AIDS). The HIV virus consists of a genomic RNA molecule associated with a reverse transcriptase (RT), protected by a capsid and an envelope. Two types, HIV-1 and HIV-2, exhibiting different prognosis and transmission rates, have been distinguished.

The major routes of HIV transmission are sexual contact, contamination by blood or blood products and mother-to-newborn transmission. The progressive deterioration of the immune defense mechanism during development of the disease facilitates opportunistic infections with fatal consequences.

The HIV 1&2 DoubleCheck™ employs HIV-derived recombinant and synthetic peptide antigens for the rapid and reliable detection of antibodies to HIV-1 and HIV-2 in human serum or plasma, without instrumentation.

Principle of the Test

The HIV 1&2 DoubleCheck™ is a dual recognition enzyme-immunoassay (EIA), based upon the specific detection of anti-HIV antibodies by antigens that bind both antibody binding sites. The test utilizes a combined application of immuno-chromatography and immuno-concentration. The Reaction Device contains two ports: a circular Specimen Port A for addition of specimen; and a larger, elliptical Reaction Port B, sensitized with HIV-antigens and containing the Internal Control (Fig. 1).

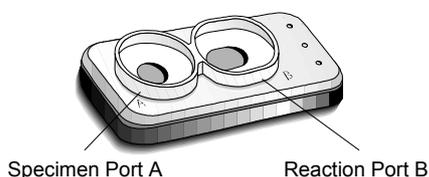


Figure 1. Reaction Device

To start the test, a diluted specimen is introduced to Port A. The fluid components migrate along the chromatographic strip and are concentrated in Port B. This results in specific binding of anti-HIV antibodies to immobilized HIV antigens (Fig. 2).

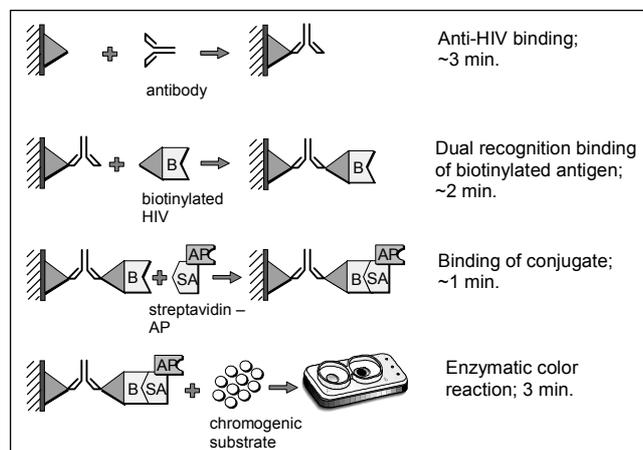


Figure 2. Principle of the Test

The antigen-antibody complex is allowed to bind biotinylated HIV antigen in a dual recognition step, followed by reaction with a streptavidin/alkaline phosphatase (AP) conjugate. Visualization of the results as gray-blue spots is accomplished by reaction with the Chromogenic Substrate. The appearance of 2 gray-blue spots in Reaction Port B indicates the presence of antibodies to HIV. In a negative result, only the Internal Control spot will be visible. The Stop Solution is added to facilitate documentation and future reference.

Kit Contents

Equipment

- 40 HIV 1 & 2 DoubleCheck™ Reaction Devices.
- 42 disposable pipettes for transferring specimens.
- 42 microtubes for diluting specimens.

Reagents

Reagent Number	Flask	Vol. (ml)	Contents
1	1 dropper bottle; white closure	5	Specimen Diluent
2	1 dropper bottle; red closure	15	Biotinylated HIV Antigens
3	1 dropper bottle; yellow closure	5	Streptavidin/AP Conjugate
4	1 dropper bottle;	50	Chromogenic Substrate
5	1 dropper bottle: blue closure	5	DoubleCheck™ Enhancer
6	1 dispenser bottle with separate dropper	50	Stop Solution

Controls

Positive Control – 1 vial (red-colored cap) of 1.5 ml diluted human plasma positive for anti-HIV, inactivated by addition of β-propiolactone and by heat treatment.

Negative Control – 1 vial (green-colored cap) of 1.5 ml diluted heat-inactivated human plasma, negative for anti-HIV.

Safety and Precautions

- This kit is for *in vitro* diagnostic use only.
- Handle the Positive Control as if potentially infectious even though it has been inactivated.
- Human source materials in the Negative Control were tested and found to be non-reactive for HIV, hepatitis B surface antigen and antibodies to hepatitis C virus. Since no test method can give complete assurance of the absence of viral contamination, all reference solutions and all human specimens should be handled as potentially infectious.
- Wear surgical gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose of all specimens, used Reaction Devices, disposable pipettes, microtubes, and other materials used with the kit as biohazardous waste.
- Do not touch the dropper tip.
- Do not mix reagents from different lots.
- Do not use the kit after expiry date.

Storage of the Kit

Store the kit at 2°-8°C. Do not freeze.

Handling of Specimens

Either serum or plasma specimens may be tested.

Specimens may be stored for 7 days at 2°-8°C before testing.

To store for more than 7 days, freeze specimens at -20°C or colder. After serum specimens have thawed, centrifuge them for clarification. Test the supernatant. Avoid repeated freezing and thawing.

Test Procedure

Preparing the Test

- Read all Test Instructions carefully before starting the test.
- Leave specimens, reagents and Reaction Devices for 3 hours at room temperature (22°-26°C) or for **10 minutes** at 37°C.
- Remove the required number of DoubleCheck™ Reaction Devices from their aluminum pouches.
- Replace the cap from Stop Solution dispenser bottle by the drop dispenser.
- Perform the test at room temperature.

Test Instructions

1. Binding of Specific Antibodies

Add **1** drop (50 µl) of Reagent #1 to a microtube. With a disposable pipette add **4** drops specimen or control fluid (150 µl) to the microtube. Discard excess fluid as biohazard waste. **Mix** contents of tube by refilling and ejecting repeatedly with the pipette.

Immediately transfer the entire contents of the microtube, using the same pipette, to **Port A** of the DoubleCheck™ Reaction Device.

Discard the pipette and microtubes as biohazard waste.

Wait until the solution has been absorbed (approximately 3 minutes).

The following steps are performed in Reaction Port B of the Doublecheck™ Reaction Device only.

2. Binding of Biotinylated Antigen

Add **6** drops of Reagent #2 (Biotinylated Antigen) to Port B. Wait until the solution has been absorbed. (approximately 2 minutes)

3. Binding of Conjugate

Add **2** drops of Reagent #3 (Streptavidin/Alkaline Phosphatase) to Port B.

Wait until the solution has been absorbed (approximately 1 minute).

4. Chromogenic Reaction

Fill Port B to the top with Reagent #4 (Chromogenic Substrate Solution).

Wait 3 minutes.

5. Addition of DoubleCheck™ Enhancer

Add **2** drops of Reagent #5 (DoubleCheck™ Enhancer) to Port B.

Wait 3 minutes.

6. Stop Reaction

Fill Port B to the top with Reagent #6 (Stop Solution).

Wait until the solution has been absorbed; then examine Port B and record results.

Interpretation of the Results

Validation

Examine the base of Port B.

In order to confirm the proper functioning of the test and to demonstrate that the results are valid, the **Internal Control** spot should appear on each device (Figures 3a and 3b; right hand spot).

The **absence** of the **Internal Control** spot (Fig. 3c) should be considered an invalid result. The test should be repeated.

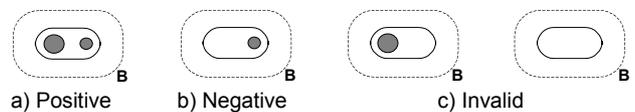


Figure 3. Validation and Interpretation

Note: In rare cases, a highly positive HIV specimen may yield a barely visible Internal Control spot against strong background. In such a event, the test should be repeated by prediluting the specimen 1:1 as follows:

With a disposable pipette add 75 µl Negative Control solution to a microtube. Add 75 µl specimen and mix contents of tube by refilling and ejecting repeatedly with the pipette. Add 1 drop (50 µl) of Reagent # 1 to this mixture. Continue according to Step 1 by mixing and transferring contents to Port A of the device.

Results

Positive Result (Fig. 3a): The presence of **two** spots indicates the presence of antibodies to HIV-1 and/or HIV-2.

Negative Result (Fig. 3b): The **sole presence** of the **Internal Control** spot indicates the absence of antibodies to HIV.

Any faintly colored spot, must be suspected to represent a reaction and must be investigated further.

Storing unused part of the kit

Return remaining control solutions, reagents and the Instructions for Use to the original kit box.

Store at 2°-8°C.

Limitations

The HIV 1 & 2 DoubleCheck™ is a screening test. Since production of antibodies to HIV may be delayed following initial exposure, anti-HIV screening tests may fail to detect early stages of HIV infection. Therefore non-reactivity with this test must not be considered conclusive evidence that the patient has not been exposed to or infected by HIV.

Presence of antibodies to HIV-1 and/or HIV-2 in the tested specimen should be confirmed by a confirmatory assay.

Performance Characteristics*

A clinical evaluation of the **HIV 1&2 DoubleCheck™** was performed under auspices of the French Health Agency ADM. Testing the ADM reference panel for HIV-1 and HIV-2-

* Detailed data available upon request

sensitivity resulted in 100% sensitivity. All six **HIV-1 subtype O-positive** specimens were also detected.

Specificity was evaluated on 3 populations. Testing of specimens from 404 blood donors yielded a specificity of 99.75%, Testing of specimens from 219 hospitalized patients and 100 pregnant women yielded a specificity of 100%.

In a separate study on 108 HIV-positive and 179 negative sera, both the sensitivity and specificity were 100%.

Bibliography

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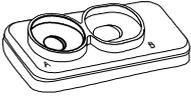
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Summary of Test Procedure

The abbreviated instructions below are for *experienced users* of the HIV 1 & 2 DoubleCheck™ Kit.

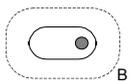
(For detailed instructions please refer to complete text inside)

Bring reagents and Reaction Devices to room temperature (22°-26°C) and perform the test at room temperature.

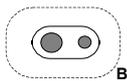
STEP:	REAGENT:	ADD:	TO:	TIME:
1. ANTIBODY BINDING	<i>Reagent #1</i> <i>Specimen</i>	1 drop (50 µl) 4 drops (150 µl); MIX 200 µl	Microtube  Mixture Transfer to ↓ Port A 	Wait until absorbed (~ 3 min.)
2. BINDING OF BIOTINYLATED ANTIGEN	<i>Reagent #2</i>	6 drops	Port B 	Wait until absorbed (~2 min.)
3. BINDING OF CONJUGATE	<i>Reagent #3</i>	2 drops	Port B 	Wait until absorbed (~1 min.)
4. CHROMOGENIC REACTION	<i>Reagent #4</i>	~1 ml	Port B 	Wait 3 min.
5. DOUBLECHECK™ ENHANCER	<i>Reagent #5</i>	2 drops	Port B 	Wait 3 min.
6. STOP REACTION	<i>Reagent #6</i>	~1 ml	Port B 	Wait until absorbed; record result

EXAMINE PORT B

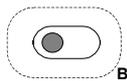
RESULTS:



Negative



Positive



Invalid

