

North Carolina HIV Prevention Program

Appendix: Quality Assurance Protocol for State Purchased Rapid HIV Testing Kits

Documentation, Policies, and Package Inserts

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Rapid Testing Kit Storage Temperature Log

Thermometer location:
Rapid test kit brands monitored:
Acceptable temperature range: 8°C to 27°C (46°F to 80°F). Recommended to keep kits at room temperature.
Month/Year

Day	Temperature	Min	Max	Initials	Day	Temperature	Min	Max	Initials
1					17				
2					18				
3					19				
4					20				
5					21				
6					22				
7					23				
8					24				
9					25				
10					26				
11					27				
12					28				
13					29				
14					30				
15					31				
16									

NOTE: Periodically (e.g., every six months) check thermometer performance and document. Min/Max thermometers maintain a record of the highest and lowest temperature recorded during an observation period and are highly recommended.

Kits should be checked at least once a week with a preference to daily monitoring. Weekly monitoring should be performed with a min/max thermometer in place.

Corrective Action

Date	Action Taken	Initials

Reviewed by:	Date:
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Rapid Testing Control Storage Temperature Log

Thermometer location:
Rapid test kit brands monitored:
Acceptable temperature range: 2°C to 8°C (35°F to 46°F)
Month/Year

Day	Temperature	Min	Max	Initials	Day	Temperature	Min	Max	Initials
1					17				
2					18				
3					19				
4					20				
5					21				
6					22				
7					23				
8					24				
9					25				
10					26				
11					27				
12					28				
13					29				
14					30				
15					31				
16									

NOTE: Periodically (e.g., every six months) check thermometer performance and document. Min/Max thermometers maintain a record of the highest and lowest temperature recorded during an observation period and are highly recommended.

Controls should be checked at least once a week with a preference to daily monitoring. Weekly monitoring should be performed with a min/max thermometer in place.

Corrective Action

Date	Action Taken	Initials

Reviewed by:	Date:
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Rapid Testing Kit Results Logs

Client ID*	Date/Time	Test Room/Area Temp	Kit name and lot#	Kit exp. date	Specimen type	Time Test Started	Time Test Interpreted	Test Result	Confirm. Test Required?	Date Results Given	Initials of Tester
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						
			Brand:		<input type="checkbox"/> Blood			<input type="checkbox"/> Non-reactive <input type="checkbox"/> Reactive <input type="checkbox"/> Invalid	<input type="checkbox"/> Yes <input type="checkbox"/> No		
			Lot:		<input type="checkbox"/> Oral						

*Unique Client ID

Reviewed by:	Date:
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Rapid Testing Control Results Logs

Date	Time	Test Area Temp	Kit name and lot#	Kit exp. date	Control lot#	Control exp. date	Date controls opened	Reason running controls*	Negative Control Results	Positive Control Results	Initials of Tester
			Brand:							HIV-1:	
			Lot:							HIV-2:	
			Brand:							HIV-1:	
			Lot:							HIV-2:	
			Brand:							HIV-1:	
			Lot:							HIV-2:	
			Brand:							HIV-1:	
			Lot:							HIV-2:	
			Brand:							HIV-1:	
			Lot:							HIV-2:	

*Options for reason running controls: new user, new shipment, new lot#, out of range kits, out of range testing area, training

Corrective Action

Date	Action Taken	Initials

Reviewed by:	Date:
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Rapid Testing Comparison Chart

		Oraquick Advance HIV 1/2 Antibody Test	Uni-Gold Recombigen HIV	Clearview Complete HIV 1/2	Determine Combo HIV	Insti HIV-1 Antibody Test*	DPP HIV 1/2 Assay*
Specimen Collection	Venipuncture Whole Blood	YES	YES	YES	YES	YES	YES
	Fingerstick Whole Blood	YES	YES	YES	YES	YES	YES
	Plasma	YES	YES	YES	YES	YES	YES
	Serum	NO	YES	YES	YES	NO	YES
	Oral Fluid	YES	NO	NO	NO	NO	YES
Complexity	Waived	Whole Blood Oral Fluid	Whole Blood	Whole Blood	Fingerstick Whole Blood	Fingerstick Whole Blood	Whole Blood Oral Fluid
	Moderate	Plasma	Plasma Serum	Plasma, Serum	Venipuncture Whole Blood, Plasma, Serum	Venipuncture Whole Blood, Plasma	Plasma, Serum
Shelf Life	Control Kits	12 months unopened 8 weeks opened	12 months unopened 1 month opened	18-24 months opened or unopened	18 months opened or unopened	12 months (freezer) 28 days (refrigerator)	24 months opened or unopened
	Test Kits	30 months	12 months	24 months	15 months	12 months	23 months
Temperature Requirements	Control Storage	2 to 8°C (35 to 46°F)	2 to 8°C (35 to 46°F)	2 to 8°C (35 to 46°F)	2 to 8°C 35 to 46°F	≤ -20 to +8°C (≤ -4F to 46°F)	2 to 8°C (35 to 46°F)
	Test Kit Storage	2 to 27°C (35 to 80°F)	2 to 27°C (35 to 80°F)	8 to 30°C (46 to 86°F)	2 to 30°C (36 to 86°F)	15 to 30°C (59 to 86°F)	2 to 30°C (36 to 86°F)
	Testing Environment	15 to 37°C (59 to 99°F)	15 to 27°C (59 to 80°F)	18 to 30°C (64 to 86°F)	15 to 30°C (59 to 86°F)	15 to 30°C (59 to 86°F)	(18 to 30°C) (64 to 86°F)
Time	Minimum Time for Development	20 minutes	10 minutes	15 minutes	20 minutes	1 minute	Blood: 10 minutes after running buffer Oral: 25 minutes after running buffer
	Read Window	20 minutes	2 minutes	5 minutes	10 minutes	5 minutes	15 minutes
Sensitivity HIV-1 <small>(95% confidence limits)</small>	Venipuncture Whole Blood	99.6%	100%	99.7%	99.9%	99.9%	99.9%
	Fingerstick Whole Blood	99.6%	100%	99.7%	99.9%	99.8%	99.8%
	Plasma	99.6%	100%	99.7%	99.9%	99.9%	99.9%
	Serum	N/A	100%	99.7%	99.9%	N/A	99.9%
	Oral Fluid	99.3%	N/A	N/A	N/A	N/A	98.9%

		Oraquick Advance HIV 1/2 Antibody Test	Uni-Gold Recombigen HIV	Clearview Complete HIV 1/2	Determine Combo HIV	Insti HIV-1 Antibody Test*	DPP HIV 1/2 Assay*
Specificity (95% confidence limits)	Venipuncture Whole Blood	100%	99.8%	99.9%	99.7%	100%	99.9%
	Fingerstick Whole Blood	100%	99.8%	99.9%	99.8%	99.5%	100%
	Plasma	99.9%	99.8%	99.9%	99.7%	100%	99.9%
	Serum	N/A	99.7%	99.9%	99.6%	N/A	99.9%
	Oral Fluid	99.8%	N/A	N/A	N/A	N/A	99.9%
Detection Time Frame** Days after HIV-1 RNA is detectable (~10 days)		23.7 days	21.6 days	19.7 days	***	13.5 days	17.5 days
HIV-2 Detection		Yes	Yes	Yes	Yes	No	Yes
Materials Provided in Test Kit (not including manufacturer supplied paperwork)		Test device, developer solution, reusable test stands, specimen collection loops	Test device, wash solution, disposable pipettes, disposable finger-stick sample collection pipettes	Sampler with test strip, buffer vial, sterile safety lancet, bandage, disposable test stands	Test device and cover, chase buffer, disposable capillary tubes, disposable workstations	Test device, 3 solution vials, safety lancet, specimen collection capillary pipette, alcohol swab,	Test device, oral fluid swab, disposable sample loops, SampleTainer bottle, Running Buffer
Available Kit Sizes		100 count 25 count	20 count	25 count	100 count 25 count	24 count 1 count	20 count
Generation of detection		2 nd generation lateral-flow	3 rd generation lateral-flow	2 nd generation lateral-flow	4 th generation lateral-flow	2 nd generation flow- through	2 nd generation dual path platform

*Not currently provided by North Carolina Communicable Disease Branch as of January 2015.

**Median of 95% confidence intervals representing the estimated ranges of days that HIV-1 tests begin to detect HIV-1 infection AFTER HIV-1 RNA is detectable. The interval between HIV infection and the appearance of HIV-1 RNA is estimates to be around 10 days, but the absolute range is not yet known. See <http://www.cdc.gov/hiv/pdf/testingAdvDisadvHIVtesting62314.pdf> for further details.

***Not available at the time of publishing

Rapid HIV Testing Training Records

Please list the name of each staff member who conducts rapid HIV testing. Indicate the date of their most recent training and who provided the training. Keep a record of this document for internal use and update as needed.

Staff Member Name	OraQuick	Clearview	Uni-Gold	Determine Combo	Whetstone	Safe Work Habits
	Date: Provided by: <input type="checkbox"/> State <input type="checkbox"/> Manufacturer <input type="checkbox"/> Internal Agency <input type="checkbox"/> Other:	Date: Provided by: <input type="checkbox"/> State <input type="checkbox"/> Manufacturer <input type="checkbox"/> Internal Agency <input type="checkbox"/> Other:	Date: Provided by: <input type="checkbox"/> State <input type="checkbox"/> Manufacturer <input type="checkbox"/> Internal Agency <input type="checkbox"/> Other:	Date: Provided by: <input type="checkbox"/> State <input type="checkbox"/> Manufacturer <input type="checkbox"/> Internal Agency <input type="checkbox"/> Other:	Date: Provided by: <input type="checkbox"/> State <input type="checkbox"/> Manufacturer <input type="checkbox"/> Internal Agency <input type="checkbox"/> Other:	Date: Provided by: <input type="checkbox"/> State <input type="checkbox"/> Manufacturer <input type="checkbox"/> Internal Agency <input type="checkbox"/> Other:
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Sample Standing Orders for HIV Rapid Tests

{Physician Letterhead}

{Date}

{Agency Name and address}

To Whom It May Concern:

The following are Standing Orders for {Name of Agency} regarding HIV pre- and post-test counseling, and, HIV rapid testing.

{Name of Executive Director} is appointed the sole person responsible for ensuring that these standing orders are carried out in full on behalf and in the authority of {Name of Physician}.

All HIV tests will be administered by and under the authority of {Name of Physician}. {Name of Executive Director} will ensure that assigned staff from {Name of Agency} have attended the Communicable Disease Branch approved HIV Counseling, Testing and Referral (CTR) training (www.whetstoneconsultations.com) and any Branch approved rapid HIV testing training.

Designated staff representing {Name of Agency} may collect appropriate specimens for HIV rapid tests and interpret rapid test results at specified nontraditional test sites in {Name of County} and during special targeted testing events.

{Name of Agency} must make post-test counseling available and all preliminary HIV-positive test results must be linked to confirmatory HIV testing. Per NC GS 130A-144(d), all clients with preliminary HIV-positive results must be given control measures. Designated staff will provide follow-up according to agency's policies and procedures.

Signed by {Name of Physician}

Rapid Testing Product Representatives

OraQuick Advance HIV 1/2 Antibody Test, OraQuick HCV Rapid Antibody Test

- J. Todd McPherson
- 484-788-5456
- tmcpherson@orasure.com

Alere Clearview Complete HIV 1/2, Alere Determine Combo HIV

- Kirk Tilly
- 704-534-0553
- kirk.tilly@alere.com

Uni-Gold Recombigen HIV

- Louis Pastors, Technical Sales Support
- 630-561-6681
- louis.pastors@trinityusa.com

- Heidi Maxwell, Order Support
- 800-325-3424 ext. 7212
- heidi.maxwell@trinityusa.com

Insti HIV 1/2 Antibody Test

- Nickie Singleton
- 470-230-7479.
- nsingleton@biolytical.com

DPP HIV 1/2 Assay

- Paul Czech
- 919-259-4771
- pczech@chembio.com

Condom Representatives

Ansell Lifestyles

- Mike Mesmer
- 732-345-5308
- mike.mesmer@ansell.com

NV Healthcare NüVo

- Ben Isaacs
- 516-766-3800
- ben@nvhealthcarellc.com

Brochures Representatives

Channing Bete

- Stacy Shiels
- 888-834-6627 ext. 5409
- sshiels@channing-bete.com

ETR

- Nancy Gonzalez-Caro
- 800-325-3048 ext. 237
- gonn@etr.org

OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test Laboratory Procedure

Rapid Antibody Test for Detection of HIV Antibodies in Oral Fluid, Fingerstick Whole Blood, Venipuncture Whole Blood and Plasma Specimens

Read the Package Insert completely before using the product. Follow the instructions carefully when performing the test. Not doing so may result in inaccurate test results. Before performing testing, all operators **MUST** read and become familiar with Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health-Care Settings.^{5,9}

Complexity	
Waived	Oral Fluid, Fingerstick Whole Blood and Venipuncture Whole Blood. Any modification by the laboratory to the test system or FDA approved test system instructions will result in the test no longer meeting the requirements for waived category.
Moderate	Plasma

I. NAME AND INTENDED USE

The **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** is a single-use, qualitative immunoassay to detect antibodies to Human immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in oral fluid, fingerstick whole blood, venipuncture whole blood and plasma specimens. The **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1 and HIV-2. This test is suitable for use in multi-test algorithms designed for statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms.

II. SUMMARY AND EXPLANATION

Acquired Immune Deficiency Syndrome (AIDS), AIDS related complex (ARC) and pre-AIDS are thought to be caused by the Human Immunodeficiency Virus (HIV). The first AIDS-related virus, HIV-1 (also known as HTLV-III, LAV-1 and ARV) has been isolated from patients with AIDS and from healthy persons at high risk for AIDS.^{1,2} Genetic analysis of HIV-1 isolates has documented the existence of subtypes. To date, eight HIV-1 subtypes (A through H), designated as Group M, have been identified world-wide in addition to the highly divergent HIV-1 isolates from AIDS patients in Cameroon, designated as Group O.³ A closely related but distinct second type of pathogenic human immunodeficiency retrovirus, designated HIV-2 (formerly LAV-2), has been isolated from West African patients with AIDS. HIV-2 has been

shown to share a number of conserved sequences with HIV-1, but serological cross-reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample.

HIV is known to be transmitted by sexual contact, by exposure to blood (including sharing contaminated needles and syringes) or by contaminated blood products, or it may be transmitted from an infected mother to her fetus during the prenatal period. Individuals infected with HIV produce antibodies against the HIV viral proteins. Testing for the presence of antibodies to HIV in bodily fluids (e.g., blood, oral fluid, and urine) is an accurate aid in the diagnosis at HIV infection. However, the implications of seropositivity must be considered in a clinical context. For example, in neonates, the presence of antibodies to HIV is indicative of exposure to HIV, but not necessarily of HIV infection, due to the acquisition of maternal antibodies that may persist for up to eighteen months. Conversely, absence of antibody to HIV cannot be taken as absolute proof that an individual is free of HIV infection or incapable of transmitting the virus. An antibody response to a recent exposure may take several months to reach detectable levels. HIV has been isolated from asymptomatic, seronegative individuals presumably before seroconversion following exposure.

The standard laboratory HIV testing algorithm used in the United States consists of screening with an enzyme immunoassay (EIA) and confirmation of repeatedly reactive EIAs using a Western blot test. Results are typically reported within 48 hours to 2 weeks, making those standard screening and supplemental tests inadequate to meet the need for rapid HIV diagnosis. The **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test** is a point-of-care test to aid in the diagnosis of infection with HIV-1 and HIV-2.

Using a rapid HIV test increases the number of HIV-infected persons who may be diagnosed. The Centers for Disease Control and Prevention (CDC) estimates that nearly one third of the estimated 900,000 HIV-infected persons in the United States do not know their HIV status. As a result, they cannot benefit from early intervention with effective antiviral therapy. Rapid HIV testing addresses this issue by providing results during the initial visit and enabling immediate counseling. Additionally, for pregnant women who do not know their HIV status at the time of delivery, rapid HIV testing permits therapy to be initiated for these mothers during labor, and to their infants post partum, substantially reducing the chance that the infants will become infected with HIV. Likewise, rapid HIV testing is instrumental in the decision to initiate treatment for health care workers after accidental exposures to body fluids from infected individuals. In the U.S., it is estimated that 600,000 to 1,000,000 "needlestick injuries" occur each year. Critical decisions about treatment depend on the availability of accurate, rapid HIV test results.

III. TEST PRINCIPLE

The **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test** is a manually performed, visually read, 20 minute immunoassay for the qualitative detection of antibodies to HIV-1 and HIV-2 in human oral fluid, whole blood obtained from a finger puncture or a venipuncture, and plasma. The **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test** is comprised of a single-use test device and a single-use vial containing a pre-measured amount of a buffered developer

solution. Each component is sealed in separate compartments of a single pouch to form the test. The **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test** utilizes a proprietary lateral flow immunoassay procedure. The device plastic housing holds an assay test strip comprised of several materials that provide the matrix for the immunochromatography at the specimen and the platform for indication of the test results.

The assay test strip, which can be viewed through the test device result window, contains synthetic peptides representing the HIV envelope region and a goat anti-human IgG procedural control immobilized onto a nitrocellulose membrane in the Test (T) zone and the Control (C) zone, respectively.

An oral fluid specimen is collected using the flat pad on the test device, followed by the insertion of the test device into the vial of developer solution. A fingerstick whole blood, venipuncture whole blood or plasma specimen is collected and transferred into the vial of developer solution, followed by the insertion of the test device. The developer solution facilitates the flow of the specimen into the device and onto the test strip. As the diluted specimen flows through the device, it rehydrates the protein-A gold colorimetric reagent contained in the device. As the specimen continues to migrate up the strip, it encounters the T zone. If the specimen contains antibodies that react with the antigens immobilized on the nitrocellulose membrane, a reddish-purple line will appear, qualitatively indicating the presence of antibodies to HIV-1 and/or HIV-2 in the specimen. The intensity of the line color is not directly proportional to the amount of antibody present in the specimen.

Further up the assay strip, the sample will encounter the C zone. This built-in procedural control serves to demonstrate that a specimen was added to the vial and that the fluid has migrated adequately through the test device. A reddish-purple line will appear in the C zone during the performance of all valid tests, whether or not the sample is positive or negative for antibodies to HIV-1 and/or HIV-2 (refer to the *Test Result and Interpretation of Test Result* section below).

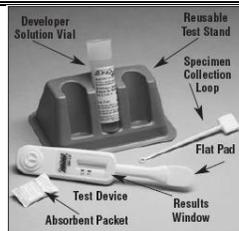
The test results are interpreted after 20 minutes but not more than 40 minutes after the introduction of the test device into the developer solution containing the test specimen. No precision pipeting, predilutions, or specialized instrumentation are required to perform the **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test**.

IV. MATERIALS AND EQUIPMENT

A. Materials provided

Each kit contains enough materials for 25 or 100 tests. The following components are included in a kit.

CONTENTS	25 COUNT KIT	100 COUNT KIT
Divided Pouch, each containing: 1 Test Device, 1 Absorbent Packet, 1 Developer Solution Vial (each vial contains 1 mL of a phosphate buffered saline solution containing polymers and an antimicrobial agent)	25	100
Reusable Test Stands	5	10
Specimen Collection Loops	25	100
Subject Information Pamphlet	25	100
Package Insert with Customer Letter	1	1



B. Materials required but not provided

- Timer or watch capable of timing 20 to 40 minutes
- Clean, disposable, absorbent workspace cover
- Biohazard waste container

Additional items required for fingerstick and venipuncture whole blood collection and plasma specimens:

- Antiseptic wipe
- Sterile lancet to obtain a fingerstick whole blood specimen, or materials required to obtain a venipuncture whole blood specimen
- Sterile gauze pads
- Latex, vinyl or nitrile disposable gloves (optional for oral fluid testing)
- Centrifuge to process a plasma specimen

C. Materials required and available as an accessory to the kit

- **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test Kit Controls.** Each Kit Control contains the following:
 - HIV-1 Positive Control (1 vial, black cap, 0.2 mL)
 - HIV-2 Positive Control (1 vial, red cap, 0.2 mL)

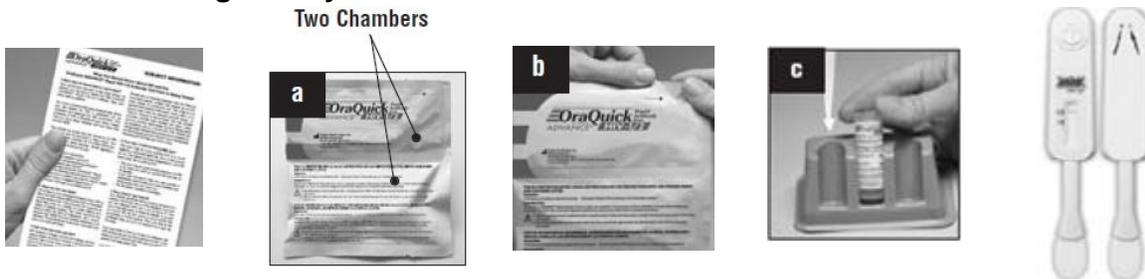
- HIV Negative Control (1 vial, white cap, 0.2 mL)
- Package Insert

V. SPECIMEN COLLECTION/PREPARATION

- Gather the materials you will need.
- Allow the test kit to come to operating temperature (15° to 37°C; 59° to 99°F) before use.
- Refer to the *External Quality Control* section in this package insert to determine when the Kit Controls should be run.
- Cover your workspace with a clean, disposable, absorbent workspace cover.
- Set an OraQuick *ADVANCE*® Reusable Test Stand (“Stand”) up on your workspace. Use only the stand provided.
- Put on your disposable gloves as required in accordance with the *Safety Precautions* section in this package insert.
- **Prior to testing, provide the “Subject Information” pamphlet to the person being tested.**
- Open the two chambers of the OraQuick *ADVANCE*® Divided Pouch (“Pouch”) by tearing at the notches on the top of each side of the Pouch (see picture a and b). To prevent contamination, leave the Test Device (“Device”) in the Pouch until you are ready to use it.
- Remove the Developer Solution Vial (“Vial”) from the Pouch. Hold the Vial firmly in your hand. Carefully remove the cap from the Vial by gently rocking the cap back and forth while pulling it off. Set the cap on your workspace cover.

Slide the Vial into the top of one of the slots in the Test Stand. **DO NOT** force the vial into the Stand from the front of the slot as splashing may occur. Make sure the Vial is pushed all the way to the bottom of the slot in the stand (*see picture c*).

NOTE: DO NOT cover the two holes in the back of the Device with labels or other materials. Doing so may cause an Invalid result.



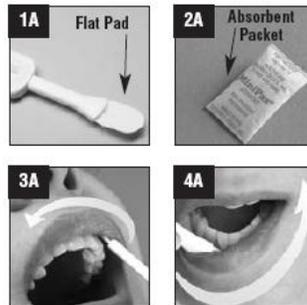
VI. ORAQUICK ADVANCE® HIV SPECIMEN COLLECTION AND TESTING PROCEDURES

The OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test can be used for testing oral fluid, fingerstick whole blood, venipuncture whole blood, and plasma specimens. Refer to the specific collection procedure below.

A. Oral Fluid Collection

1. Ensure prior to testing that the subject has not had anything to eat, drink or has chewed gum for at least 15 minutes. Have the subject wait for at least 30 minutes prior to testing if they have used any oral care products.
2. Have the person being tested remove the Device from its Pouch. **DO NOT** allow the person to touch the Flat Pad (see *picture 1A*). Check to make sure that an Absorbent Packet is included with the Device (see *picture 2A*). If no Absorbent Packet is present, discard the Device and obtain a new Pouch for testing.
3. Direct the person to place the Flat Pad above the teeth against the outer gum. Direct the person to gently swab completely around the outer gums, both upper and lower, one time around, using the Flat Pad (see *pictures 3A and 4A*). **DO NOT** allow the person to swab the roof of the mouth, the inside of the cheek or the tongue.

NOTE: Both sides of the Flat Pad may be used during this procedure.



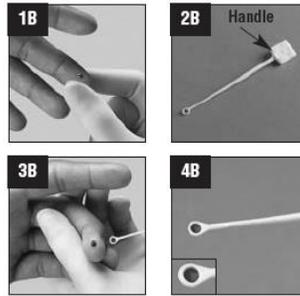
B. Fingerstick Whole Blood and Venipuncture Whole Blood Collection:

Whole blood specimens may be collected either by fingerstick (see *Step 1A*) or by venipuncture (see *Step 1B*).

▪ Fingerstick Whole Blood Collection Instructions

1. Using an antiseptic wipe, clean the finger of the person being tested. After cleansing the skin puncture site, allow the area to air dry, so the antiseptic action of the alcohol can take effect. Using a sterile lancet, puncture the skin just off the center of the finger pad. Hold the finger downward. Apply gentle pressure beside the point of the puncture. Avoid squeezing the finger to make it bleed (see *picture 1B*). Wipe away this first drop of blood with a sterile gauze pad. Allow a new drop of blood to form.
2. Pick up an unused Specimen Collection Loop (“Loop”) by the thick “handle” end (see *picture 2B*). Put the “rounded” end of the Loop on the drop of blood (see *picture 3B*). Make sure that the Loop is completely filled with blood (see *picture 4B*). **NOTE:** If the

Loop is dropped or comes in contact with any other surface, discard it in a biohazard waste container. Get a new Loop for the collection of the blood sample.

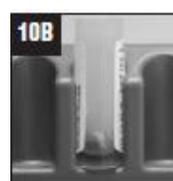
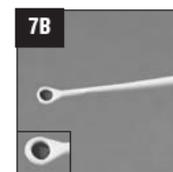
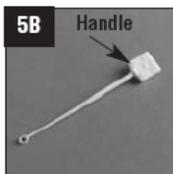


▪ **Venipuncture Whole Blood Instructions**

1. Using standard venous phlebotomy procedures, collect a whole blood sample using a tube containing any of the following anticoagulants: EDTA (lavender top), sodium heparin (green top), or sodium citrate (light blue top). **Other anticoagulants have not been tested and may give an incorrect result.** If the specimens are not tested at the time of collection, the whole blood may be stored at 2° to 30°C (36° to 86°F) for up to 5 days. Prior to testing, mix the blood tube gently by inversion several times to ensure a homogeneous sample.
2. Pick up an unused Specimen Collection Loop (“Loop”) by the thick “handle” end (see *picture 5B*). Put the “rounded” end of the Loop into the tube of blood (see *picture 6B*). Make sure that the Loop is completely filled with blood (see *picture 7B*). **NOTE:** If the Loop is dropped or comes in contact with any other surface, discard it in a biohazard waste container. Get a new Loop for the collection of the blood sample.

▪ **Mixing Instructions for Fingertick Whole Blood and Venipuncture Whole Blood**

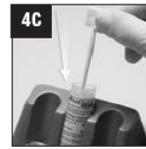
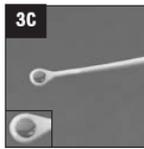
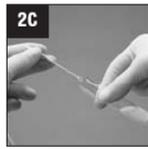
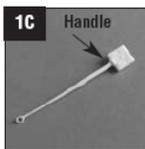
1. Immediately insert the blood-filled end of the Loop all the way into the Vial (see *picture 8B*). Use the Loop to stir the blood sample in the Developer Solution (“Solution”) (see *picture 9B*). Remove the used Loop from the Solution. Throw the used Loop away in a biohazard waste container.
2. Check the Solution to make sure that it appears pink. This means that the blood was correctly mixed into the Solution (see *picture 10B*). If the Solution is not pink, discard all test materials in a biohazard waste container. Start the test over. Use a new Pouch and a new blood sample.



C. Plasma Collection:

NOTE: Testing of plasma samples may only be performed by laboratories certified to perform Moderate Complexity tests.

1. Using standard venous phlebotomy procedures, collect a whole blood sample using a tube containing EDTA (lavender top) anticoagulant. **Other anticoagulants have not been tested and may give an incorrect result.** If the specimens are not tested at the time of collection, the specimen may be stored as whole blood for up to 5 days at 2° to 30°C (36° to 86°F) or as plasma for up to 7 days at 2° to 8°C (36° to 46°F).
2. Centrifuge the tube of blood [1000-1300 x g, for approximately 5 minutes, no refrigeration required] to separate the cells from the plasma. Carefully uncap the tube by gently rocking the stopper towards you so that it vents away from you.
 - Pick up an unused Specimen Collection Loop (“Loop”) by the thick “handle” end (see *picture 1C*). Put the “rounded” end of the Loop into the tube of plasma (see *picture 2C*). Make sure that the Loop is completely filled with plasma (see *picture 3C*). **NOTE:** If the Loop is dropped or comes in contact with any other surface, discard it in a biohazard waste container. Get a new Loop for the collection of the plasma sample.
 - **Mixing Instructions for Plasma**
 1. Immediately insert the plasma-filled end of the Loop all the way into the Vial (see *picture 4C*). Use the Loop to stir the plasma sample in the Developer Solution (“Solution”) (see *picture 5C*). Remove the used Loop from the Solution. Throw the used Loop away in a biohazard waste container.



D. Test

Refer to *Test Procedure* outlined in Section X.

VII. STORAGE AND STABILITY

Store unused **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Tests** unopened at 2° to 27°C (36° to 80°F). Do not open the Divided Pouch until you are ready to perform a test. If stored refrigerated, ensure that the Divided Pouch is brought to operating temperature (15° to 37°C, 59° to 99°F) before opening.

VIII.QUALITY CONTROL

A. Internal Quality Control (*Built-In Control Features*):

The **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** has a built-in procedural control that demonstrates assay validity. A reddish-purple line in the Control (“C”) area of the Result Window indicates that a specimen was added and that the fluid migrated appropriately through the Test Device. The Control line will appear on all valid tests, whether or not the sample is reactive or non-reactive. (Refer to *Test Result and Interpretation of Test Result* section below.)

B. External Quality Control:

OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test Kit Controls are available separately for use only with the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**. The Kit Controls are specifically formulated and manufactured to ensure performance of the Test, and are used to verify your ability to properly perform the test and interpret the results. The HIV-1 and HIV-2 Positive Controls will produce a reactive test result and have been manufactured to produce a very faint Test (“T”) line. The Negative Control will produce a non-reactive test result. (Refer to *Test Result and Interpretation of Test Result* section below.) Use of kit control reagents manufactured by any other source may not produce the required results, and therefore, will not meet the requirements for an adequate quality assurance program for the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**.

C. Run the Kit Controls under the following circumstances:

- Each new operator prior to performing testing on patient specimens,
- When opening a new test kit lot,
- Whenever a new shipment of test kits is received,
- If the temperature of the test kit storage area falls outside of 2° to 27°C (36° to 80°F),
- If the temperature of the testing area falls outside of 15° to 37°C (59° to 99°F), and
- At periodic intervals as dictated by the user facility.

Refer to the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** Kit Controls package insert for instructions on the use of these reagents. It is the responsibility of each laboratory using the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** to establish an adequate quality assurance program to ensure the performance of the device under its specific locations and conditions of use. Contact OraSure Technologies’ Customer Service if the Kit Control reagents do not produce the expected results.

IX. PRECAUTIONS

A. Safety Precautions

- Handle blood specimens and materials contacting blood specimens as if capable of transmitting infectious agents.
- Do not drink, eat, or smoke in areas where specimens are being handled or testing is being performed.
- Wear disposable gloves while handling blood specimens and performing testing of blood specimens. Change gloves and wash hands thoroughly after performing each test. Dispose of used gloves in a biohazard waste container.
- Oral fluid is not considered potentially infectious unless it contains blood.⁸ Use of gloves for oral fluid testing is optional. Test administrators with breaks in the skin (cuts, abrasions, or dermatitis) should wear gloves when performing oral fluid testing. Wash hands thoroughly after performing each oral fluid test and after contact with oral fluid.
- Dispose of all test specimens and materials used in the test procedure in a biohazard waste container. Lancets and venipuncture materials should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Liquid wastes may be mixed with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination. **NOTE: Do not autoclave solutions that contain bleach.**
- Wipe all spills thoroughly with a solution of 10% bleach or other appropriate disinfectant⁴. Bleach solutions should be made fresh each day.
- For additional information on biosafety, refer to “Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health-Care Settings”⁵ and “Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis”.^{5,9}

B. Handling Precautions

- Use all Specimen Collection Loops, Test Devices, and Developer Solution Vials only once and dispose of properly (see *Safety Precautions*). **Do not reuse any of these test components.**
- Do not use the test beyond the expiration date printed on the Divided Pouch. Always check expiration date prior to testing.
- Do not interchange Test Devices and Developer Solution Vials from kits with different lot numbers.
- Avoid microbial contamination and exercise care in handling the kit components.
- To ensure accurate results, the Test Device must be inserted into the Developer Solution Vial within 60 minutes after introducing the fingerstick whole blood, venipuncture whole blood or plasma sample.

- When collecting oral fluid specimens the Test Device must be inserted into the Developer Solution Vial within 30 minutes of collection. A Test Device containing an oral fluid specimen that is not inserted into the Developer Solution Vial within 10 minutes of collection should be either stored on a flat surface or returned to the Divided Pouch after the desiccant has been removed from the Divided Pouch. For a 10 to 30 minute delay in insertion, return the Test Device containing the oral fluid specimen to the Divided Pouch after the desiccant has been removed from the Divided Pouch. Ensure that the Divided Pouch containing the Test Device is kept in a horizontal position until the Test Device is inserted into the Developer Solution Vial.
- Adequate lighting is required to read a test result.

C. Warnings

- For *in vitro* Diagnostic Use
- Read the package insert completely before using the product. Follow the instructions carefully. Not doing so may result in inaccurate test results.
- Before performing testing, all operators MUST read and become familiar with Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health-Care Settings.^{5, 9}
- FDA has approved this kit for use with oral fluid, fingerstick whole blood, venipuncture whole blood, and plasma specimens only. Use of this test kit with specimen types other than those specifically approved for use with this device may result in inaccurate test results.
- This test should be performed at temperatures in the range of (15° to 37°C, 59° to 99°F). If stored refrigerated, ensure that the Divided Pouch is brought to operating temperature (15° to 37°C, 59° to 99°F) before performing testing.
- If the test kit is stored at temperatures outside of ambient temperature (2° to 27°C, 36° to 80°F), or used outside of the operating temperature (15° to 37°C, 59° to 99°F), use the Kit Controls to ensure performance of the test.
- Individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.
- Individuals undergoing preventive treatment for HIV may produce false negative results.

D. Restrictions

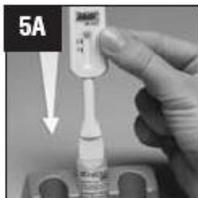
- Sale of the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** is restricted to clinical laboratories:
- that have an adequate quality assurance program, including planned systematic activities to provide adequate confidence that requirements for quality will be met; and
- where there is assurance that operators will receive and use the instructional materials.
- The **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** is approved for use only by an agent of a clinical laboratory.

- Test subjects must receive the “Subject Information” pamphlet prior to specimen collection and appropriate information when test results are provided.
- The **OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test** is not approved for use to screen blood or tissue donors.

X. TEST PROCEDURE

A. Oral Fluid Test Procedure

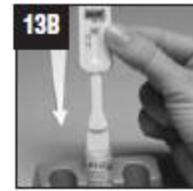
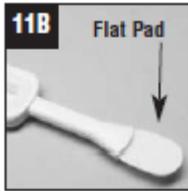
1. Insert the Flat Pad of the Device all the way into the Vial (see *picture 5A*). Make sure that the Flat Pad touches the bottom of the Vial. The Result Window on the Device should be facing towards you (see *picture 6A*).
2. Start timing the test (see *picture 7A*). **DO NOT** remove the Device from the Vial while the test is running. Pink fluid will appear and travel up the Result Window. The pink fluid will gradually disappear as the test develops (see *picture 8A*). Read the results after 20 minutes but not more than 40 minutes in a fully lighted area.
3. Refer to the *Test Result and Interpretation of Test Result* section in this package insert.
4. Dispose of the used test materials in a biohazard waste container.
5. When using gloves, change your gloves between each test to prevent contamination. Throw away the used gloves in a biohazard waste container.
6. Use a freshly prepared 10% solution of bleach to clean up any spills.



B. Fingertick and Venipuncture Whole Blood Test Procedure

1. Remove the Device from the Pouch. **DO NOT** touch the Flat Pad (see *picture 11B*). Check to make sure that an Absorbent Packet is included with the Device (see *picture 12B*). If no Absorbent Packet is present, discard the Device and obtain a new Pouch for testing.
2. Insert the Flat Pad of the Device all the way into the Vial containing the blood sample (see *picture 13B*). Make sure that the Flat Pad touches the bottom of the Vial. The Result Window on the Device should be facing towards you (see *picture 14B*).
3. Start timing the test (see *picture 15B*). **DO NOT** remove the Device from the Vial while the test is running. Pink fluid will appear and travel up the Result Window. The pink fluid will gradually disappear as the test develops (see *picture 16B*). Read the results after 20 minutes but not more than 40 minutes in a fully lighted area.
4. Refer to the *Test Result and Interpretation of Test Result* section in this package insert.
5. Dispose of the used test materials in a biohazard waste container.

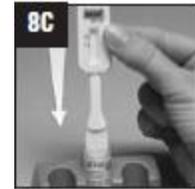
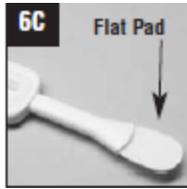
- When using gloves, change your gloves between each test to prevent contamination. Throw away the used gloves in a biohazard waste container.
- Use a freshly prepared 10% solution of bleach to clean up any spills.



C. Plasma Test Procedure

NOTE: Testing of plasma samples may only be performed by laboratories certified to perform Moderate Complexity tests.

- Remove the Device from the Pouch. **DO NOT** touch the Flat Pad (see *picture 6C*). Check to make sure that an Absorbent Packet is included with the Device (see *picture 7C*). If no Absorbent Packet is present, discard the Device and obtain a new Pouch for testing.
- Insert the Flat Pad of the Device all the way into the Vial containing the blood sample (see *picture 8C*). Make sure that the Flat Pad touches the bottom of the Vial. The Result Window on the Device should be facing towards you (see *picture 9C*).
- Start timing the test (see *picture 10C*). **DO NOT** remove the Device from the Vial while the test is running. Pink fluid will appear and travel up the Result Window. The pink fluid will gradually disappear as the test develops (see *picture 11C*). Read the results after 20 minutes but not more than 40 minutes in a fully lighted area.
- Refer to the *Test Result and Interpretation of Test Result* section in this package insert.
- Dispose of the used test materials in a biohazard waste container.
- When using gloves, change your gloves between each test to prevent contamination. Throw away the used gloves in a biohazard waste container.
- Use a freshly prepared 10% solution of bleach to clean up any spills.



XI. TEST RESULT AND INTERPRETATION OF TEST RESULT

Refer to the **Result Window on the Test Device**.

Reactive: A test is **Reactive** if:

- a reddish-purple line appears next to the triangle labeled “C” **and**
- a reddish-purple line appears next to the triangle labeled “T”. One of these lines may be darker than the other.

NOTE: The test is **Reactive** if a **complete** reddish-purple line appears next to the “T” triangle **and** next to the “C” triangle, no matter how faint these lines are.

- A **Reactive** test result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The test result is interpreted as **PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies**. Follow CDC guidelines to inform the test subject of the test result and its interpretation.^{6,7}
- The diagrams below show examples of **Reactive** test results.

REACTIVE



Non-Reactive: A test is **Non-Reactive** if:

- a reddish-purple line appears next to the triangle labeled “C”, and **NO** line appears next to the triangle labeled “T”.
- A **Non-Reactive** test result means that HIV-1 and HIV-2 antibodies were not detected in the specimen. The test result is interpreted as **NEGATIVE for HIV-1 and HIV-2 antibodies**. Follow CDC guidelines to inform the test subject of the test result and its interpretation.^{6,7}

- The diagram below shows an example of a **Non-Reactive** test result.

NON-REACTIVE



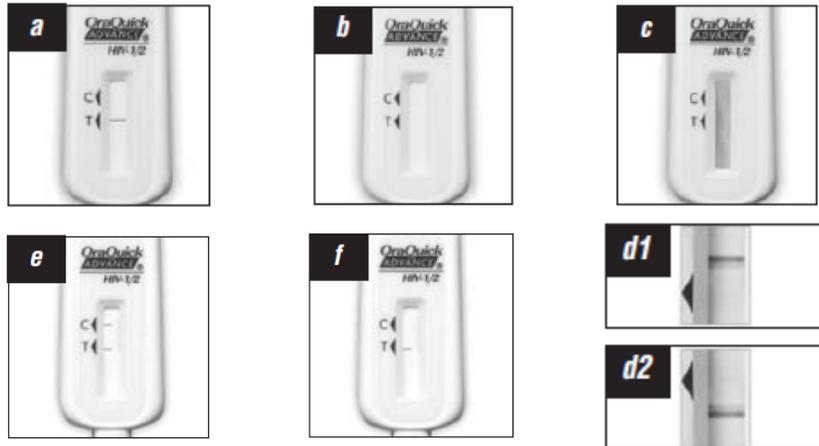
Invalid: A test is **Invalid** if:

- **NO** reddish-purple line appears next to the triangle labeled “C” (see picture a and b), or
- a red background in the Result Window makes it difficult to read the result after 20 minutes (see picture c), or
- if any of the lines are NOT inside the “C” or “T” triangle areas (see picture d1 and d2)
- any partial lines on one side of the “C” or “T” triangle areas (see picture e and f)

An **Invalid** test result means that there was a problem running the test, either related to the specimen or to the Test Device. An **Invalid** result cannot be interpreted. **Repeat the test with a new Divided Pouch and a new oral fluid, fingerstick or venipuncture whole blood, or plasma sample. Contact OraSure Technologies’ Customer Service if you are unable to get a valid test result upon repeat testing.**

- The diagram below shows examples of **Invalid** test results.

INVALID



XII. INTERFERENCE

Interfering Substances and Unrelated Medical Conditions – Sensitivity

To assess the impact of unrelated medical conditions or interfering substances on the sensitivity of the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**, 200 serum/plasma specimens from a variety of medical conditions unrelated to HIV-1 infection and 125 specimens with interfering substances were spiked with an HIV-1 positive specimen to give a level of reactivity in the low positive range (see list of medical conditions and interfering substances in Table 10 below). All spiked specimens gave reactive results.

In addition, a study was performed to assess the potential effect of anticoagulants on assay sensitivity. Venipuncture whole blood collected from 24 subjects, in each of 3 tubes containing one of three anticoagulants (EDTA, sodium heparin, and sodium citrate) was spiked with an HIV-1 positive specimen or an HIV-2 positive specimen to give a level of reactivity in the low positive range. The HIV-1 positive samples and the HIV-2 positive samples were then aliquoted and stored refrigerated (2° to 8°C), at room temperature (18°C) or at elevated temperatures (30 to 33°C) and tested over a 7-day period. There was no anticoagulant-specific effect observed on assay performance with samples held up to 7 days at 2° to 30°C.

As part of the oral fluid clinical studies, information was collected from the participants regarding concurrent diseases or medical conditions, oral pathologies, non-HIV viral infections, and other factors (e.g., use of tobacco products, mouthwash within 24 hours of testing, concomitant medications, dental fixtures, and food or drink immediately prior to testing). None of these disease states, medical conditions or other factors interfered with test sensitivity. In a separate study of 40 individuals, consumption of alcohol, brushing of teeth, use of mouthwash or smoking tobacco 5 minutes prior to testing, were shown to have no effect on test sensitivity. Nonetheless, it is recommended that subject observes a wait period prior to oral fluid collection, according to the Oral Fluid Collection Procedure of this package insert.

Interfering Substances and Unrelated Medical Conditions – Specificity

To assess the impact of unrelated medical conditions or interfering substances on the specificity of the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**, 321 serum/plasma specimens from a variety of medical conditions unrelated to HIV infection and 119 specimens with interfering substances were analyzed. The results of this study are shown in Table 10. One specimen from subjects known to be positive for EBV, for HBV, or for rheumatoid factor, one from a multiparous woman, and three specimens from known HAV infected subjects gave false positive results.

In addition, a study was performed to assess the potential effect of anticoagulants on assay specificity. Venipuncture whole blood was collected from 24 HIV negative subjects, in each of 3 tubes containing one of the following anticoagulants: EDTA, sodium heparin, and sodium citrate. The samples were then aliquoted and stored either refrigerated (2° to 8°C), at room temperature

(18°C) or at elevated temperatures (30 to 33°C) and tested over a 7-day period. There was no anticoagulant-specific effect observed on assay performance with samples held up to 5 days at 2 to 30°C (refer to Table 10).

TABLE 10		
OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test Reactivity with Specimens from Individuals with Potentially Interfering Medical Conditions and Specimens with Interfering Substances		
Medical Condition (n = 321)	OraQuick ADVANCE® Results	
	Reactive	Non-Reactive
Multiparous women	1 ²	14
Anti-nuclear antibody (ANA)	0	17
Lupus	0	15
Rheumatoid factor	1 ²	17
Cytomegalovirus (CMV)	0	15
Epstein Barr virus (EBV)	1 ²	14
Hepatitis A virus (HAV)	3 ¹	17
Hepatitis B virus (HBV)	1 ²	16
Hepatitis C virus (HCV)	0	15
Human T-cell Lymphotropic virus Type I (HTLV-I)	0	15
Human T-cell Lymphotropic virus Type II (HTLV-II)	0	15
Rubella	0	15
IgG gammopathies	0	13
IgM gammopathies	0	12
Syphilis	0	15
Toxoplasmosis	0	15
Tuberculosis	0	15
Influenza	0	10
Multiple transfusions	0	10
Hemophiliac	0	10
Herpes Simplex virus	0	5
Cirrhosis	0	5
Dialysis patient	0	4
Colon cancer	0	4
HTLV I/II	0	2
Chlamydia	0	3
Anti-scl or anti-rnp antibody	0	3
Breast cancer	0	1
Anti-DNA antibody	0	1
Gonorrhea	0	1
Interfering Substances (n = 211)		
Elevated Bilirubin	0	20
Elevated Hemoglobin	0	20
Elevated Triglycerides	0	20
Elevated Protein	0	20
Bacterially Contaminated	0	25
Visual Hemolysis (hemolytic)	0	5
Icteric	0	5
Lipemic	0	4
Sodium Heparin ³	0	24
EDTA ³	0	24
Sodium Citrate ³	0	24

¹ A total of 3 of the 20 HAV specimens were OraQuick ADVANCE® falsely reactive. Two of the 3 specimens were OraQuick ADVANCE® non-reactive at the 20 to 25 minute read time and reactive at the 55 to 60 minute read time. The remaining specimen was reactive at both read times.

² One of the specimens was OraQuick ADVANCE® non-reactive at the 20 to 25 minute read time and reactive at the 55 to 60 minute read time.

³ The OraQuick ADVANCE® assay maximum read time for these specimens was 40 minutes. Based upon specimen storage for 5 days at 2 to 30°C.

As part of the oral fluid clinical studies, information was collected from the participants regarding concurrent diseases or medical conditions, oral pathologies, non-HIV viral infections, and other

factors (e.g., use of tobacco products, mouthwash within 24 hours of testing, concomitant medications, dental fixtures, and food or drink immediately prior to testing). None of these disease states, medical conditions or other factors interfered with test specificity. In a separate study of 40 individuals, consumption of alcohol, brushing of teeth, use of mouthwash or smoking tobacco 5 minutes prior to testing, were shown to have no effect on test specificity.

XIII.LIMITATIONS

- The OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test must be used in accordance with the instructions in this package insert to obtain an accurate result.
- Reading test results earlier than 20 minutes or later than 40 minutes may yield erroneous results.
- This test is approved by FDA for use with oral fluid, fingerstick whole blood, venipuncture whole blood, and plasma specimens only. Use of other types of specimens, testing of venipuncture whole blood specimens collected using a tube containing an anticoagulant other than EDTA, sodium heparin, or sodium citrate, or testing of plasma specimens collected using a tube containing an anticoagulant other than EDTA may not yield accurate results.
- Individuals infected with HIV-1 or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.
- Individuals undergoing preventive treatment for HIV may produce false negative results.
- Clinical data has not been collected to demonstrate the performance of the OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test in persons under 12 years of age.
- A reactive result using the OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test suggests the presence of HIV-1 and/or HIV-2 antibodies in the specimen. OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
- For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
- A non-reactive result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to recent exposure may take several months to reach detectable levels.
- A person who has antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.

XIV.SPECIFICITY

A. Oral Fluid

- A specificity study was performed at four clinical trial sites using freshly obtained oral fluid specimens collected from 605 previously unscreened individuals at low risk for HIV-1 infection. All of the 605 specimens were correctly non-reactive using the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**. Of the 3077 HIV antibody-negative specimens from the four study sites that examined populations at high risk for HIV-1 infection, the OraQuick **ADVANCE[®]** test was non-reactive for 3069. The results are summarized in Table 7.

Performance of the OraQuick ADVANCE [®] Rapid HIV-1/2 Antibody Test on Oral Fluid Specimens from Individuals Presumed to be Negative for HIV Infection				
Test Group	Total Samples	OraQuick ADVANCE [®] Non-Reactive	Licensed EIA Non-Reactive	True Negative ¹
Low-Risk	605	605	599 ²	605
High-Risk	3150	3069 ³	3076 ⁴	3077
TOTAL	3755	3674	3675	3682

¹ Confirmation performed by licensed HIV-1 Western blot, with confirmation of indeterminate Western blot results by RIPA or IFA.

² Six specimens were EIA false positive, five with a negative Western blot and one with an indeterminate blot which was confirmed negative by IFA.

³ One additional specimen was OraQuick ADVANCE[®] false negative (see Table 1).

⁴ One specimen was EIA false positive with a negative Western blot.

- Combining the number of OraQuick ADVANCE[®] non-reactive results obtained from the study of the low-risk populations with the number of OraQuick ADVANCE[®] non-reactive results obtained from the study of the high-risk populations, the specificity of the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** in these studies was calculated to be $3674/3682 = 99.8\%$ (95% C.I. = 99.6% - 99.9%).

B. Plasma

- A specificity study was performed at seven clinical trial sites using EDTA-plasma specimens collected from 1102 previously unscreened individuals at low risk for HIV infection. All of the specimens, except for one, gave non-reactive results using the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**. In addition, 519 of the 520 HIV antibody-negative specimens from study sites that examined populations at high risk for HIV-1 infection also gave non-reactive results using the OraQuick ADVANCE[®] test. The results of this study are shown in Table 8.

Performance of the OraQuick ADVANCE [®] Rapid HIV-1/2 Antibody Test on Plasma Specimens from Individuals Presumed to be Negative for HIV Infection				
Test Group	Total Samples	OraQuick ADVANCE [®] Reactive	Licensed EIA Non-Reactive	True Negative ¹
Low-Risk	1102	1101	1096 ²	1102
High-Risk	534	519	516 ³	520
TOTAL	1636	1620	1612	1622

¹ Confirmation performed by licensed HIV-1 Western blot, with confirmation of indeterminate Western blot results by RIPA or IFA.

² Six specimens were EIA false positive, five with a negative Western blot and one with an indeterminate blot which was confirmed negative by IFA.

³ Four specimens were EIA false positive, with 1 negative and 3 indeterminate by Western blot, that confirmed negative by IFA.

- Combining the number of OraQuick *ADVANCE*[®] non-reactive results obtained from the study of the low-risk populations with the number of OraQuick *ADVANCE*[®] non-reactive results obtained from the study of the high-risk populations, the specificity of the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** in these studies was calculated to be 1620/1622 = 99.9% (95% C.I. = 99.6% -99.9%).

C. Fingerstick Whole Blood

- A specificity study was performed at eight clinical trial sites using freshly obtained fingerstick whole blood samples from 1250 previously unscreened individuals at low risk for HIV-1 infection. In the course of this study, two specimens were confirmed to have antibodies to HIV-1 and were removed from the specificity calculation. All of the remaining specimens gave non-reactive results using the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test**. In addition, all of the 608 HIV-1 antibody-negative specimens from the study sites that examined populations at high risk for HIV-1 infection also gave non-reactive results using the OraQuick *ADVANCE*[®] test. The results of this study are shown in Table 9.

Table 9				
Performance of the OraQuick <i>ADVANCE</i> [®] Rapid HIV-1/2 Antibody Test on Fingerstick Whole Blood Specimens from Individuals Presumed to be Negative for HIV Infection				
Test Group	Total Samples	OraQuick <i>ADVANCE</i> [®] Non-Reactive	Licensed EIA Non-Reactive	True Negative ³
Low-Risk	1250 ¹	1248	1247 ²	1248
High-Risk	625	608	605	608
TOTAL	1875	1856	1852	1856

¹ Two specimens in the low-risk study that gave reactive results using the OraQuick *ADVANCE*[®] test, repeatedly reactive results using a licensed EIA, and positive results using a licensed Western blot were removed from the calculation of specificity.

² One specimen was EIA repeatedly reactive, Western blot negative.

³ True negative status based on negative or indeterminate test results using a licensed Western blot.

- Combining the number of OraQuick *ADVANCE*[®] non-reactive results obtained from the study of the low-risk populations with the number of OraQuick *ADVANCE*[®] non-reactive results obtained from the study of the high-risk populations, the specificity of the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** in these studies was calculated to be 1856/1856 = 100% (95% C.I. = 99.7% - 100%).

XV.PERFORMANCE CHARACTERISTICS

Sensitivity Detection of Antibodies to HIV-1 in Specimens from individual infected with HIV-1

A. Oral Fluid

- A sensitivity study was performed at eight clinical trial sites using freshly obtained oral fluid specimens collected from 767 individuals reported to be infected with HIV-1. Of the 767 specimens that were identified as seropositive using licensed confirmatory

testing, 762 gave a reactive result on the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**. The results of this study are shown in Table 1.

- A separate study was performed at four clinical trial sites using freshly obtained oral fluid specimens collected from 3150 previously unscreened individuals from populations at high risk for HIV-1 infection. The results of this study are also shown in Table 1. Of the 73 specimens that were identified as seropositive using licensed confirmatory testing, 72 were reactive using the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**.

Table 1				
Detection of Antibody to HIV-1 in Oral Fluid Specimens from HIV-1 Seropositive Individuals				
Test Group	Total Samples	OraQuick ADVANCE [®] Reactive	Licensed EIA Repeatedly Reactive	True Positive ¹
Known HIV-1 Positive	767	762	764	767
High-Risk	3150	72 ²	74 ³	73
TOTAL	3917	834	838	840

¹ Confirmation performed by licensed HIV-1 Western blot, with confirmation of indeterminate Western blot results by licensed immunofluorescence assay (IFA).

² Eight additional specimens were OraQuick ADVANCE[®] false positive (see Table 7).

³ One specimen was EIA false positive, with a negative Western blot.

- Combining the number of OraQuick ADVANCE[®] reactive results obtained from the study of confirmed positives with the number of OraQuick ADVANCE[®] reactive results obtained from the study of high-risk populations, the sensitivity of the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** in these studies was calculated to be $834/840 = 99.3\%$ (95% C.I. = 98.4% - 99.7%).

B. Plasma

- A sensitivity study was performed at eleven clinical trial sites using EDTA-plasma specimens collected from 891 individuals reported to be infected with HIV-1. Of the 891 specimens that were identified as seropositive using licensed confirmatory testing, 887 gave a reactive result on the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**. The results of this study are shown in Table 2.
- A separate study was performed at six clinical trial sites using EDTA-plasma specimens collected from 533 previously unscreened individuals from populations at high risk for HIV-1 infection. The results of this study are also shown in Table 2. All of the 14 specimens that were identified as seropositive using licensed confirmatory testing, were reactive using the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**.

Table 2				
Detection of Antibody to HIV-1 in Plasma Specimens from HIV-1 Seropositive Individuals				
Test Group	Total Samples	OraQuick ADVANCE [®] Reactive	Licensed EIA Repeatedly Reactive	True Positive ¹
Known HIV-1 Positive	891	887	891	891
High-Risk	533	14 ²	14	14
TOTAL	1424	901	905	905

¹ Confirmation performed by licensed HIV-1 Western blot, confirmation of indeterminate Western blot results by radioimmunoprecipitation assay (RIPA) or licensed IFA.

² One additional specimen was OraQuick ADVANCE[®] false positive (see Table 8).

- Combining the number of OraQuick *ADVANCE*[®] reactive results obtained from the study of confirmed positives with the number of OraQuick *ADVANCE*[®] reactive results obtained from the study of high-risk populations, the sensitivity of the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** in these studies was calculated to be $901/905 = 99.6\%$ (95% C.I. = 98.9% - 99.8%).

C. Fingerstick Whole Blood

- A sensitivity study was performed at eight clinical trial sites using freshly obtained fingerstick whole blood samples from 481 individuals known to be infected with HIV-1 and 40 AIDS patients. Of the 521 specimens that were repeatedly reactive using a licensed EIA and positive by Western blot, 519 gave a reactive result on the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test**. The results of this study are shown in Table 3.
- A separate study was performed at seven clinical trial sites using 625 freshly obtained fingerstick whole blood samples from previously unscreened individuals from populations at high risk for HIV-1 infection. The results of this study are also shown in Table 3. Of the 625 specimens tested, 20 were repeatedly reactive using a licensed EIA, of which 17 were positive by Western blot. These same 17 specimens gave a reactive result using the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test**.

Total Group	Total Samples	OraQuick <i>ADVANCE</i> [®] Reactive	Licensed EIA Repeatedly Reactive	True Positive ¹
AIDS	40	40	40	40
Known HIV-1 Positive	481	479	481	481
High-Risk	625	17	20 ²	17
TOTAL	1146	536	541	538

¹ Confirmation performed by licensed HIV-1 Western blot, with confirmation of indeterminate Western blot results by RIPA.

² Two specimens were negative and one was indeterminate on Western blot with a negative RIPA.

- Combining the number of OraQuick *ADVANCE*[®] reactive results obtained from the study of confirmed positives with the number of OraQuick *ADVANCE*[®] reactive results obtained from the study of high-risk populations, the sensitivity of the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** in these studies was calculated to be $536/538 = 99.6\%$ (95% C.I. = 98.5% - 99.9%).

Detection of Antibodies To HIV-2 In Specimens From Individuals Infected With HIV-2

A total of 324 serum/plasma specimens reported to be HIV-2 antibody positive were obtained from various repository sources. Specimens were tested by licensed anti-HIV-1/2 EIA, licensed anti-HIV-2 EIA, licensed HIV-1 Western blot, an HIV-2 Western blot and HIV-2 specific PCR. A total of 6 specimens were not demonstrated to be positive for antibodies to HIV-1 or HIV-2, all of which were OraQuick *ADVANCE*[®] non-reactive. Two of the 6 negative specimens were

repeatedly reactive by licensed anti-HIV-1/2 EIA, negative by licensed anti-HIV-2 EIA, and indeterminate by licensed HIV-1 Western blot and by an HIV-2 Western blot.

Of the remaining 318 specimens, 151 were positive on an HIV-2 Western blot and 50 were positive using an HIV-2 specific PCR. One hundred and twenty-two specimens gave confirmatory results consistent with HIV-1 infection and were excluded from the analysis. One specimen was categorized as a dual infection based on additional testing by co-culture, and was not included in the sensitivity analysis. One specimen, while indeterminate on HIV-1 and HIV-2 Western blots, gave a positive result on an HIV-2 radioimmunoprecipitation assay (RIPA) and is also considered to be positive for antibodies to HIV-2. OraQuick *ADVANCE*[®] detected 201/201 (100%) of the specimens from individuals confirmed as positive for HIV-2 antibodies (see Table 6).

In a separate study, a total of 499 plasma specimens collected from an HIV-2 endemic area (Ivory Coast) were prepared as contrived whole blood and tested by OraQuick *ADVANCE*[®], licensed anti-HIV-1/2 EIA, licensed anti-HIV-2 EIA, licensed HIV-1 Western blot, and an HIV-2 Western blot. Table 6 shows a summary of the results. OraQuick *ADVANCE*[®] was reactive with all of the 27 specimens that were repeatedly reactive by licensed anti-HIV-1/2 EIA, licensed anti-HIV-2 EIA and positive on licensed HIV-1 Western blot, and with all three specimens that were confirmed as positive for HIV-2 only by an HIV-2 Western blot. Two specimens were OraQuick *ADVANCE*[®] false positive.

TABLE 6				
Detection of Antibody to HIV-2 in Samples from HIV-2 Seropositive Individuals and Individuals at High Risk of HIV-2 Infection				
Test Group	Total Samples	OraQuick <i>ADVANCE</i> [®] Reactive	Licensed anti-HIV-2 EIA Repeatedly Reactive or HIV-2 PCR Positive	True HIV-2 Positive ¹
Known HIV-2 Positive	324 ²	201	201 ³	201 ⁴
High-Risk	499	32	33	3
TOTAL	823	233	234	204

¹ Confirmation performed by HIV-2 Western blot, with RIPA confirmation of Indeterminate Western blot results.

² One hundred and twenty-two specimens gave confirmatory results consistent with HIV-1 infection and were excluded from the analysis. In addition, one specimen was categorized as a dual infection based on additional testing by co-culture, and was not included in the sensitivity analysis.

³ 151 specimens were tested with an anti-HIV-2 EIA alone. HIV-2 DNA or RNA PCR was performed on the remaining 50 specimens instead of EIA. All results were positive.

⁴ One specimen was confirmed to be HIV-2 positive based on the positive results of an HIV-2 specific RIPA.

Combining the number of OraQuick *ADVANCE*[®] reactive results obtained from the study of confirmed positives with the number of OraQuick *ADVANCE*[®] reactive results obtained from the study of the high risk population, the sensitivity of the OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test for the detection of antibodies to HIV-2 in these studies was calculated to be 204/204 = 100% (95% C.I. = 98.2% - 100%).

In addition, 3 HIV-2 infected individuals located in the USA were tested by fingerstick whole blood and oral fluid OraQuick *ADVANCE*[®] tests. Fingerstick whole blood and oral fluid samples from all three subjects were reactive on the OraQuick *ADVANCE*[®] test.

Reactivity with HIV-1 Specimens From Various Geographic Regions

- To assess the sensitivity of the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** for HIV-1 variants from various geographic regions, 215 confirmed HIV-1 antibody-positive serum/plasma specimens were obtained from various parts of the world. Of these 215 specimens, 214 were reactive using the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test**. One confirmed HIV-1 antibody-positive specimen from China was non-reactive using the OraQuick ADVANCE[®] test. An additional 13 specimens representing HIV-1 Subtypes A, B, C, D, F, and G, and Group O were tested and reactive on OraQuick ADVANCE[®].

Reactivity with HIV-1 Seroconversion Panels

- Eleven HIV-1 seroconversion panels were tested in comparison with licensed anti-HIV EIA tests. Each panel consisted of sequential serum/plasma specimens obtained from a single individual during seroconversion. The eleven seroconversion panels consisted of 69 specimens. The results of this study are shown in Table 4. In this study, the **OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test** was demonstrated to be capable of detecting seroconversion similar to currently available FDA licensed EIAs.

Specimen Information		OraQuick ADVANCE [®] Test	Licensed Anti-HIV EIA Tests				
Panel	Relative Day of Bleed		EIA #1	EIA #2	EIA #3	EIA #4	EIA #5
K	1	NR	NR	NR	NR	NR	NR
	7	NR	NR	NR	NR	NR	NR
	9	NR	NR	NR	NR	NR	NR
	14	R	NR	RR	NR	NR	NR
	16	R	NR	RR	NR	NR	NR
	21	R	NR	RR	NR	RR	RR
	23	R	RR	RR	RR	RR	RR
	30	R	RR	RR	RR	RR	RR
	34	R	RR	RR	RR	RR	RR
37	R	RR	RR	RR	RR	RR	
N	1	R	RR	RR	NR	NR	NR
	5	R	RR	RR	NR	RR	NR
	8	R	RR	RR	NR	RR	NR
	26	R	RR	RR	RR	RR	RR
	32	R	RR	RR	RR	RR	RR
Q	1	NR	NR	NR	NR	NR	NR
	54	NR	NR	NR	NR	NR	NR
	58	NR	NR	NR	NR	NR	NR
	61	NR	NR	RR	NR	NR	NR
	66	R	NR	RR	NR	NR	NR
	68	R	RR	RR	NR	NR	NR
	73	R	RR	RR	RR	RR	RR
R (M)	3	NR	NR	RR	NR	NR	NR
	8	NR	NR	RR	NR	NR	NR
	14	R	RR	RR	RR	RR	RR
	16	R	RR	RR	RR	RR	RR

	22	R	RR	RR	RR	RR	RR
S	1	NR	NR	NR	NR	NR	NR
	10	R	RR	RR	NR	NR	NR
	12	R	RR	RR	NR	RR	NR
W	1	NR	NR	NR	NR	NR	NR
	8	NR	NR	NR	NR	NR	NR
	13	NR	NR	NR	NR	NR	NR
	15	NR	NR	NR	NR	NR	NR
	29	NR	NR	NR	NR	NR	NR
	31	NR	NR	NR	NR	NR	NR
	36	NR	NR	NR	NR	NR	NR
	38	NR	NR	NR	NR	NR	NR
	48	NR	NR	RR	NR	NR	NR
	85	R	RR	RR	RR	RR	RR
	87	R	RR	RR	RR	RR	RR
	146	R	RR	RR	RR	RR	RR
162	R	RR	RR	RR	RR	RR	
AB	1	NR	NR	NR	NR	NR	NR
	29	NR	NR	RR	NR	NR	NR
	34	R	RR	RR	NR	NR	NR
	36	R	RR	RR	NR	NR	RR
	41	R	RR	RR	RR	RR	RR
AC	1	NR	NR	NR	NR	NR	NR
	112	NR	NR	RR	NR	NR	NR
	121	R	RR	RR	RR	RR	RR
	126	R	RR	RR	RR	RR	RR
	131	R	RR	RR	RR	RR	RR
AE	1	NR	NR	NR	NR	NR	NR
	4	NR	NR	NR	NR	NR	NR
	8	NR	NR	RR	NR	NR	NR
	11	NR	RR	RR	NR	RR	NR
AF	1	NR	NR	NR	NR	NR	NR
	3	NR	NR	NR	NR	NR	NR
	8	NR	NR	NR	NR	NR	NR
	10	NR	NR	NR	NR	NR	NR
	16	NR	NR	NR	NR	NR	NR
	29	R	NR	RR	NR	NR	NR
	34	R	RR	RR	NR	RR	RR
	36	R	RR	RR	RR	RR	RR
43	R	RR	RR	RR	RR	RR	
AI	1	NR	NR	NR	NR	NR	NR
	8	R	RR	RR	NR	NR	RR
	12	R	RR	RR	NR	RR	RR

NR = Non-Reactive; R = Reactive; RR = Repeatedly Reactive

Reactivity with HIV-1 Low Titer Panels

- Two low titer HIV-1 antibody panels were tested in comparison with licensed anti-HIV EIA tests. The low titer antibody panels consisted of 30 serum/plasma specimens. The results of this study are shown in Table 5. In this study, the OraQuick **ADVANCE**[®] Rapid HIV-1/2 Antibody Test was demonstrated to be capable of detecting antibodies to HIV-1 similar to currently available FDA licensed EIAs.

TABLE 5							
Comparison of the OraQuick <i>ADVANCE</i> [®] Rapid HIV-1/2 Antibody Test and Licensed Anti-HIV EIA Tests Using Low Titer HIV-1 Antibody Panels							
Specimen Information		OraQuick <i>ADVANCE</i> [®] Test	Licensed Anti-HIV EIA Tests				
Panel	Member		EIA #1	EIA #2	EIA #3	EIA #4	EIA #5
LT106	1	R	RR	RR	RR	RR	RR
	2	NR	NR	RR	NR	NR	NR
	3	R	RR	RR	RR	RR	RR
	4	R	RR	RR	RR	RR	RR
	5	R	RR	RR	RR	RR	RR
	6	NR	NR	NR	NR	NR	NR
	7	R	RR	RR	RR	RR	RR
	8	NR	RR	RR	NR	NR	NR
	9	R	RR	RR	RR	RR	RR
	10	R	RR	RR	RR	RR	RR
	11	R	RR	RR	NR	NR	RR
	12	R	RR	RR	NR	NR	RR
	13	R	RR	RR	RR	RR	RR
	14	R	RR	RR	RR	RR	RR
	15	R	RR	RR	RR	RR	RR
LT107	1	NR	NR	RR	RR	NR	NR
	2	R	NR	RR	RR	RR	NR
	3	R	NR	RR	NR	NR	NR
	4	R	RR	RR	RR	RR	NR
	5	NR	NR	NR	NR	NR	NR
	6	R	RR	RR	RR	RR	NR
	7	NR	NR	RR	RR	NR	NR
	8	NR	NR	RR	NR	RR	NR
	9	NR	NR	RR	NR	NR	NR
	10	R	RR	RR	RR	RR	RR
	11	R	RR	RR	RR	RR	RR
	12	NR	NR	RR	NR	NR	NR
	13	NR	NR	RR	RR	NR	NR
	14	R	RR	RR	RR	RR	RR
	15	R	RR	RR	RR	RR	RR

NR = Non-Reactive; R = Reactive; RR = Repeatedly Reactive

XVI. REPRODUCIBILITY

The reproducibility of the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** was tested at 3 sites using 3 lots of the device on 3 different days with 9 operators (3 per site). A blind-coded panel was tested that consisted of 5 contrived blood specimens (4 antibody-positive and 1 antibody-negative). Test results were recorded at 20 to 25 minutes and at 55 to 60 minutes. A total of 405 tests were performed (135/site), with a total of 81 tests per panel member. The overall reproducibility of the **OraQuick *ADVANCE*[®] Rapid HIV-1/2 Antibody Test** was 405/405 = 100%. Concordance between the specified assay read time limits was 99.8% (404/405); a single HIV-1 low positive panel member that was non-reactive at the 20 to 25 minute read time was reactive at the 55 to 60 minute read time.

XVII. RESULTS OF UNTRAINED USER STUDY:

An “Untrained User” study was conducted in which participants were given only the test instructions and asked to perform testing of a blinded panel comprised of 6 randomized specimens of three different levels (Negative, Low Positive and High Positive OraQuick

ADVANCE[®] test reactivity) consisting of human plasma. The participants were not given any training on the use of the test or the interpretation of the test results, nor were they allowed to observe the performance of the Kit Controls by the Study Coordinator. The study protocol stipulated that professionally trained medical laboratory personnel or persons with prior experience using the OraQuick ADVANCE[®] device were excluded from participation. A total of 100 participants were enrolled from a total of four sites, representing a diverse demographic (educational, ethnic, age, gender, etc.) population. The rate of correct results for the overall study was 98.6% (592/600). Refer to the table below for a summary of the performance relative to the specimen type. The eight incorrect results were attributed to six participants. Of these six participants, four obtained 5 out of 6 correct results, and two participants obtained 4 out of 6 correct results.

Untrained Users Rate of Correct Test Results			
Negative	Low Positive	High Positive	Total
98.5% (197/200) 95% C.I. (95.7% - 99.7%)	98.0% (196/200) 95% C.I. (95.0% - 99.5%)	99.5% (199/200) 95% C.I. (97.3% - 99.9%)	98.6% (592/600) 95% C.I. (97.4% - 99.4%)

There were 1.7% (10/600) Invalid results reported, with 5 of the 10 Invalid results attributed to one participant. All tests were successfully repeated, with 8/10 of the repeat test results interpreted correctly. The 2 incorrect repeat results were attributed to one participant. While most participants were able to obtain valid results with the first attempt, one of the 100 participants experienced five Invalid test results out of six tests performed. Operator error was observed in some cases to be attributed to specimen vial mixups. These findings support the need for training of non-laboratory personnel in the handling of multiple samples in a laboratory setting where specimens are tested in batch mode. As part of the Untrained User study, a Participant Feedback Questionnaire was completed. All participants rated the test as 'easy to use' and felt 'able to perform the test correctly'.

XVIII. REFERENCES

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Uni-Gold™ Recombigen® HIV-1/2 Laboratory Procedure

Product No. 1206506

CLIA COMPLEXITY:

**WAIVED FOR WHOLE BLOOD FINGERSTICK AND VENIPUNCTURE SAMPLES
MODERATE COMPLEXITY FOR SERUM AND PLASMA SAMPLES**

INTENDED USE

Uni-Gold™ Recombigen® HIV-1/2 is a single use rapid immunoassay, for the qualitative detection of antibodies to HIV-1/2 in serum, plasma and whole blood (venipuncture and fingerstick). Uni-Gold™ Recombigen® HIV-1/2 is intended for use in point of care settings as an aid in diagnosis of infection with HIV-1 and/or HIV-2. This test is suitable for use in appropriate multi-test algorithms designed for the statistical validation of rapid HIV test results.

RESTRICTIONS

- Sale of Uni-Gold™ Recombigen® HIV-1/2 is limited to clinical laboratories that have an adequate quality assurance program, including planned systematic activities to provide adequate confidence that requirements for quality will be met where there is assurance that operators will receive and use the instructional materials.
- Uni-Gold™ Recombigen® HIV-1/2 is approved for use only by an agent of a clinical laboratory.
- The test subjects must receive the "Subject Information Leaflet" prior to specimen collection, and appropriate information when test results are provided.
- Uni-Gold™ Recombigen® HIV-1/2 is not approved for use to screen donors of blood, plasma, cells or tissues.

BACKGROUND

HIV is the causative agent of AIDS (Acquired Immunodeficiency Syndrome). AIDS is the end stage of a drawn out process in which the immune system of an infected person and its ability to control infections or malignant proliferative disorders are progressively destroyed (1). HIV is mainly transmitted by unprotected sexual intercourse or from mother to child (1). Most frequently, HIV infection is diagnosed by tests that assess whether an individual's immune system has produced an HIV-specific immune response (antibodies to HIV) (1).

In the USA the standard laboratory test algorithm (set of different tests) may take 48 hours to one week before results may be made available. This algorithm consists of screening with an enzyme immunoassay (EIA) followed by confirmation by Western Blot (WB) or immuno-fluorescent (IFA) methods.

During the last 20 years, HIV infection and severe HIV-related diseases (e.g. AIDS) have become a leading cause of illness and death in the United States. Approximately 800,000-900,000 persons in the United States are infected with HIV and approximately 275,000 of these persons might not know they are infected (2).

Approximately 25 million persons each year in the United States are tested for HIV. Publicly funded counseling and testing programs conduct approximately 2.5 million of these tests each year. In 1995, 25% of these individuals testing HIV positive and 33% of persons testing HIV negative at publicly funded clinics did not return for their test results. Rapid tests to detect HIV antibody can be performed within 20 minutes, enabling health-care providers to supply definitive negative and preliminary positive results to patients at the time of testing, potentially increasing the overall effectiveness of counseling and testing programs. In comparison, results from enzyme immunoassays (EIAs) currently used for HIV screening often are not available for 1-2 weeks (3). Using rapid tests, during 1995, a total of 697,495 more persons would have learned their HIV status (3).

Many advances have been made in HIV/AIDS prevention and treatment, including the development of effective antiretroviral therapies that have reduced HIV-related illness and death. Early knowledge of HIV infection is now recognized as a critical component in controlling the spread of HIV infection (2). Rapid HIV testing allows clients to receive results the same day in a single visit, which is useful in urgent medical circumstances and settings where clients tend not to return for HIV test results (e.g., some STD clinics) (2). Advances in these areas have resulted in revised recommendations for HIV screening of pregnant women (4,5), treating opportunistic infections and other sexually transmitted and blood-borne diseases and managing occupational and non-occupational exposures and prophylaxis (6,7).

PRINCIPLES OF THE PROCEDURE

Uni-Gold™ Recombigen® HIV-1/2 was designed as a rapid immunoassay and is intended to detect antibodies to HIV-1 and/or HIV-2 in human serum, plasma and whole blood (venipuncture and finger stick). Uni-Gold™ Recombigen® HIV-1/2 uses proteins representing regions of the HIV virus. If antibodies to HIV-1 and/or HIV-2 are present in the sample, they combine with these proteins and a color reagent and this complex binds to the proteins in the test forming a visible pink/red band in the test region of the device adjacent to the word 'Test'. The control line should always appear as a visible pink/red band in the control region of the device to indicate that the test device is functioning correctly. A reactive result is indicated by a pink/red band in the test region of the device. A non-reactive result occurs in the absence of detectable levels of antibodies to HIV-1 and/or HIV-2 in the specimen; consequently no visually detectable band develops in the test region of the device.

SPECIMEN COLLECTION AND STORAGE

For venipuncture whole blood and plasma: EDTA, acid citrate dextran (ACD) or heparin should be used as the anticoagulant. Other anticoagulants have not been tested and may give incorrect results.

Whole blood collected by fingerstick:

Whole blood samples collected by fingerstick should be used on the Uni-Gold™ Recombigen® HIV-1/2 **immediately** after collection.

Whole blood collected by venipuncture:

Using standard phlebotomy procedures, collect a venipuncture whole blood specimen using a blood collection tube containing either EDTA, acid citrate dextran (ACD) or heparin. **Other anticoagulants have not been tested and may give incorrect results.**

It is recommended that specimens should be tested immediately but can be tested within 8 hours of collection if stored at ambient temperature (15°C- 27°C/ 59.0°F – 80.6°F). If specimens are not to be tested within 8 hours of collection, a plasma sample should be generated and stored at 2-8°C/ 35.6 – 46.4°F for up to five (5) days to allow testing. For long term storage plasma specimens should be frozen at -20°C or below. Grossly hemolysed or lipemic samples should not be used. Avoid multiple freeze thaw cycles. **(note: Plasma may only be tested in laboratories certified to run moderate complexity tests).**

Serum and Plasma (note: Serum and Plasma may only be tested in laboratories certified to run moderate complexity tests):

Using standard phlebotomy procedures, collect a venipuncture whole blood specimen using a blood collection tube. If collecting plasma use a blood collection tube containing either EDTA, acid citrate dextran (ACD) or heparin. **Other anticoagulants have not been tested and may give incorrect results.** Centrifuge the tube of blood (1000-1300 x g, for approximately 5 minutes, no refrigeration required) to separate the cells from the plasma. Carefully uncap the tube by gently rocking the stopper towards you so that it vents away from you.

Specimens may be tested immediately upon receipt or stored at 2-8°C/ 35.6 –46.4°F for up to five (5) days to allow testing. Specimens should be stored at -20°C or below if storage is necessary for more than five (5) days. Grossly hemolysed or lipemic samples should not be used. Avoid multiple freeze thaw cycles.

MATERIALS PROVIDED

Each kit contains:

- a) 20 Test Devices (individually pouched)
- b) Wash solution 5.0 ml
- c) 20 Disposable Pipettes for use with serum, plasma or venipuncture whole blood. To be used also with Controls (Catalog number 1206530)
- d) 20 Disposable Fingerstick Sample Collection and Transfer Pipettes for use with fingerstick whole blood
- e) 20 Subject Information Leaflets
- f) 1 Package Insert

Materials required and available as an accessory to the kit

Uni-Gold™ Recombigen® HIV Controls Kit. Catalog number 1206530.

Each pack of Kit Controls contains HIV-1 Positive Control, 1 vial (red cap) (0.5ml), HIV-2 Positive Control, 1 vial (green cap) (0.5ml), and Negative Control, 1 vial (black cap) (0.5ml) and a package insert.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer or stopwatch

Blood collection devices, for testing of venipuncture whole blood, serum or plasma

Biohazard disposal container

Disposable gloves

For Fingerstick samples the following additional material are required.

- Adhesive bandages
- Lancet capable of producing a 50µl droplet
- Sterile wipes and sterile gauze pads

WARNINGS

For *in vitro* diagnostic use

1. Read the package insert completely before using the product. The instructions must be followed carefully as not doing so may result in inaccurate results.
2. Before performing testing all operators must read and become familiar with the Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus and other Blood-Borne Pathogens in Health-Care settings (8).
3. The FDA has approved this kit for use with serum, plasma and whole blood (venipuncture and fingerstick) specimens. Use of the kit with specimens other than those specifically approved for use with this device may result in inaccurate test results.
4. This test kit is CLIA-waived for use only with fingerstick whole blood and venipuncture whole blood samples.
5. Uni-Gold™ Recombigen® HIV-1/2 is for diagnostic use only and is not to be used for screening donors of blood, plasma, cells or tissues.
6. Perform test at room temperature (15°C – 27°C / 59.0°F – 80.6°F).

PRECAUTIONS

Safety Precautions

1. Standard precautions for handling infectious agents should be observed when using this kit.
2. Wear standard protective clothing such as a lab coat and disposable gloves when handling specimens and assay reagents in accordance with local regulations.
3. Wash hands thoroughly after use.
4. In the case of Wash Solution contact with eyes, rinse immediately with plenty of water and seek medical advice.

Appropriate biosafety practices should be followed when handling specimens and reagents. These precautions include, but are not limited to, the following:

1. Do not smoke, eat, drink, apply cosmetics or handle contact lenses in areas where specimens are handled.
2. Dispose of all specimens, used devices and pipettes as though they are capable of transmitting infection. The preferred methods of disposal are by autoclaving at 121°C for a minimum of 60 minutes or by incineration. Disposable materials may be incinerated. Liquid waste may be mixed with appropriate chemical disinfectants. A solution of 10% bleach is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions containing bleach. For additional information on biosafety refer to "Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B virus and Other Blood-Borne Pathogens in Health Care Settings"(8).

3. When disposing of wash buffer, avoid contact with acid to prevent liberation of a toxic gas.
4. All spills should be wiped thoroughly using a suitable disinfectant such as a solution of 10% bleach.
5. Use a separate disposable pipette and device for each specimen tested.
6. Do not pipette by mouth.

Handling Precautions

1. Do not use any device if the pouches have been perforated.
2. Each device is for single use only.
3. Do not mix reagents from different kit lots.
4. Do not use the kit past the expiration date (this date is printed on the box).
5. Adequate lighting is required to read the test results.
6. Read results 10 minutes following the addition of Wash Solution. Do not read results more than 12 minutes following the addition of Wash Solution.
7. Lancets should be placed in a puncture resistant container prior to disposal.

STORAGE INSTRUCTIONS

Uni-Gold™ Recombigen® HIV-1/2 device and Wash Solution should be stored between 2°C-27°C / 35.6°F – 80.6°F.

Kit components are stable until expiration date when stored as directed.

If stored refrigerated, ensure that the pouched device is brought to room temperature (15°C – 27°C / 59.0°F – 80.6°F before opening).

Do not use beyond expiration date.

Do not freeze the kit.

Store the separately supplied Uni-Gold™ Recombigen® HIV Controls Kit at 2°C-8°C/35.6°F-46.4°F.

TEST PROCEDURE AND INTERPRETATION FOR CLIA WAIVED AND CLIA MODERATE SETTINGS

Test Procedure For Fingerstick Whole Blood

1. Ensure that the Subject Information Leaflet has been given to the subject.
2. Allow the kit (unopened devices and Wash Solution) to reach room temperature (15°C – 27°C / 59.0°F – 80.6°F) (at least 20 minutes) if previously stored in the refrigerator. Once at room temperature remove the required number of Uni-Gold™ Recombigen® HIV devices from their pouches.

PERFORM ONLY ONE TEST AT A TIME.

3. Lay the device on a clean flat surface.
4. Label the device with the appropriate patient information.
5. Sample collection and addition to device:
 - Using an antiseptic wipe, clean the finger of the person being tested. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad.
 - Using a sterile lancet capable of producing a 50µl blood let, puncture the skin just off the centre of the finger pad. Hold the finger downward. Apply gentle pressure beside the point of the puncture. Avoid squeezing the finger to make it bleed. Wipe away the first drop of blood with a sterile gauze pad. Allow a new drop of blood to form. If blood flow is inadequate the subject's finger may have to be gently massaged at the finger base to produce a droplet of sufficient volume. Avoid 'milking' the finger.

- Collect the blood into the fingerstick sample transfer pipette provided following the procedure presented below.
 - a. Hold the Pipette bulb gently in a horizontal position to the sample to be collected. This is important, as the specimen may not be adequately drawn in the pipette if the pipette is held in a vertical position.
 - b. Place the tip of the Pipette into the sample, taking care not to squeeze the bulb. Maintain this position until the flow of sample into the Pipette has stopped. The sample should fill to the mark on the Pipette, (Figure 1). If sample is not collected to the mark, the Pipette should be safely discarded and another specimen should be collected from another finger by repeating the sample collection process. The sample should be used immediately.
 - c. Squeeze the bulb until the sample is fully discharged into the Uni-Gold™ Recombigen® HIV-1/2 sample port. Should the sample not fully discharge, cover the small opening at the mark on the Pipette with a gloved finger. Then squeeze the bulb until the sample is fully discharged. Allow the sample to absorb into the paper in the sample port. Ensure air bubbles are not introduced into the sample port.
 - d. Dispose the Pipette in biohazard waste.
- 6. Holding the dropper bottle of Wash Solution in a vertical position, add four (4) drops of Wash Solution to the Sample Port.
- 7. Set the timer for 10 minutes and start timing the test.
- 8. Read test results after 10 minutes but not more than 12 minutes incubation time.
- 9. Refer to the Test Results and Interpretation of Whole Blood Samples below. Note there is a different interpretation for Whole Blood Samples from that for Plasma or Serum Samples.
- 10. If testing whole blood check for full red color in sample port. The sample port must contain red color for test to be valid. A red/pink line must appear adjacent to the word control. A red/pink line may appear adjacent to the word test. If no red color is seen in the sample port repeat test with fresh device.

Test Procedure Venipuncture Whole Blood

1. Ensure that the Subject Information Leaflet has been given to the subject.
2. Allow the kit (unopened devices and wash solution) to reach room temperature (15°C – 27°C / 59.0°F – 80.6°F) (at least 20 minutes) if previously stored in the refrigerator. Once at room temperature remove the required number of Uni-Gold™ Recombigen® HIV-1/2 devices from their pouches. Perform no more than 10 tests at one time.
3. Lay the devices on a clean flat surface.
4. Label each device with the appropriate patient information / ID.
5. Draw up adequate sample to the first graduation on the pipette using one of the disposable pipettes included in the kit. Use only the pipette included in the kit and do not reuse.
6. Holding the pipette vertically over the sample port, add one (1) free falling drop of sample carefully. Do not add the full volume contained within the pipette. Allow the sample to absorb into the paper in the sample port. Ensure air bubbles are not introduced into the sample port. Discard the pipette in a biohazard waste container.
7. Holding the dropper bottle of Wash Solution in a vertical position, add four (4) drops of Wash Solution to the Sample Port.
8. Set the timer for 10 minutes and start timing the test.
9. Read test results after 10 minutes but not more than 12 minutes incubation time.
10. Refer to the Test Results and Interpretation of Whole Blood Samples below. Note there is a different interpretation for Whole Blood Samples from that for Plasma or Serum Samples.
11. If testing whole blood check for full red color in sample port. The sample port must contain red color for test to be valid. A red/pink line must appear adjacent to the word control. A

red/pink line may appear adjacent to the word test. If no red color is seen in the sample port repeat test with fresh device.

INTERPRETATION FOR WHOLE BLOOD SAMPLE

FOR A TEST TO BE VALID A CONTROL LINE MUST BE PRESENT AND THE SAMPLE PORT MUST CONTAIN FULL RED COLOR

Invalid Results FOR A TEST TO BE VALID A CONTROL LINE MUST BE PRESENT AND THE SAMPLE PORT MUST CONTAIN FULL RED COLOUR				
				
REPORT AS INVALID	REPORT AS INVALID	REPORT AS INVALID	REPORT AS INVALID	REPORT AS INVALID
Test line present No control line present Full red color at Sample Port	No test line present No control line present Full red color at Sample Port	No test line present Control line present No red color at Sample Port	No test line present Control line present Not full red color at Sample Port	Test line present Control line present No red color at Sample Port
No pink/red line appears in the device window adjacent to word "Control" whether or not a pink/red line appears in the device window adjacent to word "Test". The test should be repeated in duplicate with fresh devices.	No pink/red line appears in the device window adjacent to word "Control" whether or not a pink/red line appears in the device window adjacent to word "Test". The test should be repeated in duplicate with fresh devices.	Red color is not seen in the Sample Port. The test should be repeated in duplicate with fresh devices.	Red color is not seen in full sample well. White of sample pad remains. The test should be repeated in duplicate with fresh devices.	Red color is not seen in the Sample Port. The test should be repeated in duplicate with fresh devices.

Valid Results	
	
REPORT AS PRELIMINARY POSITIVE	REPORT AS NEGATIVE
Test line present Control line present Full red color at Sample Port	No test line present Control line present Full red color at Sample Port
Reactive Test Result A pink/red line of any intensity appears in the device window adjacent to word "Test" AND a second pink/red line of any intensity appears adjacent to word "Control" AND a full red color appears in the Sample Port. This indicates a Reactive result that is interpreted as Preliminary Positive for HIV-1 and/or HIV-2 antibodies.	Non-Reactive Test Result A pink/red line of any intensity appears in the device window adjacent to word "Control" AND a full red color appears in the Sample Port, but no pink/red line appears in the device window adjacent to "Test". This indicates a Non-Reactive result that is interpreted as Negative for HIV-1 and/or HIV-2 antibodies.

**TEST PROCEDURE SERUM, PLASMA AND CONTROLS:
SERUM AND PLASMA SUITABLE FOR CLIA MODERATE SETTING ONLY**

Test Procedure

1. Ensure that the Subject Information Leaflet has been given to the subject.
2. Allow the kit (unopened devices and wash solution) to reach room temperature (15°C – 27°C / 59.0°F – 80.6°F) (at least 20 minutes) if previously stored in the refrigerator. Once at room temperature remove the required number of Uni-Gold™ Recombigen® HIV-1/2 devices from their pouches. Perform no more than 10 tests at onetime.
3. Lay the devices on a clean flat surface.
4. Label each device with the appropriate patient information / ID.
5. Draw up adequate sample to the first gradation on the Pipette using one of the disposable pipettes included in the kit. Use only the Disposable Pipette included in the kit and do not reuse. If Kit Controls are being run, these must be used as described in the package insert provided with the Kit Controls.
6. Holding the Disposable Pipette vertically over the sample port, add one (1) free falling drop of sample carefully. Do not add the full volume contained within the Disposable Pipette Allow the sample to absorb into the paper in the sample port. Ensure air bubbles are not introduced into the sample port. Discard the Disposable Pipette in a biohazard waste container.
7. Holding the dropper bottle of Wash Solution in a vertical position, add four (4) drops of Wash Solution to the Sample Port
8. Set the timer for 10 minutes and start timing the test.

9. Read test results after 10 minutes but not more than 12 minutes incubation time.
10. Refer to the interpretation guide for serum and plasma. A red/pink line must appear adjacent to the word control. A red/pink line may appear adjacent to the word test.

INTERPRETATION FOR SERUM PLASMA SAMPLE SERUM AND PLASMA SAMPLES SUITABLE FOR CLIA MODERATE SETTING ONLY

TEST RESULTS AND INTERPRETATION OF RESULTS

Reactive Test Result

A pink/red line of any intensity appears in the device window adjacent to word "Test" and a second pink/red line of any intensity appears adjacent to word "Control".



This indicates a Reactive result that is interpreted as Preliminary Positive for HIV-1 and/or HIV-2 antibodies.

Non-Reactive Test Result

A pink/red line of any intensity appears in the device window adjacent to word "Control", but no pink/red line appears in the device window adjacent to "Test".



This indicates a Non-Reactive result that is interpreted as Negative for HIV-1 and/or HIV-2 antibodies.

Invalid Result

No pink/red line appears in the device window adjacent to word "Control" whether or not a pink/red line appears in the device window adjacent to word "Test". This is an Invalid result that cannot be interpreted.



OR



The test should be repeated in duplicate with fresh devices.

QUALITY CONTROL

Built-In Control Features:

The Uni-Gold™ Recombigen® HIV-1/2 test has a built in procedural control that demonstrates assay validity. A red / pink line appearing adjacent to the word 'control' indicates that the test is running correctly. In addition, when using whole blood samples, there must be a red color in the sample port to validate the addition of the sample. The control line will appear on all valid tests, whether or not the sample is Reactive or Non-Reactive (refer to the test results and interpretation sections).

External Quality Control:

Uni-Gold™ Recombigen® HIV Controls Kit (Product Code: 1206530) are available separately for use only with the Uni-Gold™ Recombigen® HIV-1/2 test. The Kit Controls are used to verify your ability to perform the test and interpret the test result. The Positive Control will produce a Reactive test result and has been manufactured to produce a very faint pink/red Test line. The Negative Control will produce a Non-Reactive test result (refer to the test results and interpretation section). Note that a red color at the sample port will not be seen if using the Uni-Gold™ Recombigen® HIV Controls Kit (Product Code: 1206530).

Run the Kit Controls under the following circumstances:

- All new operators performing testing on patient specimens
- Each new kit lot
- Whenever a new shipment of test kits is received
- If the temperature of the test kit storage area falls outside of 2°C-27°C / 35.6°F –80.6°F
- If the temperature of the testing area falls outside of 15°C – 27°C / 59.0°F –80.6°F
- At periodic intervals as specified in your Quality Assurance program.

The Kit Controls must give the expected reactive or non-reactive results, otherwise the test results are not valid. Refer to the Uni-Gold™ Recombigen® HIV Controls Kit package insert for instructions on the use of these reagents. It is the responsibility of each laboratory using the Uni-Gold™ Recombigen® HIV-1/2 test to establish an adequate quality assurance program to assure the performance of the device under its specific locations and conditions of use. Contact Trinity Biotech Customer Service if the Kit Controls do not produce the expected results.

LIMITATIONS

1. Uni-Gold™ Recombigen® HIV-1/2 must be used in accordance with the instructions in this package insert to obtain an accurate result.
2. Uni-Gold™ Recombigen® HIV-1/2 is designed to detect antibodies to HIV-1 and/or HIV-2 in undiluted whole blood (venipuncture and fingerstick) serum, and plasma. For venipuncture whole blood and plasma, EDTA, acid citrate dextran (ACD) or heparin should be used as the anticoagulant. Other anticoagulants have not been tested and may give incorrect results. Other body fluids may not give accurate results and must not be used.
3. Immunosuppressed or immunocompromised individuals infected with HIV-1 and/or HIV-2 may not produce antibodies to the virus. Testing with any kit designed to detect antibodies may give negative results in this incidence and would not be a reliable test method for such patients.
4. The intensity of a pink/red line at the "Test" region is not an indication of the level of antibody in the specimen.
5. A Reactive result by Uni-Gold™ Recombigen® HIV-1/2 suggests the presence of anti-HIV-1 and/or HIV-2 antibodies in the specimen. Uni-Gold™ Recombigen® HIV-1/2 is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. AIDS and AIDS-related conditions are clinical symptoms and their diagnosis can only be established clinically.
6. Reading test results earlier than 10 minutes or later than 12 minutes may give incorrect results.
7. A Non-Reactive result with Uni-Gold™ Recombigen® HIV-1/2 does not exclude the possibility of infection with HIV. A false negative result may occur in the following circumstances:
 - Recent infection. Antibody response to a recent exposure may take several months to reach detectable levels.

- The test procedure has not been correctly followed.
 - Antibodies to a variant strain of HIV-1 and/or HIV-2 in the patient that do not react with specific antigens utilized in the assay configuration.
 - Improper specimen handling.
 - Failure to add sample.
8. A person who has antibodies to HIV-1 and/or HIV-2 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.

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9. CDC Revised guidelines for HIV counseling MMWR Recommendations and Reports, 2001; 50 (RR-19)

Clearview[®] COMPLETE HIV 1/2 Laboratory Procedure

This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete product insert. **Any modifications to this document are the sole responsibility of the Facility.**

This test is Waived for fingerstick whole blood and venipuncture whole blood.

This test is Moderately Complex for serum and plasma samples.

1. Intended Use

The Clearview[®] COMPLETE HIV 1/2 assay is a single-use immunochromatographic test for the detection of antibodies to Human Immunodeficiency Virus Types 1 (HIV-1) and Type 2 (HIV-2) in fingerstick whole blood, venous whole blood, and serum or plasma specimens. The Clearview[®] COMPLETE HIV 1/2 assay is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1 and HIV-2. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms.

2. Test Principle

The Clearview[®] COMPLETE HIV 1/2 assay employs a unique combination of a specific antibody binding protein which is conjugated to colloidal gold dye particles, and HIV-1/2 antigens which are bound to the solid phase membrane. The venous or capillary (fingerstick) whole blood, serum or plasma is applied to the capillary tip of the Sampler of test device. The Sampler is inserted into the Buffer, which is provided in a sealed vial. The Buffer facilitates the lateral flow of the specimen and test reagents and promotes the binding of the antibodies to the antigen. The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the TEST area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device

3. Specimen Collection/Treatment

A. Specimen:	<u>Waived:</u> Fingerstick and venous whole blood. <u>Moderate:</u> Serum/plasma
B. Specimen Collection:	<u>Fingerstick:</u> Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze and avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Collect the sample from the second drop touching the disposable Sample Loop provided to the drop of blood until the Sample Loop is full. Test immediately.

	<p><u>Venous:</u> Draw blood following laboratory procedures for obtaining venous blood. Collect sample in a tube containing citrate, heparin, or EDTA. Be sure the tube of blood is well mixed before sampling. Venous whole blood may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 to 8°C (36 to 46°F) following collection. Test within 3 days of collection.</p> <p><u>Serum/Plasma:</u> Note: Serum and Plasma may only be tested in laboratories certified to run moderately complexity tests. Draw blood following laboratory procedures for obtaining serum or plasma specimens. Collect specimen in a tube not containing any anticoagulant (serum), or in a tube containing citrate, heparin, or EDTA (plasma). Collect specimen in a clean container following standard laboratory procedures. Serum and plasma specimens may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 and 8°C (36 to 46°C) following collection. These specimens should be tested within 3 days of collection. If specimens are not tested within 3 days of collection, serum or plasma specimens should be frozen at -20°C (-4°F) or colder.</p>
C. Specimen Transport:	If specimens are to be shipped, they should be packaged in compliance with regulations covering the transportation of etiologic agents. Venous whole blood, serum and plasma specimens should be shipped refrigerated with cold packs or wet ice.
D. Handling Precautions:	Patient samples, controls, and test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards.

4. Reagents and Equipment

A. Reagents and Materials Provided

Component	Content	Quantity
Pouches	Sampler with a test strip inside, Buffer Vial attached to the sampler (~ 350 µL), sterile safety lancet, bandage, and desiccant packet	25
Disposable Test Stands		25
Subject Information Notice		25
Product Insert		1

B. Accessories Available and Required

Clearview[®] HIV Reactive/Nonreactive Controls (catalog # 92112). Each package contains:

- 1 HIV 1 Reactive Control (0.25 mL)
- 1 HIV 2 Reactive Control (0.25 mL)
- 1 Nonreactive Control (0.25 mL)
- 1 Product Insert for Catalog # 92112

C. Reagents and Materials not Provided

- Clock, watch, or other timing device
- Pipettor capable of delivering 2.5 µL of sample (for other than fingerstick or venipuncture whole blood specimens)
- Disposable gloves

- Sterile gauze
- Antiseptic wipes
- Biohazard disposal container
- Collection device for specimens (other than fingerstick whole blood specimens)

D. Storage and Stability

The Clearview[®] COMPLETE HIV 1/2 assay should be stored in its unopened pouch at 8 to 30°C (46 to 86°F). Do not freeze. Do not open the pouch until you are ready to perform a test. When stored as indicated, test devices are stable until the expiration date marked on the pouch.

5. Quality Control

Built In Control Feature:

The control line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. A pink/purple line will appear in the Control (C) area if the test has been performed correctly and the device is working properly. (Please see section: Interpretation of Test Results).

External Quality Control:

Good Laboratory Practices (GLP) necessitates testing external control material along with the test samples to ensure proper performance of the test kit. Clearview[®] HIV Reactive/Nonreactive Controls (catalog # 92112) are available separately for use with the Clearview[®] COMPLETE HIV 1/2 assay. The HIV Controls are used to verify the operator's ability to properly perform the test and to interpret the results. Each Reactive Control will produce a REACTIVE Test Result and has been manufactured to produce a faint line in the TEST (T) area. The Nonreactive Control will produce a NONREACTIVE Test Result. Run the Controls as per the TEST PROCEDURE and follow the instructions as described in the INTERPRETATION OF TEST RESULTS sections of the Product Insert. It is the responsibility of each facility using the Clearview[®] COMPLETE HIV 1/2 assay to establish an adequate quality assurance program to ensure the performance of the device under specific locations and conditions of use.

RUN THE KIT CONTROLS UNDER THE FOLLOWING CIRCUMSTANCES:

- **Each new operator prior to performing tests on patient specimens**
- **When opening a new test Kit lot**
- **Whenever a new shipment of test Kits is received**
- **If the temperature of the testing storage area falls outside of 8 to 30°C (46 to 86°F)**
- **If the temperature of the testing area falls outside of 18 to 30°C (64 to 86°F)**
- **At periodic intervals as indicated by the user facility**

If the HIV Control reagents do not produce the expected results, contact Alere[™] Technical Service at 877-441-7440.

6. RESTRICTIONS

- **Sale of the Clearview[®] COMPLETE HIV 1/2 assay is restricted to clinical laboratories that have an adequate quality assurance program, including planned systematic activities that provide adequate confidence that requirements for quality will be met and where there is assurance that operators will receive and use the instructional material.**
- **The Clearview[®] COMPLETE HIV 1/2 assay is approved for use only by an agent of a clinical laboratory.**

- **Non-clinical testing sites that offer waived rapid HIV tests must either have their own CLIA Certificate of Waiver or have an agreement to work under the CLIA Certificate of an existing laboratory.**
- **Test subjects must receive the “Subject information Notice” prior to specimen collection and appropriate information when test results are provided.**
- **The Clearview[®] COMPLETE HIV 1/2 assay is not approved for use to screen blood, plasma, cell or tissue donors**

7. Warnings

For *IN VITRO* diagnostic use

1. Read the Product Insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate Test Results.
2. Users of this test should follow the CDC Universal Precautions for prevention of transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other bloodborne pathogens.¹
3. Use of this test Kit with specimen types other than those specifically approved for use with this device may result in inaccurate test results.
4. This test is CLIA-waived for use only with fingerstick whole blood and venipuncture whole blood samples.
5. This test should be performed at 18 to 30°C (64 to 86°F). If stored refrigerated, ensure that the pouch is brought to operating temperature before performing testing.
6. If the test Kit is stored at temperatures outside the storage temperature 8 to 30°C (46 to 86°F), or used outside the operating temperature 18 to 30°C (64 to 86°F), use the Kit Controls to ensure proper performance of the test.
7. Individual infected with HIV-1 and/or HIV-2 who is receiving highly active antiretroviral therapy (HAART) may produce false negative results.

8. Safety Precautions

1. Handle the specimens and materials contacting specimens as if capable of transmitting infection.
2. Do not eat, drink or smoke in the area where specimens and kit reagents are handled. Avoid any contact with hands, eyes or mouth during specimen collection and testing.
3. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when handling specimens.
4. Dispose of all specimens and materials used in the test procedure in a biohazard waste container. Lancets should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Liquid wastes may be mixed with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions that contain bleach.
5. For additional information on biosafety, refer to “Universal Precautions for prevention of transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other blood borne pathogens.”¹ and “Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposure to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis.”¹³
6. Use 10% bleach or other appropriate disinfectant to wipe all spills. The bleach solution should be made fresh each day.

9. Handling Precautions

1. The Clearview[®] COMPLETE HIV 1/2 assay device has a sample filter in the lower part of the device and an absorbent pad in the upper part of the device within the barrel that encloses the

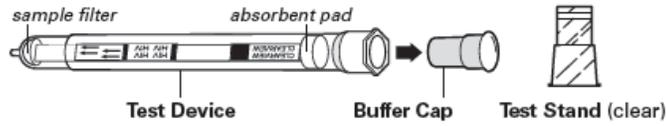
test strip. Confirm the presence of the sample filter and absorbent pad prior to performing the test. If either is missing, DO NOT USE.

2. Do not use any device if the pouch has been perforated. Do not use the device if the Desiccant Packet is missing.
3. Each device is for single use only.
4. Do not use the reagents beyond the expiration date printed on the pouch. Always check expiration date prior to testing.
5. Do not mix reagents from different lot numbers of Kits.
6. To ensure accurate Test Results, the Sampler must be inserted into the Buffer Vial immediately after the sample application.
7. Adequate lighting is required to read the Test Results.

10. Test Procedure for CLIA Waived and CLIA Moderate Settings

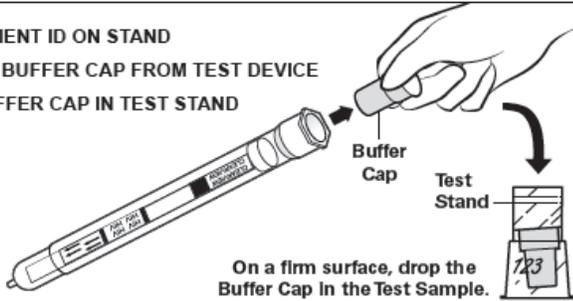
All components for the Clearview[®] COMPLETE HIV 1/2 assay test are ready to use as supplied. Follow directions as indicated. If the specimen to be tested is refrigerated, remove it from the refrigerator and allow it to come to a temperature of 18 to 30°C (64 to 86° F) prior to testing.

1. OPEN POUCH, REMOVE AND IDENTIFY COMPONENTS



Identify Test Device, Buffer Cap and Test Stand
Note: If Desiccant Packet is missing or if absorbent pad (at top of Sampler) is missing or if sample filter (at bottom of Sampler) is missing, DO NOT USE. Discard device and use a new device.

2. WRITE PATIENT ID ON STAND
3. SEPARATE BUFFER CAP FROM TEST DEVICE
4. PLACE BUFFER CAP IN TEST STAND



On a firm surface, drop the Buffer Cap in the Test Sample.

5. SPECIMEN APPLICATION WAIVED PROCEDURES

For fingerstick whole blood, touch blood drop with Sampler tip until the tip is full.

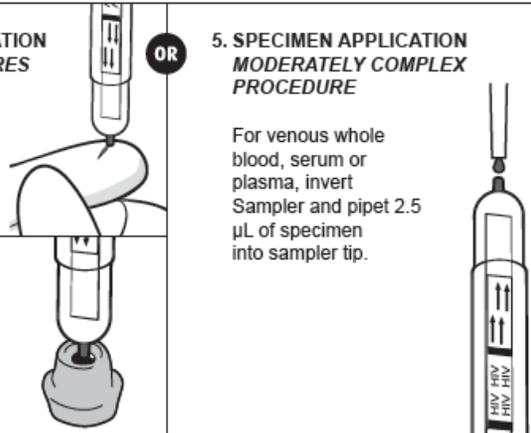
OR

Alternately, pick up a drop of whole blood from the inside of a blood tube cap.

OR

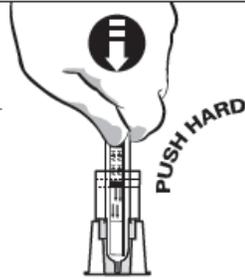
5. SPECIMEN APPLICATION MODERATELY COMPLEX PROCEDURE

For venous whole blood, serum or plasma, invert Sampler and pipet 2.5 µL of specimen into sampler tip.



6. START THE TEST

- With Buffer Cap in Stand, firmly press the Device tip through foil cover.
- Push hard until Device is fully seated in the Buffer Cap.



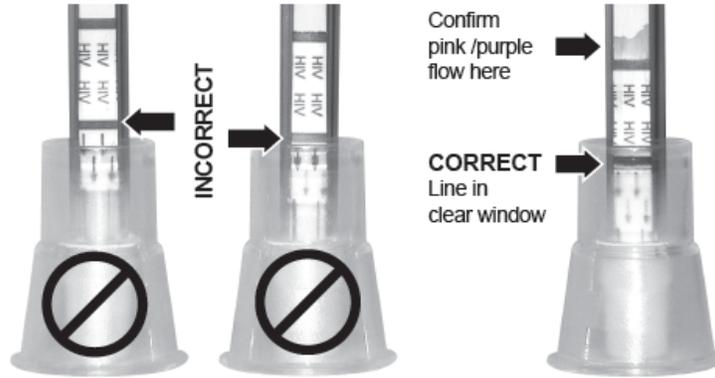
It will “snap” 3 times when properly seated.

- Snap 1: through foil
- Snap 2: into cap
- Snap 3: fully seated

7. CONFIRM DEVICE IS FULLY SEATED

- A. The blue line directly above the arrows must line up with the clear line in the Stand
- B. You will see pink/purple Buffer solution begin to flow upwards

IF YOU DO NOT SEE PINK/PURPLE FLOW WITHIN 3 MINUTES, PUSH AGAIN!
(then start timer)



8. START TIMING - WAIT FOR 15 MINUTES

NOTE: the Sampler/ Buffer Vial should be kept upright in the Test Stand

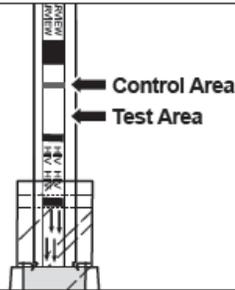


READ TEST RESULTS

Read the test between 15 and 20 minutes.

NOTE: Reactive Test Results (See Interpretation of Test Results section) may be observed and read earlier than 15 minutes. To verify a Nonreactive Test Result, wait the entire 15 minutes after starting the test.

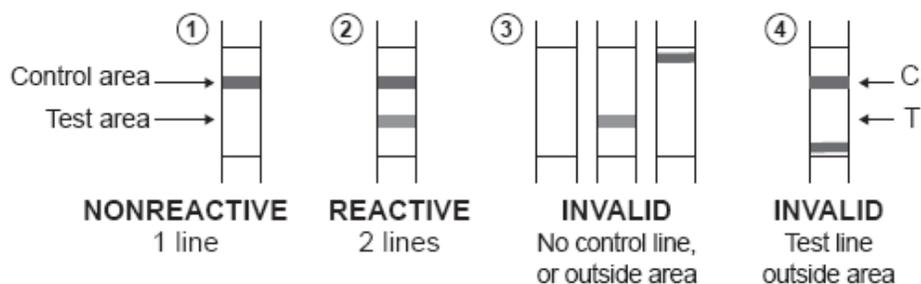
Do not read results after 20 minutes.



11. Interpretation of Results for CLIA Waived and CLIA Moderate Settings

When the Clearview[®] COMPLETE HIV 1/2 assay is properly performed, the appropriate pink/purple lines will become visible. These are:

1. **The CONTROL LINE** - which appears closer to the top of the test strip, indicates that specimen was adequately applied, and there was proper hydration and migration of reagents. The control line will become visible within 15 minutes after starting the test regardless of the HIV antibody status of the specimen.
2. **The TEST LINE** - which appears closer to the bottom of the test strip (below the control line) indicates the presence of HIV-specific antibodies. The test line will only become visible within 15 minutes after starting a valid test when HIV specific antibodies are present at detectable levels in the specimen.



NONREACTIVE (diagram 1):

One pink/purple line in the CONTROL area, with no line in the TEST area indicates a NONREACTIVE Test Result. A NONREACTIVE Test Result means that HIV-1 and HIV-2 antibodies were not detected in the specimen. The Test Result is interpreted as NEGATIVE for HIV-1 and HIV-2 antibodies. However, this does not exclude possible infection with HIV. Follow CDC guidelines to inform the test subject of the Test Result and its interpretation.^{7,14}

REACTIVE (diagram 2):

Two pink/purple lines, one in the TEST area and one in the CONTROL area indicate a REACTIVE Test Result. The line in the TEST area may look different from the line in the CONTROL area. Intensities of the Test and Control Lines may vary. Test Result with visible lines in both TEST and CONTROL areas, regardless of intensity, is considered REACTIVE. A REACTIVE Test Result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The Test Result is interpreted as Preliminary POSITIVE for HIV-1 and/or HIV-2 antibodies. Follow CDC guidelines to inform the test subject of the Test Result and its interpretation.^{7,14}

INVALID (diagram 3):

A pink/purple line should always appear in the CONTROL area, whether or not a line appears in the TEST area. If there is no distinct pink/purple line visible in the CONTROL area (see diagrams 1 and 2), then the test is INVALID. Any line that appears outside of the Control Area or Test Area (see diagram 3) is an INVALID test. An INVALID test cannot be interpreted. It is recommended that the INVALID test be repeated with a new device.

INVALID (diagram 4):

One pink/purple line in the CONTROL area, with Test line outside the TEST area, then test is INVALID. It is recommended that the INVALID test be repeated with a new device.

12. Limitations

- The Clearview[®] COMPLETE HIV 1/2 must be used in accordance with the instructions in the Product Insert to obtain accurate results.
- The Clearview[®] COMPLETE HIV 1/2 assay must be used with capillary (fingerstick) or venous whole blood, serum or plasma only. Use of other types of specimens or testing of venipuncture whole blood specimens collected using a tube containing an anticoagulant other than citrate, heparin or EDTA may not yield accurate results. For serum samples, collect blood without anticoagulant.
- Reading Nonreactive Test Results earlier than 15 minutes or any Test Results later than 20 minutes may yield erroneous results.
- Do not open the sealed foil pouch until just prior to use.
- Do not use Kit contents beyond labeled expiration date.
- For the collection of the fingerstick whole blood specimen, ensure that finger is completely dry before performing fingerstick.
- Read results in a well-lit area.
- A Reactive Test Result using the Clearview[®] COMPLETE HIV 1/2 test suggests the presence of antibodies to HIV-1 and/or HIV-2 in the specimen. The Clearview[®] COMPLETE HIV 1/2 assay is intended as an aid in the diagnosis of infection with HIV 1/2. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
- For a Reactive Test Result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
- A person who has antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV.
- A Nonreactive Test Result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to recent exposure may take several months to reach detectable levels.
- This assay has not been evaluated for newborn screening, cord blood specimens, individuals less than 13 years of age.

13. Performance Characteristics

NOTE: For complete details and performance of this product, refer to the Product Insert provided with the test kit.

14. References

1. Centers for Disease Control and Prevention (CDC) Universal Precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. *MMWR* 1988; 37(24):377-388.
2. Essex, M. (1999) Human immunodeficiency viruses in the developing world. *Adv Virus Res* 53: 71-88.
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7. CDC. Revised Guidelines for HIV Counseling, Testing and Referral and Revised Recommendations for HIV Screening of Pregnant Women. *MMWR* 2001; 50(19):32-35.
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11. Kenealy, W., Reed, D., Cybulsky, R., et.al. (1987) Analysis of human serum antibodies to human immunodeficiency virus (HIV) using recombinant ENV and GAG antigens. *AIDS Res Human Retrovir* 3:95-105.
12. Kovacs, A., Xu, J., Rasheed, S., et al. (1995) Comparison of a rapid non-isotopic polymerase chain reaction assay with four commonly used methods for the early diagnosis of human immunodeficiency Virus type 1 infection in neonates and children. *Pediatr Infect Dis J* 14: 948-954.
13. CDC: Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposure to HBV, HCV, and Recommendations for Postexposure Prophylaxis. *MMWR* 2001; 50(RR-11):1-42.
14. CDC: Approved of a New Rapid Test for HIV Antibody. *MMWR* 2002; 51 (46) 1051-1052.

Guidance on using Determine Combo in point-of-care settings

The Determine HIV-1/2 Ag/Ab Combo (Determine Combo) is a CLIA waived rapid test capable of detecting HIV-1/2 antibodies and the HIV-1 p24 antigen. The p24 antigen is a part of the HIV virus that can be detected before antibodies develop and therefore use of this test may allow for an earlier diagnosis of HIV infection. Acceptable clinical specimens include whole blood, serum, and plasma.

Agencies that use Determine Combo must have measures in place to ensure the client can receive confirmatory testing the same day the Determine test is given. The method of confirmation will depend on the results of the Determine Combo test.

In accordance with CDC guidance, the following algorithm should be implemented. For an antigen positive/antibody negative result, a blood draw must be performed to confirm HIV diagnosis. Due to the highly infectious nature of acute HIV infection, clients with a positive antigen but negative antibody test who endorse recent or current symptoms of acute HIV should be immediately referred to care without waiting for confirmatory testing results.

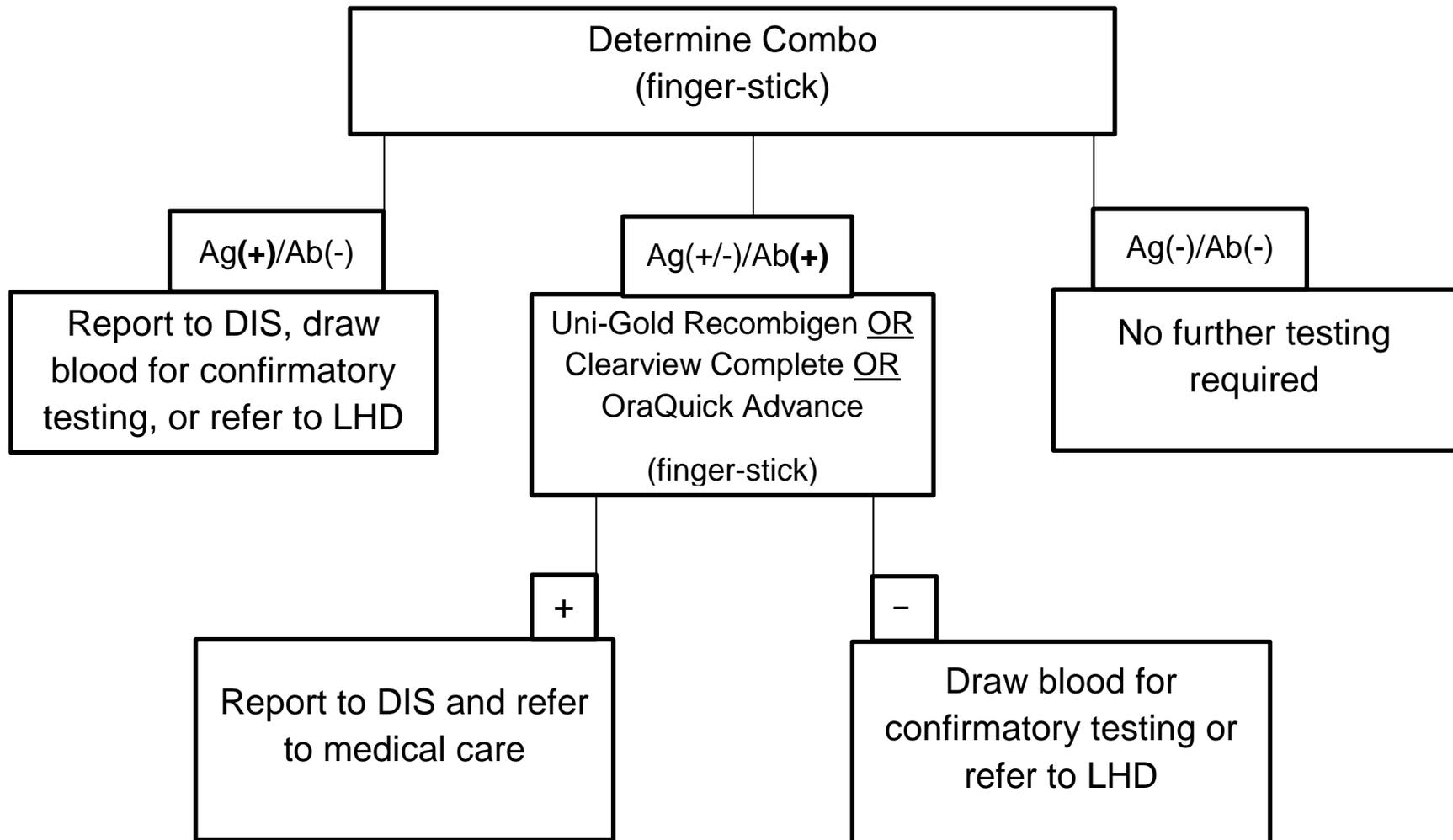
For an antigen negative/antibody positive OR antigen positive/antibody positive result, additional testing is required to confirm the diagnosis. This can be done by either a blood draw or completion of the dual rapid algorithm. OraQuick Advance, Clearview Complete, or Uni-Gold Recombigen finger-stick tests may be used as the second rapid test in the dual rapid algorithm.

A positive second rapid HIV antibody test confirms the HIV diagnosis, at which point the client should be referred to medical care and reported to the DIS for partner notification services.

If the result of the second rapid HIV test is negative, the client should receive a blood draw to determine the actual infection status. In the case of an antigen negative/antibody positive test and a negative second rapid test, counselors must explain to the client that their results are presumptively positive but further testing is required to confirm the HIV diagnosis.

Counselors must still use the three-month window in rapid testing counseling and retest guidance.

For agencies that have phlebotomy capability, samples must be processed via 4th generation HIV screening assays like those available through the State Laboratory of Public Health. For agencies that do not have phlebotomy capability or cannot draw blood in outreach settings, measures must be in place to ensure the client can receive a blood draw the same day. This may include partnering with another agency that can draw blood on site, or implementing protocols to ensure the client is seen by the health department or another primary care provider. Agencies may also implement the dual rapid algorithm, but only in results scenarios listed above for early referral to care.



Alere Determine™ HIV-1/2 Ag/Ab Combo Laboratory Procedure

This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete Alere Determine™ HIV-1/2 Ag/Ab Combo package insert. **Any modifications to this document are the sole responsibility of the Facility.**

CLIA Complexity: Waived (For Finger stick Whole Blood Only)

- Any modification by the laboratory to the Alere Determine™ HIV-1/2 Ag/Ab Combo test or the FDA approved Alere Determine™ HIV-1/2 Ag/Ab Combo test instructions will result in the test no longer meeting the requirements for waived category.
- A CLIA Certificate of Waiver is required to perform the test in a waived setting.
- Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA or from your state health department.

CLIA Complexity: Nonwaived Moderate (For Venous Whole Blood, Serum and Plasma Samples)

1. Intended Use

Alere Determine™ HIV-1/2 Ag/Ab Combo is an *in vitro*, visually read, qualitative immunoassay for the simultaneous detection of Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen (Ag) and antibodies (Ab) to HIV Type 1 and Type 2 (HIV-1 and HIV-2) in human serum, plasma, capillary (fingerstick) whole blood or venipuncture (venous) whole blood. It is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1 and HIV-2, including an acute HIV-1 infection, and may distinguish acute HIV-1 infection from established HIV-1 infection when the specimen is positive for HIV-1 p24 antigen and negative for anti-HIV-1 and anti-HIV-2 antibodies. The test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV test are available, this test can be used in appropriate multi-test algorithms.

Alere Determine™ HIV-1/2 Ag/Ab Combo is not intended for newborn screening or for use with cord blood specimens or specimens from individuals less than 12 years of age.

Alere Determine™ HIV-1/2 Ag/Ab Combo is not intended for use in screening blood, plasma, cell, or tissue donors.

2. Test Principle

Alere Determine™ HIV-1/2 Ag/Ab Combo is an immunochromatographic test for the simultaneous and separate qualitative detection of free HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2. The test device is a laminated strip that consists of a Sample Pad containing monoclonal biotinylated anti- HIV-1 p24 antibody, a Conjugate Pad containing monoclonal anti-HIV-1 p24 antibody-colloidal selenium and HIV-1 and HIV-2 recombinant antigen-colloidal selenium, and a nitrocellulose membrane with an immobilized mixture of recombinant and synthetic peptide HIV-1 and HIV-2 antigens in the Lower Test Area, immobilized streptavidin in

the Upper Test Area, and an immobilized mixture of anti-HIV-1 antibody, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody in the Control Area.

A specimen (venipuncture or capillary whole blood, serum, or plasma) is applied to the Sample Pad (followed by Chase Buffer for venipuncture or fingerstick whole blood specimens) and migrates by capillary action through the Conjugate Pad and then through the nitrocellulose membrane.

If HIV-1 p24 antigen is present in the specimen, it binds with the monoclonal biotinylated anti-HIV-1 p24 antibody from the Sample Pad and then with monoclonal anti-HIV-1 p24 antibody-colloidal selenium from the Conjugate Pad to form a complex (biotinylated antibody-antigen-colloidal selenium- antibody). This complex migrates through the solid phase by capillary action until it is captured by immobilized streptavidin at the Upper Test Area (labeled “Ag”) where it forms a single pink/red “Ag” line. If HIV-1 p24 antigen is not present in the specimen or is below the limit of detection of the test, no pink/red Ag line is formed. NOTE: The monoclonal biotinylated anti-HIV-1 p24 antibody used in this assay does not cross react with HIV-2 p26 antigen.

If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to recombinant gp41 (HIV-1) and gp36 (HIV-2) antigen-colloidal selenium conjugates from the Conjugate Pad. The complex migrates through the solid phase by capillary action until it is captured by immobilized HIV-1 and HIV-2 synthetic peptide antigens and recombinant gp41 antigen at the Lower Test Area (labeled “Ab”) and forms a single pink/red “Ab” line. If antibodies to HIV-1 and/or HIV-2 are absent or are below the detection limit of detection of the test, no pink/red Ab line is formed.

To ensure assay validity, a procedural “Control” line containing a mixture of anti-HIV-1 antibody, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody is incorporated in the nitrocellulose membrane. For a test result to be valid there must be a visible pink/red Control line. During the testing procedure the colloidal selenium conjugates released from the Conjugate Pad will be captured by the antibodies and antigens immobilized in the Control Area and form a pink/red Control line for samples that are either positive or negative. NOTE: A pink/red Control line may appear even when a test sample has not been applied to the Test Unit.

3. Specimen Collection/Treatment

A. Specimen:	Prior to specimen collection, provide test subjects with the Subject Information Notice. Alere Determine™ HIV–1/2 Ag/Ab Combo can be used for testing fingerstick whole blood, venous whole blood, serum, or plasma specimens.
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B. Specimen Transport:	If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Venous whole blood, serum, and plasma specimens should be shipped refrigerated with cold packs or wet ice.
C. Specimen Storage:	<p>Serum and plasma specimens may be stored at room temperature (15-30°C) for up to two days before testing. If testing will not be performed within two days of sample collection, serum and plasma specimens should be stored at 2- 8°C if the test is to be run within 7 days of collection. If testing is delayed more than 7 days, the specimen should be frozen (-20°C or colder). Mix specimen well by gentle inversion of the tube immediately before testing.</p> <ul style="list-style-type: none"> • Avoid repeated freeze/thaw cycles. Specimens that have been frozen and thawed more than 3 times cannot be used. • All frozen specimens must be centrifuged at 10,000g for 5 min at room temperature. Carefully remove the 50 µL test sample from the supernatant. If a lipid layer is formed on the surface of the liquid, ensure that the sample is taken from the clear liquid below that layer. <p>Whole blood collected by venipuncture may be stored at room temperature (15- 30°C) for up to two days before testing. If testing will not be performed within two days of sample collection, whole blood collected by venipuncture should be stored at 2- 8°C if the test is to be run within 6 days of collection. Do not freeze whole blood specimens. If stored at 2- 8°C, bring specimen to room temperature before testing. Mix specimen well by gentle inversion of the tube immediately before testing.</p> <ul style="list-style-type: none"> • Whole blood collected by fingerstick should be tested immediately.
D. Handling Precautions:	<ol style="list-style-type: none"> 1. Handle the samples, material contacting samples, and kit controls as if capable of transmitting infection. 2. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when handling patient samples. 3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where samples and kit reagent materials are handled. Avoid any contact between hands, eyes, or mouth during sample collection and testing

4. Reagents and Equipment

A. Reagents and Materials Provided

1. Aluminum ziplock pouch containing Alere Determine™ HIV-1/2 Ag/Ab Combo Cards. Each Card consists of 5 or 10 Test Units which can be separated from each other by tearing along the perforated lines. Each Test Unit has a cover that is to be removed for sample application and visualization of test results.
2. Desiccant Package
3. Chase Buffer: 1 in the 25 Test Units kit, 2 in the 100 Test Units kit. Containing sodium chloride, disodium hydrogen phosphate, and Nipasept as a preservative.
4. Quick Reference Card
5. Package Insert
6. Subject Information Notices: 25 in the 25 Test Units kit, and 100 in the 100 Test Units kit.
7. Customer Letter

8. Disposable Capillary Tubes: For collection and transfer of fingerstick samples. 25 in the 25 Test Units kit, and 100 in the 100 Test Units kits
 9. Disposable Workstations: 25 in the 25 Test Units kit, and 100 in the 100 Test Units kit.
- Alere Determine™ HIV-1/2 Ag/Ab Combo Controls (Catalog #7D2628). Each package contains:
 - HIV-1 p24 Antigen Control: 1.5 mL, HIV-1 viral lysate in defibrinated pooled normal human plasma; negative for antibodies to HIV-1, HIV-2 and HCV; negative for HBsAg.
 - HIV-1 Reactive Control: 1.5 mL, human plasma positive for anti-HIV-1 antibodies, diluted in defibrinated pooled normal human plasma; negative for antibodies to HIV-2 and HCV; negative for HBsAg.
 - HIV-2 Reactive Control: 1.5 mL, human plasma positive for anti-HIV-2 antibodies, diluted in defibrinated pooled normal human plasma; negative for antibodies to HIV-1 and HCV; negative for HBsAg and HIV-1 p24.
 - Nonreactive Control: 1.5 mL, defibrinated normal human plasma; negative for antibodies to HIV-1, HIV-2, and HCV; negative for HBsAg and HIV-1 p24.
 - 40 Disposable Pipettes – for use testing the external controls only. The disposable pipettes are not to be used for testing patient samples.
 - Package Insert

B. Materials Available as an Accessory to the Kit

- Finger stick Sample Collection Kit (Catalog #2604US199). Each Collection Kit contains:
 - 100 Sterile Safety Lancets
 - 100 Adhesive Bandages
 - 100 Ethanol Swabs
 - 100 Gauze Pads

C. Materials Required, but not Provided

- Clock, watch, or other timing device
- Precision pipette capable of delivering 50 µL of sample with disposable tips, to be used in lieu of the disposable capillary tubes supplied with the kit (for other than fingerstick whole blood specimens)
- Disposable gloves
- Sterile gauze (for fingerstick whole blood specimens)
- Antiseptic wipes
- Biohazard disposal container
- Collection devices for specimens (other than fingerstick whole blood specimens)
- Sterile lancet capable of producing 50 µL of blood

D. Storage and Stability

Alere Determine™ HIV-1/2 Ag/Ab Combo Test Cards and Chase Buffer must be stored at 2-30°C (36-86°F) until expiration date.

5. Quality Control

A. Internal Quality Control

To ensure assay validity, a procedural “Control” line containing a mixture of anti-HIV-1 antibody, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody is incorporated in the nitrocellulose membrane. For a test result to be valid there must be a visible pink/red Control line. During the testing procedure the colloidal selenium conjugates released from the Conjugate Pad will be captured by the antibodies and antigens immobilized in the Control Area and form a pink/red Control line for samples that are either positive or negative. *NOTE: A pink/red Control line may appear even when a test sample has not been applied to the Test Unit.*

B. External Quality Control

Alere Determine™ HIV-1/2 Ag/Ab Combo Controls should be tested under the following circumstances:

- *Each new untrained operator prior to performing tests on patient specimens*
- *When opening a new test kit lot*
- *Whenever a new shipment of test kits is received*
- *If the temperature of the test storage area falls outside of 2 to 30°C (36 to 86°F)*
- *If the temperature of the testing area falls outside of 15 to 30°C (59 to 86°F)*
- *At periodic intervals indicated by the testing facility*

Controls should be tested in the same manner as serum or plasma samples in the following Test Procedure.

Good Laboratory Practices (GLP) necessitates testing external control material along with the test samples to ensure proper performance of the test kit. Alere Determine™ Combo HIV-1, HIV-2, p24 Reactive and Nonreactive Controls are available separately for use with Alere Determine™ HIV-1/2 Ag/Ab Combo. The HIV Controls are used to verify proper functioning of the test and the operator’s ability to properly perform the test and to interpret the results.

The HIV-1 and HIV-2 Reactive Controls will produce a REACTIVE test result and have been manufactured to produce a visible Test “Ab” line. The HIV-1 p24 Antigen Control will produce a REACTIVE test result and has been manufactured to produce a visible Test “Ag” line. The Nonreactive Control will produce a NONREACTIVE Test Result. Run the Controls as per the TEST PROCEDURE for serum/plasma samples (the use of Chase Buffer is not required) and interpret results as described in INTERPRETATION OF TEST RESULTS sections of the Product Insert. It is the responsibility of each facility using Alere Determine™ HIV–1/2 Ag/Ab Combo to establish an adequate quality assurance program to ensure the performance of the device under specific locations and conditions of use.

Please refer to the Alere Determine™ HIV-1/2 Ag/Ab Combo Package Insert for pictorial examples of REACTIVE, NONREACTIVE and INVALID Test Results.

6. Precautions

A. Safety Precautions

1. Handle the samples, material contacting samples, and kit controls as if capable of transmitting infection.
2. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when handling patient samples.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where samples and kit reagent materials are handled. Avoid any contact between hands, eyes, or mouth during sample collection and testing.
4. Do not touch the Sample Pad.
5. Decontaminate and dispose of all specimens, reagents, disposable workstations, and other potentially contaminated materials in a biohazard waste container in accordance with local regulations. Lancets should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Liquid wastes may be mixed with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions that contain bleach. The workstations are for single use only. The used workstation and Test Unit should be regarded as potentially infectious material. They should be disposed of together, without trying to remove the Test Unit from the workstation, in a biohazard waste container as indicated above.
6. Clean and disinfect all spills of specimens or reagents using 10% bleach or other appropriate disinfectant. The bleach solution should be made fresh every day.
7. For additional information refer to: Centers for Disease Control and Prevention: Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendation for Post-exposure Prophylaxis (see list of references below).

B. Handling Precautions

1. If Desiccant Packet is missing, DO NOT USE. Discard Test Cards (all Test Units) and use a new Test Card.
2. Do not use any Test Units from Test Cards if the pouch has been perforated.
3. Each Test Unit, lancet and disposable capillary tube for collection and transfer of fingerstick samples is for single use only.
4. Do not use kit components beyond the expiration date printed on the label. Always check expiration date prior to testing.
5. Adequate lighting is required to read a test result.

7. Test Procedure

NOTE: *Alere Determine™ HIV-1/2 Ag/Ab Combo Controls should be tested prior to testing patient specimens when a new untrained operator performs testing, a new test kit lot is to be used, a new shipment of test kits is received, if the temperature of the test storage area falls outside of 2 to 30°C (36 to 86°F), if the temperature of the testing area falls outside of 15 to 30°C (59 to 86°F), and at periodic intervals indicated by the testing facility. Controls should be tested in the same manner as serum or plasma samples in the following Test Procedure.*

Kit Component Preparation

1. Open the aluminum pouch containing the Alere Determine™ HIV-1/2 Ag/Ab Combo Cards.
2. Remove the desired numbers of test units from the 5 or 10-Test Unit Card by bending and tearing at the perforation.
8. *NOTE: Removal of the test units should start from the right side of the Card to preserve the lot number which appears on the left side of the Card.*
3. Return the unused test units to the aluminum pouch and close the pouch with the ziplock.
9. *NOTE: Store the unused cards and test units only in the aluminum pouch containing the desiccant package. Carefully close the ziplock, so that the cards are not exposed to ambient humidity during storage.*
4. Remove the protective foil cover from each Test Unit. Lay the Test Unit flat in the workstation. The test should be initiated within 2 hours after removing the protective foil cover from each Test Unit. Do NOT touch the Sample Pad with your fingers.
10. *NOTE: Use of the workstation is optional. If the workstation is not used, place the Test Unit on a flat surface.*

For serum or plasma samples:

1. Apply 50 µL of sample (precision pipette) by touching the tip of the pipette to the Sample Pad (marked by the arrow symbol). Do not add Chase Buffer when using serum or plasma specimens.
2. Read the test result between 20 and 30 minutes after the addition of the Sample. Do not read Test Results after 30 minutes.

For whole blood (venipuncture) samples:

1. Using a precision pipette with a disposable tip, apply 50 µL of sample by touching the tip of the pipette to the Sample Pad (marked by the arrow symbol).
2. When all of the blood is transferred to the Sample Pad, wait one minute to ensure the Chase Buffer does not overflow the Sample Pad.
3. Add one drop of Chase Buffer to the Sample Pad.
4. Read the test result between 20 and 30 minutes after the addition of Chase Buffer. Do not read Test Results after 30 minutes.

For whole blood (fingerstick) samples using disposable Capillary Tube provided with the kit:

Caution: The Capillary Tube must be used to collect the fingerstick sample.

To optimize whole blood circulation:

- Warm the hand by washing in warm water (or holding it in a heating pad or hand warmer).
- Massage the finger with a downward motion several times before performing the fingerstick. Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet capable of producing 50 µL of blood, puncture the skin just off the center of the finger pad and wipe away the first drop with sterile gauze.

To collect an adequate sample volume:

- Quickly express blood down the fingertip by gently squeezing across the entire finger, to the last joint (not to the end of the fingertip).
- Do not squeeze or “milk” the fingertip to accelerate bleeding.
- Collect the second drop of blood by holding the capillary tube HORIZONTALLY, and touch the tip of the capillary tube to the blood sample.

NOTE: Filling of the capillary is automatic – do NOT squeeze the bulb while sampling. Maintain this position until the flow of the sample has reached the fill line and stopped.

To add sample to the test strip:

1. Touch the tip of the Capillary Tube containing the blood sample to the Sample Pad (marked by the arrow symbol) and gently squeeze the bulb. Avoid air bubbles. Wait until all the blood is transferred from the Capillary Tube to the Sample Pad.

Caution: Do not lift the Capillary Tube from the Sample Pad before all the blood has been transferred—a bubble may form which will prevent the complete transfer of sample. If a sample won't expel, cover the small opening at the mark on the capillary with a gloved finger. Then squeeze the bulb until the sample is fully dispensed onto the Sample Pad.

2. When all of the blood is transferred to the Sample Pad, wait one minute to ensure the Chase Buffer does not overflow the Sample Pad.
3. Add one drop of Chase Buffer to the Sample Pad.
4. Read the test result between 20 and 30 minutes after the addition of the Chase Buffer. Do not read Test Results after 30 minutes.

NOTE: Discard the used pipette tips, Capillary Tube, Test Units and any other test materials into a biohazard waste container.

8. Interpretation of Test Results

NOTE: When testing whole blood samples, a faint pink background may be visible on the test membrane.

ANTIBODY REACTIVE (Two Lines - Control and Ab Line)

A **PINK/RED** Control line appears in the Control Area AND a **PINK/RED** Ab line must appear in the Lower Test Area of the Test Unit. The intensity of the Ab and Control lines may vary. Any visible **PINK/RED** color in both the Control and Lower Test Areas, regardless of intensity, is considered REACTIVE. A Reactive test result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies.

ANTIGEN (HIV-1 p24) REACTIVE (Two Lines - Control and Ag Line)

A **PINK/RED** Control line appears in the Control Area AND a **PINK/RED** Ag line must appear in the Upper Test Area of the Test Unit. The intensity of the Ag and Control lines may vary. Any visible **PINK/RED** color in both the Control and Upper Test Areas, regardless of intensity, is considered REACTIVE. A Reactive test result means that HIV-1 p24 antigen has

been detected in the specimen. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 p24 antigen.

NOTE: A test result that is PRELIMINARY POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV-1 infection in the test subject. In this case the acute HIV-1 infection is distinguished from an established HIV-1 infection in which antibodies to HIV-1 are present.

ANTIBODY REACTIVE AND ANTIGEN (HIV-1 p24) REACTIVE (Three Lines - Control, Ab and Ag Lines)

A **PINK/RED** Control line appears in the Control Area AND a **PINK/RED** Ab line must appear in the Lower Test Area AND a **PINK/RED** Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ab, Ag and Control lines may vary. Any visible **PINK/RED** color in the Control Area, the Lower Test Area and the Upper Test Area, regardless of intensity, is considered REACTIVE. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies and HIV-1 p24 antigen.

NONREACTIVE (One Line – Control Line)

A **PINK/RED** Control line appears in the Control Area of the Test Unit, and no **PINK/RED** Ab or Ag line appears in the Lower Test Area and the Upper Test Area of the Test Unit, respectively. A NONREACTIVE test result means that HIV-1 or HIV-2 antibodies and HIV-1 p24 antigen were not detected in the specimen.

INVALID (No Control Line)

If there is no **PINK/RED** Control line in the Control Area of the Test Unit, even if a **PINK/RED** line appears in the Lower Test Area or the Upper Test Area of the Test Unit, the result is INVALID and the test should be repeated. If the problem persists, contact Alere™ Technical Support.

9. Limitations

1. Alere Determine™ HIV-1/2 Ag/Ab Combo must ONLY be used with capillary (fingerstick) or venous (venipuncture) whole blood, serum or EDTA plasma. Using other types of samples or testing of venipuncture whole blood and plasma samples collected using a tube containing an anticoagulant other than EDTA may not yield accurate results. For serum samples, collect blood without anticoagulant.
2. Alere Determine™ HIV-1/2 Ag/Ab Combo must be used in accordance with the instructions in the Package Insert to obtain accurate results.
3. This assay does not detect or has not been validated to detect HIV-2 antigen.
4. A Reactive result using Alere Determine™ HIV-1/2 Ag/Ab Combo suggests the presence of HIV-1 p24 antigen and/or antibodies to HIV-1 and/or HIV-2 in the sample. The Reactive result is interpreted as Preliminary Positive for HIV-1 p24 antigen and/or antibodies to HIV-1 and/or HIV-2. Alere Determine™ HIV-1/2 Ag/Ab Combo is intended as aid in the diagnosis of infection with HIV-1/2. AIDS-related conditions are clinical syndromes, and their diagnosis can only be established clinically.
5. For a Reactive result, the intensity of the test line does not necessarily correlate with the titer of antigen or antibody in the sample.
6. Reactive test results should be confirmed by additional testing using other tests.

7. A Nonreactive result does not preclude the possibility of exposure to HIV or infection with HIV.
8. A person who has HIV-1 p24 antigen or antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus. However, a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV.
9. This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 12 years of age.
10. Specimens from individuals infected with HIV-1 and/or HIV-2 who are receiving antiretroviral (ART) therapy may produce false negative test results.
11. Specimens from individuals with Toxoplasma IgG, human anti-mouse antibodies, rheumatoid factor, elevated triglycerides (above 600 mg/dL), herpes simplex virus infection, hospitalized and cancer patients may give false positive test results.

10. Performance Characteristics

The clinical performance of Alere Determine™ HIV-1/2 Ag/Ab Combo was established in a prospective clinical study conducted at 11 clinical trial sites located in the United States from 2010 – 2011. In this study, venous whole blood, serum and plasma specimens were evaluated from individuals either known to be infected with HIV-1 as confirmed by FDA-licensed confirmatory assays and/or FDA- approved NAT assays, at low risk for HIV infection, or at high risk for HIV infection. A subset of individuals also provided capillary fingerstick samples for evaluation. In addition, a CLIA waiver study was done where the performance of Alere Determine™ HIV-1/2 Ag/Ab Combo was evaluated in a prospective study conducted at 9 geographically diverse sites located in the United States. At each site, Alere Determine™ HIV-1/2 Ag/Ab Combo was conducted by operators who had no laboratory experience and were representative of users at CLIA waived testing sites (intended use). Refer to the Package Insert for detailed performance characteristics.

11. References

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