REVEAL® G4 RAPID HIV-1 antibody test

Cat. No. 815311007583 – Reveal G4 POC

Cat. No. 815311007576 – Reveal G4 LAB+

Cat. No. 815311000591 – Reveal G4 LAB S/P

2°C 30°C Temperature Limit (2-30°C or 35-85°F)

V For *In Vitro* Diagnostic Use

Complexity: Moderate

This package insert must be read carefully and completely prior to use of Reveal® G4 Rapid HIV-1 Antibody Test (Reveal G4). Instructions must be followed carefully. If directions are not followed exactly, inaccurate test results may occur. Before performing Reveal G4, operators must be 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*.[1]

NAME AND INTENDED USE

Reveal G4 Rapid HIV-1 Antibody Test (Reveal G4) is a single use, qualitative immunoassay to detect antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human whole blood (venipuncture and fingerstick), serum, and plasma. Reveal G4 is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1. This test is suitable for use in multi-test algorithms designed for statistical validation of rapid HIV test results. When multiple rapid tests are available, this test should be used in appropriate multi-test algorithms.

RESTRICTIONS

- Sale of Reveal G4 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.
- Reveal G4 is approved for use only by an agent of a clinical laboratory.
- Test subjects must receive the Subject Information Brochure prior to specimen collection and appropriate information when test results are provided.
- Reveal G4 is not approved for use to screen donors of blood, plasma, cells or tissues.

SUMMARY AND EXPLANATION OF THE TEST

Infection with Human Immunodeficiency Virus (HIV) causes Acquired Immune Deficiency Syndrome (AIDS). Of the two types of HIV (HIV type 1 and HIV type 2), HIV-1 is far more prevalent within North America and in most regions worldwide.[2] HIV is known to be transmitted through contact with the body fluids of an infected individual. Sexual contact, exposure to blood through contaminated syringes and needles, through transfusion, or from an infected mother during the birthing process or breastfeeding are the major modes of HIV transmission.[3]

Infection with HIV-1 and/or HIV-2 elicits an immune response resulting in the production of corresponding anti-HIV antibodies. Antibody detection tests for HIV-1/HIV-2 antibodies provide a means

to aid in the diagnosis of HIV-infected individuals.[4,5] However, when utilizing HIV antibodies to diagnose HIV infection, corresponding clinical factors must also be considered. Following a recent exposure to HIV, it may take several months for the antibody response to reach detectable levels, during which time testing for antibodies to HIV will not be indicative of true infection status. On the other hand, newborns of HIV-infected mothers may carry maternal antibodies to HIV for up to eighteen months, which may not necessarily indicate the true infection status of the newborn.[4]

Conventional laboratory testing for antibodies to HIV utilizes enzyme immunoassays (EIAs), followed by confirmation of repeatedly reactive EIAs using supplemental tests such as the Western blot test, both of which are complex, multi-step procedures. Rapid immunoassay technology has proven to be extremely useful in the diagnosis of infection and is widely utilized as a screening tool. Although use of an EIA screening test is well-suited for batch testing, the turnaround time could be several days to a few weeks. Additionally, the complexity and cost of EIA screen testing and the required equipment may prohibit its universal utilization in medical settings with limited resources and personnel.[6]

Rapid, less complex HIV testing could improve the delivery of medical care and HIV prevention services with substantial time and cost savings.[6,7] Realizing the utility of rapid tests, the World Health Organization (WHO) recommends the use of alternative testing strategies using rapid and simpler HIV tests.[5] Similar recommendations were made by the United States Centers for Disease Control and Prevention (CDC) upon determining that large numbers of patients tested for HIV using conventional methods did not return to the medical facility to obtain test results.[8] From a public health perspective, this high non-return rate has great implications for the health and welfare of an HIV- infected individual and his/her contacts.[7,8] Reveal G4 is a rapid, flow-through diagnostic immunoassay developed to utilize the performance characteristics of a conventional diagnostic immunoassay while simplifying the test procedure to eliminate the requirement for expensive equipment and highly trained personnel and decrease turnaround time.

BIOLOGICAL PRINCIPLES OF THE TEST

Reveal G4 is a manually performed, visually interpreted, rapid vertical flow immunoassay.

Reveal G4 is comprised of a single-use, leak-proof plastic test cartridge containing an immunoreactive test membrane. The immunoreactive test membrane is comprised of a combination of synthetic peptides corresponding to conserved regions of HIV structural proteins coated onto a membrane matrix, which functions to capture anti-HIV-1 antibodies present in human whole blood (venipuncture and fingerstick), serum, and plasma when a drop of the specimen is applied. In addition, the test membrane has a procedural and reagent Control Line comprised of protein A.

Following the application of the sample, captured anti-HIV-1 antibodies are visualized through a reaction with the InstantGold™ cap, a plastic cap housing a filter medium impregnated with a proprietary protein A-colloidal gold conjugate which reacts to form color in the test and control regions so that the test result can be visualized. Universal Buffer, a solution composed of Tris-buffered saline, lysing agents, synthetic polymers and anti-microbial agents (Preservative: 0.05% Proclin 950) is used in the test procedure.

A Reactive test result occurs only when the protein A portion of the conjugate binds to the captured antibodies, producing a distinctive red dot in the test (T) zone and a vertical red Control Line in the

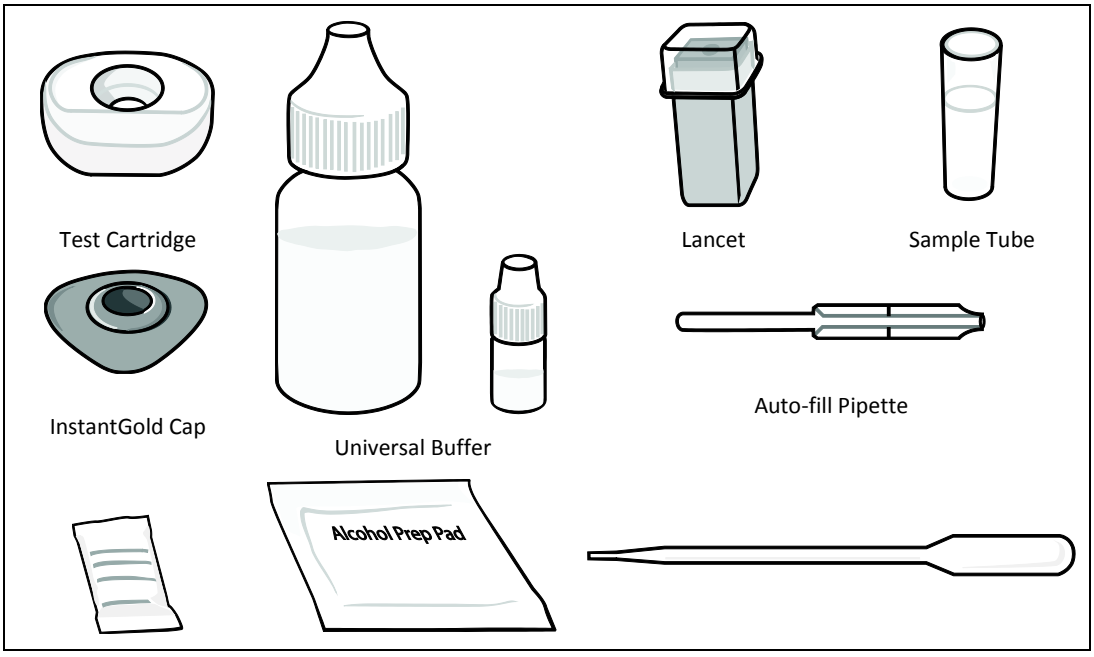
control (C) zone of the test membrane upon completion of the test procedure. In contrast, a Non-Reactive test result, due to the absence of the HIV-1 antibody/antigen complex, is indicated by the presence of only the vertical red Control Line on the test membrane. If the vertical red Control Line is not present, or is incomplete, the test result is considered invalid and testing must be repeated with a new cartridge (refer to TEST RESULTS AND INTERPRETATION OF RESULTS section below).

The test results are to be read and interpreted immediately following completion of the test procedure. Precision pipetting or specialized equipment are not required to perform Reveal G4.

MATERIALS PROVIDED

Reveal G4 POC **Cat. No. 815311007583** *For Fingerstick Whole Blood*	**Reveal G4 LAB+** **Cat. No. 815311007576** *For Venipuncture Whole Blood/Serum/Plasma*	**Reveal G4 LAB S/P** **Cat. No. 815311000591** *For Serum/Plasma*
20 Mylar pouches each containing: 1 test cartridge 1 InstantGold cap 1 silica gel packet 1 auto-fill pipette 1 sample tube 1 vial Universal Buffer 1 lancet (sterile) 1 alcohol swab 1 test tray 1 package insert 20 Subject Information Brochures 1 Customer Letter	50 Mylar pouches each containing: 1 test cartridge 1 InstantGold cap 1 silica gel packet 2 bottles Universal Buffer 50 transfer pipettes 50 sample tubes 1 package insert 50 Subject Information Brochures 1 Customer Letter	50 Mylar pouches each containing: 1 test cartridge 1 InstantGold cap 1 silica gel packet 2 bottles Universal Buffer 50 transfer pipettes 1 package insert 50 Subject Information Brochures 1 Customer Letter

Test Components



Silica Gel Packet	Alcohol Swab	Transfer Pipette

MATERIALS REQUIRED AND AVAILABLE AS AN ACCESSORY

HIV-1 Antibody Test Controls, Cat. No. 815311007590: Each box contains 1 Mylar pouch with 1 HIV-1 Positive Test Control, 1 HIV-1 Negative Test Control, and 1 silica gel packet, 10 transfer pipettes, 1 vial Reconstitution Buffer and 1 package insert.

ADDITIONAL COMPONENTS AVAILABLE

Universal Buffer, Cat. No. 815311007606: Additional bottles of Universal Buffer may be purchased, subject to availability.

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment such as disposable gloves, laboratory coat, and eye protection
- Absorbent cotton for fingerstick or venipuncture wound closure
- Permanent marking pen
- Appropriate biohazard waste containers and disinfectants

WARNINGS

V For *In Vitro* Diagnostic Use

- Read the package insert completely and carefully prior to use of Reveal G4. If the directions are not followed exactly, inaccurate test results may occur.
- Before performing Reveal G4, operators must be familiar with *Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health Care Settings*. [1]
- The United States Food and Drug Administration has approved this test for use with human whole blood (venipuncture and fingerstick), serum, and plasma specimens only. Use of this test with specimens other than those specifically approved for use with Reveal G4 may result in inaccurate test results.
- Perform Reveal G4 at room temperature (15-27°C, 60-80°F).
- Perform Reveal G4 on a flat work surface to ensure that reagents and specimens uniformly flow through the test device.

PRECAUTIONS

Safety Precautions

- Handle all specimens, HIV-1 Antibody Test Controls, and materials contacting specimens as if capable of transmitting infectious agents. It is recommended that all specimens and test reagents be handled in accordance with biosafety containment level 2 practices as described in *Canadian Laboratory Biosafety Standards & Guidelines*, the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*, the WHO *Biosafety Manual*, or the CDC Universal Precautions.[9,10,11,12]
- Do not smoke, eat, or drink in areas where specimens or test reagents are handled. Do not pipette by mouth.

- Wear disposable gloves, laboratory coat and eye protection throughout the test procedure. Change gloves and wash hands thoroughly after performing each test. Dispose of used gloves in a biohazard waste container.
- Dispose of all test specimens and materials used in the test in a biohazard waste container. The recommended method of disposal is autoclaving for a minimum of 1 hour at 121°C or by incineration. Add an equal volume of freshly prepared 5% sodium hypochlorite solution (household bleach) to liquid waste and allow it to soak for at least 1 hour for disinfection. Do not autoclave solutions that contain bleach.
- Wipe spills promptly with a 1% sodium hypochlorite solution (five-fold v/v dilution of household bleach, prepared fresh daily) or other appropriate disinfectant.[14] Contaminated materials should be disposed of in a biohazard waste container.
- 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*[1] and *Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis*.[14]

Handling Precautions

- Use test components only once, excluding bottles of Universal Buffer in LAB+ and LAB S/P products, and dispose of properly (see Safety Precautions). Do not reuse these components.
- Do not use Reveal G4 or any of its components beyond the expiration date. The expiration date can be found on the test pouch or box. Always check the expiration date prior to testing. If Reveal G4 is expired, discard and obtain a new Reveal G4 test.
- Do not interchange InstantGold caps, Universal Buffer or test devices from different lots.
- Exercise care in handling test components and do not touch the immunoreactive test membrane to prevent contamination.
- Adequate lighting is required to read the test result.

STORAGE INSTRUCTIONS

- Store Reveal G4 tests in a dry area at 2-30°C (35-85°F).
- Keep the Mylar pouches sealed until immediately prior to use. Ensure that the Mylar pouch is intact prior to opening. If the pouch is not intact, discard and obtain a new pouch.
- If tests and reagents are stored at refrigerated temperatures, allow all test components and specimens to equilibrate to room temperature (15-27°C, 60-80°F) for 30-60 minutes prior to opening the packages.

SPECIMEN HANDLING/COLLECTION AND USE

Provide the *Subject Information Brochure* to the test subject prior to specimen collection.

Serum and Plasma (Cat. No. 815311007576 or Cat. No. 815311000591)

1. Plasma obtained using EDTA, heparin, or sodium citrate as anticoagulant is suitable for testing.

2. Fresh serum and plasma specimens may be tested immediately upon receipt or stored at 2-8°C (35-45°F) for up to five (5) days prior to testing. Serum and plasma specimens should be stored at -20°C (-4°F) or below if storage is necessary for more than five (5) days.
3. Particulate matter can block the test membrane or cause high background color making interpretation of results difficult. Cloudy or viscous specimens should not be used for testing.
4. If serum or plasma specimens are to be shipped, dispatch by the fastest means available. Package specimens in compliance with statutory regulations governing transportation of dangerous goods.
5. Serum or plasma specimens may be shipped overnight at ambient temperature. However, if the transit time is expected to exceed 24 hours and/or the ambient temperature is >35°C (95°F), specimens should be shipped at 2-8°C (35-45°F).
6. For serum or plasma that has been previously frozen:
    a. Thaw completely at room temperature (15-27°C, 60-80°F) and mix thoroughly by gently tapping the bottom of the capped tube.
    b. Centrifuge an aliquot of the specimen in a small, capped tube at room temperature (15-27°C, 60-80°F) at 3361 g (radius of rotor 8.35 cm = 6000 rpm) for at least five (5) minutes and use only the clear supernatant for testing.
7. Avoid multiple freeze-thaw cycles. Serum and plasma specimens should not be frozen and thawed more than twice prior to use with Reveal G4.
8. Proceed to GENERAL TEST PREPARATION.

Venipuncture Whole Blood (Cat. No. 815311007576)

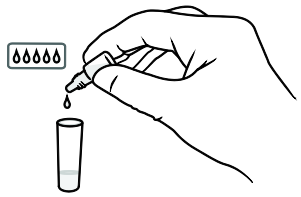
1. Use standard venous phlebotomy procedures to collect a whole blood sample in a tube containing K_2EDTA anticoagulant. If specimens are not tested at the time of collection, they may be stored at 2-8°C (35-45°F) for up to five (5) days prior to testing. Prior to testing, mix the blood by gentle inversion several times.*
2. Place the sample tube in a secured rack on a flat surface and add five (5) drops from the bottle of Universal Buffer to the sample tube.
3. Using the transfer pipette provided, collect whole blood from the specimen collection tube. Add one (1) drop of whole blood to the sample tube prepared in Step 2.
4. Hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish color.
5. Proceed to GENERAL TEST PREPARATION.

*If storage is necessary for over five (5) days, plasma should be separated from the whole blood specimen and the plasma should be stored at -20°C (-4°F) or below.

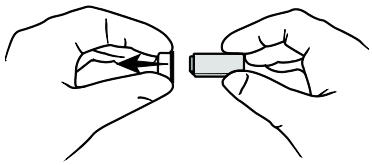
Fingerstick Whole Blood (Cat. No. 815311007583)

1. Place the sample tube into the hole of the test tray.

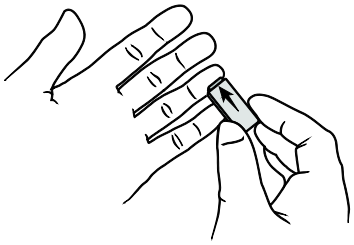
2. Add five (5) drops from the vial of Universal Buffer to the sample tube.



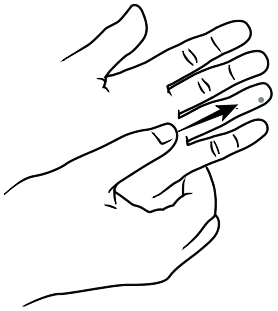
3. Using an alcohol swab, clean the index finger. Allow the finger to dry thoroughly.
4. Remove the protective cap from the sterile lancet provided with the test. Do not use lancet if damaged.



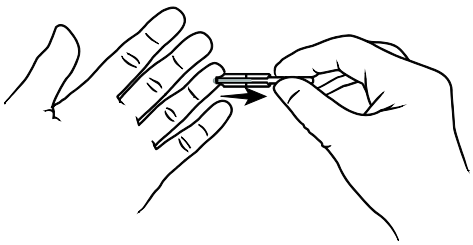
5. Firmly press the lancet against the puncture site to activate the device and puncture the skin.



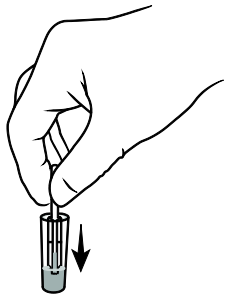
6. Point the lanced finger downward, apply gentle pressure massaging the lanced finger vertically from the base of the finger towards the lanced fingertip to form a drop of blood. Avoid squeezing the fingertip to make it bleed.



7. Use the auto-fill pipette provided to collect a drop of blood from the fingerstick site. To do this, touch the tip of the pipette to the blood sample in a horizontal position. The blood sample will be automatically drawn to the black fill line and stop. Do not squeeze the pipette bulb during filling.



8. Place the tip of the auto-fill pipette into the Universal Buffer in the sample tube (prepared in Step 2). Squeeze the bulb to empty the blood sample into the tube. Discard the auto-fill pipette.



9. Hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish color.

10. Proceed to GENERAL TEST PREPARATION

GENERAL TEST PREPARATION

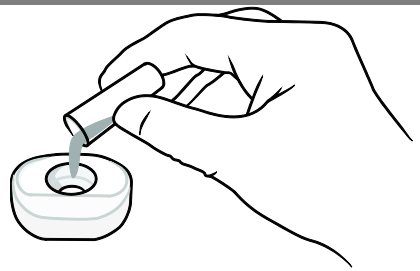
1. Allow all test components and specimens to equilibrate to room temperature (15-27°C, 60-80°F) for 30-60 minutes prior to opening the container or Mylar pouch.
2. Using the notched corners, tear open the required number of Mylar pouches.
    a. Ensure that a silica gel packet is present in each pouch. If the silica gel packet is not present, discard that pouch and all of its contents and open a new pouch.
    b. Inspect each test cartridge to ensure that a faint blue line is visible in the Control zone (under the C on the test cartridge). If this blue line is not visible, discard that pouch and all of its contents and open a new pouch.
    c. Inspect each InstantGold cap to ensure that the blue plastic casing is snapped securely around the rose-colored filter medium. If this is not the case, discard that pouch and all of its contents and open a new pouch.
3. Align the test cartridges in front of the specimens to be tested. Label test cartridges on the white plastic casing with a permanent marking pen. DO NOT LABEL OR MAKE ANY MARKS ON THE IMMUNOREACTIVE TEST MEMBRANE.
4. Proceed to TEST PROCEDURE.

TEST PROCEDURE

- **All solutions must be completely absorbed into the test membrane before proceeding to the next step in the test procedure.**
- **Once the assay has been started, all subsequent steps should be completed without interruption.**
- **Perform the test on a flat work surface to ensure that reagents and specimens uniformly flow through the test device.**
- **Hold the Universal Buffer bottle at a slight angle from vertical when dispensing drops of buffer.**
- **Do not let the buffer bottle drop tip touch the immunoreactive membrane.**
- **Read the test results immediately. Failure to do so may result in inaccurate test results.**
- **Follow CDC guidelines to inform the test subject of the test result and its interpretation.** [14]
- **After recording test results, dispose of test materials in biohazard waste container.**

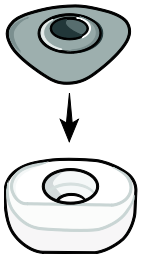
WHOLE BLOOD PROCEDURE - (Cat. No. 815311007583 or 815311007576)

1



Pour the entire contents of the sample tube into the center of the test cartridge. Allow the specimen to absorb completely.

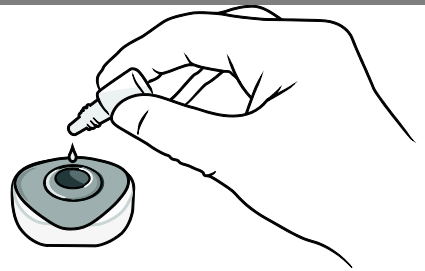
2



Place the InstantGold cap on the test cartridge.

Select the catalog number that corresponds to the test format being used and proceed to the next step.

3 – Cat. No.815311007583 (POC)



Dispense the remaining buffer form the vial of Universal Buffer onto the InstantGold cap and allow the solution to absorb completely.

Remove the InstantGold cap and wait for the solution to absorb completely.

Read test results immediately.

OR

3 – Cat. No.815311007576 (LAB+)

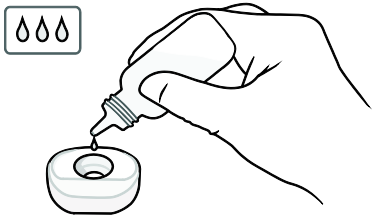
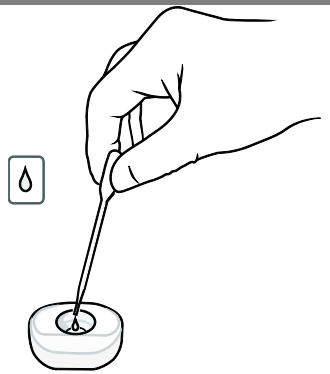
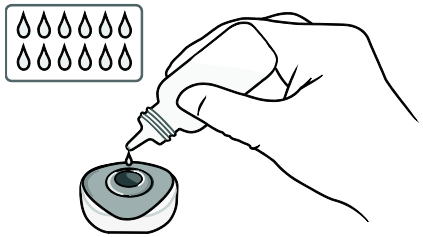
Dispense twelve (12) drops of Universal Buffer onto the InstantGold cap, wait for the solution to absorb completely.

Remove the InstantGold cap and wait for the solution to absorb completely.

Add three (3) drops of Universal Buffer to clarify results.

Read test results immediately.

SERUM/PLASMA PROCEDURE - (Cat. No. 815311007576 or 815311000591)

1	2	3
		
Apply three (3) drops of Universal Buffer to the center of the test cartridge. Allow the buffer to absorb completely.	Apply one (1) drop of serum or plasma specimen to the center of the test cartridge. Allow the specimen to absorb completely.* Apply three (3) drops of Universal Buffer to the center of the test cartridge. Allow the buffer to absorb completely. Place the InstantGold cap on the test cartridge. *If the serum or plasma specimen is not absorbed within 30 seconds, centrifuge an aliquot of the specimen in a small, capped tube at room temperature (15-27°C, 60-80°F) at 3361g (radius of rotor 8.35cm=6000 rpm) for at least five (5) minutes. Test the clear supernatant using a new test cartridge. If slow absorption persists after centrifugation, the specimen may not be suitable for use.	Dispense twelve (12) drops of Universal Buffer onto the InstantGold cap, wait for the solution to absorb completely. Remove the InstantGold cap and wait for the solution to absorb completely. Optional – Add three (3) drops of Universal Buffer to clarify results. Read test results immediately.

QUALITY CONTROL

Built-in Control Features

Reveal G4 includes a built-in procedural and reagent Control Line that demonstrates the validity of the test procedure and reagent function. A vertical red line under the "C" (Control Area) on the test cartridge indicates that specimen has been added to the test cartridge, and that the test reagents are functioning properly. The Control Line will appear on all valid tests, regardless of whether the test result is Reactive or Non-Reactive (see Test Results and Interpretation of Results section below).

External Quality Control

HIV-1 Antibody Test Controls (Cat. No. 815311007590) are available separately for use only with Reveal G4. The test controls are used to monitor proper test performance. The Positive Test Control and the Negative Test Control are to be run using separate test cartridges. The Positive Test Control will produce a Reactive test result indicated by both the red dot in the test zone beside the T on the test and a vertical red Control Line under the C on the test upon completion of the test procedure. The expected test result using the Positive Test Control may be less intense than test results obtained using clinical specimens. In contrast, a Non-Reactive test result is obtained with the Negative Test Control and is indicated by the presence of only the vertical red Control Line under the C on the test. Use of test controls manufactured by other any other source may not produce the required results, and therefore would not meet the requirements for an adequate quality assurance program for Reveal G4.

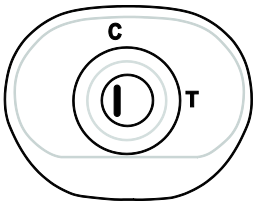
Run the HIV-1 Antibody Test Controls under the following circumstances:

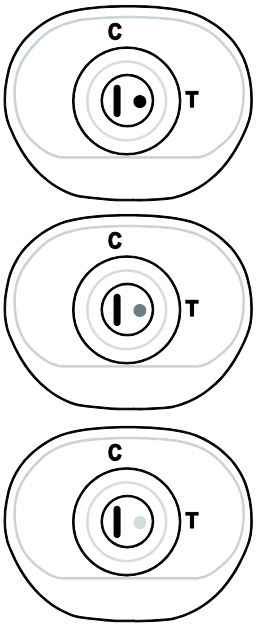
- **With each new operator prior to performing testing on patient specimens.**
- **When beginning testing with a new lot of test devices.**
- **On each new shipment of tests received.**
- **If the temperature in the storage area for the tests falls outside of the 2-30°C (35-85°F) range.**
- **If the temperature in the testing area falls outside of the 2-30°C (35-85°F) range.**
- **At periodic intervals as required by the user facility.**

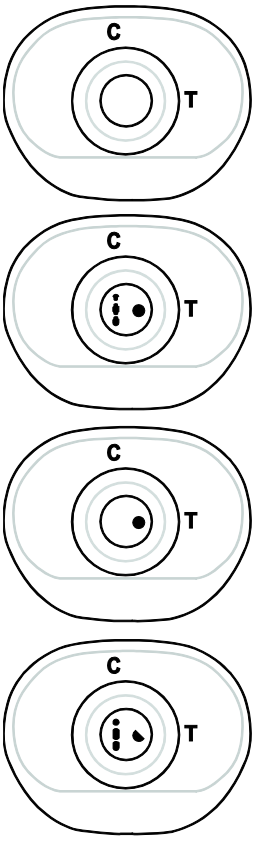
Refer to the HIV-1 Antibody Test Controls package insert for additional information on the use of these reagents. It is the responsibility of each laboratory using Reveal G4 to establish an adequate quality assurance program to ensure the proper performance of the device under its conditions of use. Contact MedMira's Customer Service Department if the HIV-1 Antibody Test Controls do not produce the expected results.

TEST RESULTS AND INTERPRETATION OF RESULTS

NON-REACTIVE Probable Non-Exposure to HIV	REACTIVE Probable Exposure to HIV	INVALID
The diagram below is an example of a Non-Reactive test result. The presence of a vertical red Control Line under the C and the absence of a red dot next to the T on the test indicate that anti-HIV-1 antibodies were not detected. The test result is interpreted as NEGATIVE for HIV-1 antibodies. A uniform, faint pinkish background may be visible on the test membrane.	The diagrams below are examples of a Reactive test result. The presence of both a vertical red Control Line under the C and a red dot next to the T on the test indicate that anti-HIV-1 antibodies have been detected in the specimen. The intensity of the line and the dot may vary. Any visible dot next to the T must be considered to be a Reactive result, regardless of how faint the dot appears.	The diagrams below are examples of an Invalid test result. The absence of the vertical red Control Line, or the presence of a broken line under the C, even if there is a red dot beside the T, indicates that there has been a problem, either with the test device or the specimen, during the Test Procedure. An Invalid test result cannot be interpreted. If an Invalid test result is obtained, the Test Procedure should be repeated using a new test and specimen.







LIMITATIONS OF THE TEST

1. Reveal G4 must be used in accordance with this package insert to ensure accurate results.
2. The FDA has approved Reveal G4 for use with fingerstick whole blood, venipuncture whole blood, serum, and plasma specimens only. Use of other types of specimens may not yield accurate results.
3. Test results are to be read and interpreted immediately following completion of the Test Procedure. A delay in reading test results may yield inaccurate results.
4. Specimens (including hemolyzed specimens) that, after centrifugation, do not pass through the membrane within thirty (30) seconds, (see SERUM/PLASMA TEST PROCEDURE, step 2) are unsuitable for testing with Reveal G4.
5. Lipemic samples or specimens contaminated with bacteria may not pass through the membrane within thirty (30) seconds, and therefore may be unsuitable for testing with Reveal G4.
6. Limited studies were conducted to determine the potential effect of interfering substances and unrelated medical conditions on the performance of Reveal G4.
7. The specificity of Reveal G4 for serum specimens in low-risk populations has not been evaluated.
8. Limited studies were conducted to determine the performance of Reveal G4 on fresh serum and plasma specimens.
9. A Reactive test result using Reveal G4 suggests the presence of anti-HIV-1 antibodies in the specimen. Reveal G4 is intended to be used as an aid in the diagnosis of infection with HIV-1. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. Results of Reveal G4 should not be used in isolation, but in conjunction with the clinical status, history, and risk factors of the individual being tested.
10. The intensity of the red dot (Reactive test result) does not necessarily correlate with the antibody titer of the specimen.
11. A person who has antibodies to HIV-1 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. Clinical correlation is indicated with appropriate counselling, medical evaluation, and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.
12. A Non-Reactive test result with Reveal G4 indicates the absence of detectable antibodies to HIV in the specimen. However, a Non-Reactive test result does not exclude the possibility of exposure to, or infection with HIV. Following a recent exposure to HIV, it may take several months for the antibody response to reach detectable levels, during which time testing for antibodies to HIV will not be indicative of true infection status. A comprehensive risk history and clinical evaluation should be considered before concluding that an individual is not infected with HIV.

PERFORMANCE CHARACTERISTICS

Note: Test performance was evaluated using the Reveal Rapid HIV-1 Antibody Test. Additional studies have demonstrated that Reveal G4 performs equivalently.

SENSITIVITY

Serum Specimens

Sensitivity studies were performed using repository HIV-1 antibody positive serum specimens from individuals known to be infected with HIV-1 and freshly collected serum specimens from routine clinical settings in high-risk populations at four clinical sites. Serum specimens were obtained from 483 individuals known to be infected with HIV-1, as well as 2914 serum specimens from previously unscreened individuals from high-risk populations. All 483 serum specimens from known HIV-1 antibody positive individuals that were Repeatedly Reactive using an FDA-licensed HIV-1/2 EIA and were HIV-1 antibody Western blot positive gave Reactive results with the Reveal Rapid HIV-1 Antibody Test. Of the 2914 previously unscreened serum specimens from individuals from a high-risk population, 124 were Repeatedly Reactive using an FDA-licensed HIV-1/2 EIA, 123 were confirmed positive by Western blot (1 indeterminate result), and 122 were Reactive using the Reveal Rapid HIV-1 Antibody Test. The results of these studies are shown in Table 1A.

Table 1A. Detection of Anti-HIV-1 Antibodies in Serum Specimens Obtained From Known HIV-1 Antibody Positive Individuals and Routine Clinical Settings in HIV High-Risk Populations

Test Group	Total Samples	Reveal Reactive	Licensed EIA Repeatedly Reactive	Western Blot Positive
Known HIV-1 Antibody Positive	483	483	483	483
High-Risk Population	2914	122	124*	123
Total	3397	605	607	606

* One specimen was Western blot indeterminate.

The overall sensitivity of the Reveal Rapid HIV-1 Antibody Test for the detection of anti-HIV-1 antibodies in serum specimens in these studies was calculated to be 605/606 = 99.8% (95% confidence interval; 99.2-100%), combining the number of Reveal Rapid HIV-1 Antibody Test Reactive results obtained from the study of known HIV-1 antibody positive serum specimens with the number of Reveal Rapid HIV-1 Antibody Test Reactive results obtained from the studies of high-risk populations.

Plasma Specimens

Sensitivity studies were performed using repository HIV-1 antibody positive plasma specimens from individuals known to be infected with HIV-1 and repository plasma specimens from clinically diagnosed AIDS patients. Plasma specimens were obtained from 397 individuals known to be infected with HIV-1, as well as 107 specimens from clinically diagnosed AIDS patients from one clinical site. All 397 plasma specimens were Repeatedly Reactive using an FDA-licensed HIV-1/2 EIA, 395 were Western blot positive (2 Western blot indeterminate) and 395 gave Reactive results with the Reveal Rapid HIV-1 Antibody Test. Of the 107 specimens from clinically diagnosed AIDS patients, 107 were Repeatedly Reactive using an FDA-licensed HIV-1/2 EIA, 104 were positive by Western blot (3 Western blot indeterminate) and 103 were Reactive using the Reveal Rapid HIV-1 Antibody Test. The results of these studies are in Table 1B.

1B. Detection of Anti-HIV-1 Antibodies in Plasma Specimens Obtained From Known HIV-1 Antibody Positive Individuals and Clinically Diagnosed AIDS Patients in Routine Clinical Settings

Test Group	Total Samples	Reveal Reactive	Licensed EIA Repeatedly Reactive	Western Blot Positive
Known HIV-1 Antibody Positive	397	395	397*	395
Clinically Diagnosed AIDS Patients	107	103	107**	104
Total	504	498	504	499

*Two specimens were Western blot indeterminate, ** Three specimens were Western blot indeterminate.

The overall sensitivity of the Reveal Rapid HIV-1 Antibody Test for the detection of anti-HIV-1 antibodies in plasma specimens in these studies was calculated to be 498/499 = 99.8% (95% confidence interval; 99.0-100%), combining the number of Reveal Rapid HIV-1 Antibody Test Reactive results obtained from the study of known HIV-1 antibody positive plasma specimens with the number of Reveal Rapid HIV-1 Antibody Test Reactive results obtained from clinically diagnosed AIDS patients.

Whole Blood Specimens

A sensitivity study was performed at four US clinical trial sites using freshly obtained matching fingerstick whole blood, K_2-EDTA whole blood, and K_2-EDTA plasma specimens collected from 536 known HIV-1 positive individuals and from 557 individuals at high risk for HIV-1 infection. The mean age of study participants was 41.85 ± 12.42 years; 69.11% of participants were male and 30.89% were female. From ethnicity and race data collected at enrollment, 39.55% of subjects were Hispanic or Latino (60.45% were Non-Hispanic); 31.16% were African American, 27.97% were Caucasian, 7.58% reported being of mixed race, and the remaining 33.29% reported other race categories. Reveal G4 sensitivity was assessed in fingerstick and venipuncture K_2-EDTA whole blood specimens, while the matched K_2-EDTA plasma specimens were used to complete the FDA approved anti-HIV-1 reference testing algorithm.

Fingerstick Whole Blood Specimens

Sensitivity studies were performed using fingerstick whole blood specimens collected from 536 individuals known to be infected with HIV-1 and 556 individuals from populations at high risk for HIV-1 infection. Of the 536 specimens from known HIV-1 positive individuals, 528 were confirmed positive by HIV-1 Western blot (two were indeterminate). Of the 528 samples that were confirmed anti-HIV-1 positive, 526 were Reactive with Reveal G4. Two specimens were false Non-Reactive with Reveal G4. Within the high risk group, 27 out of the 556 specimens were confirmed to be anti-HIV-1 positive, and of these 27 were Reactive with Reveal G4. One additional fingerstick specimen from the high risk population was Reveal G4 false reactive. The results of these studies are shown in Tables 1C and 1D.

Table 1C. Detection of Anti-HIV-1 Antibodies in Fingerstick Whole Blood Specimens Obtained From Known HIV-1 Positive Individuals and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	Reveal G4 Result (Fingerstick)			Reference Test Result			
		Reactive	Non-Reactive	Invalid[1]	Reactive[2]	Non-Reactive	Indeter-minate	True Positive[2]
Known HIV-1 Antibody Positive	536	528[3]	8[4]	0	528	6	2	528
At-Risk of HIV Infection	556	28[5]	526	2	27	529	0	27

Total	1092	556[3]	534	2	555	535	2	555

[1] Invalid results were not included in the calculation of sensitivity. The two specimens which gave invalid results on the Reveal G4 device were Non-Reactive on the reference test.

[2] Confirmed by licensed HIV-1 Western Blot. Subjects that had indeterminate Western blot results were eliminated from the analysis, since true anti-HIV-1 status could not be determined.

[3] The two subjects with Western blot indeterminate results were Reactive on Reveal G4, but were excluded from the analysis as their true anti-HIV-1 status could not be determined.

[4] There were two false Non-Reactive results noted; both from known HIV-positive individuals. Both of these individuals reporting taking multi-therapy concomitant antiretroviral medication.

[5]One specimen from an individual in the At Risk group was false Reactive with Reveal G4.

Table 1D. Results for Fingerstick Whole Blood Specimens from Known HIV-1 Positive Individuals and Individuals at High Risk of HIV Infection

		Reference Test Result		
Reveal G4 Test Result (Fingerstick)	**Reactivity**	True Positive	Negative	TOTAL
	Reactive[1]	553	1	554
	Non-Reactive[2]	2	532	534
	Invalid	0	2	2
	TOTAL	555	535	1090

[1] The one specimen that gave a false Reactive result on Reveal G4 was from an individual at risk of HIV infection.

[2] The two false Non-Reactive specimens on Reveal G4 were from known HIV-1 positive individuals. Both were taking multi-therapy concomitant antiretroviral medication.

The overall sensitivity of the Reveal G4 Rapid HIV-1 Antibody Test in fingerstick whole blood specimens for the confirmed anti-HIV-1 positives from the combined high risk and known HIV-positive populations was calculated to be 553/555 = 99.64% (95% CI = 98.70 – 99.96).

Venipuncture Whole Blood Specimens

Sensitivity studies were performed using venipuncture whole blood specimens collected from 536 individuals known to be infected with HIV-1 and 557 individuals at high risk for HIV-1 infection. Of the 536 venipuncture specimens collected from known HIV-1 positive individuals, 528 were confirmed anti-HIV-1 positive by the FDA approved reference method (HIV-1 Western blot positive) and a further two were HIV-1 Western blot indeterminate. Of these, 526 were Reactive with Reveal G4. Two specimens were false Non-Reactive with Reveal G4. There were two invalid results obtained, both of which were from HIV-1 positive subjects; these were eliminated from the sensitivity calculation. Within the high risk group, 27 specimens were confirmed anti-HIV-1 positive; of those, 26 were Reactive with Reveal G4. One specimen was false Non-Reactive with Reveal G4. The results of these studies are in Tables 1E and 1F.

Table 1E. Detection of Anti-HIV-1 Antibodies in Venipuncture Whole Blood Specimens Obtained from Known HIV-1 Positive Individuals and Individuals at High Risk of HIV Infection

Test Group	Total Specimens	Reveal G4 Result (Venipuncture)			Reference Test Result			
		Reactive	Non-Reactive	Invalid[1]	Reactive[2]	Non-Reactive	Indeter-minate	True Positive[2]
Known HIV-1 Antibody Positive	536	526[3]	8[4]	2	528	6	2	528
At-Risk of HIV Infection	557	26	531	0	27	530	0	27

Total	1093	552	539	2	555	536	2	555

[1] Although there were eight initially invalid test results with Reveal G4, when repeat testing was performed per protocol, only 2 invalid results remained.

[2] Confirmed by licensed HIV-1 Western Blot. Subjects that had indeterminate Western blot results were eliminated from the analysis, since true anti-HIV-1 status could not be determined.

[3] The two subjects with Western blot indeterminate results were Reactive on Reveal G4, but were excluded from the analysis as their true anti-HIV-1 status could not be determined.

[4] Of the three false Non-Reactive results observed, two were from known HIV-positive individuals. The third false Non-Reactive result was from an individual at risk of HIV infection; the site noted that this could have been an operator error, as the operator first noted the test result as difficult to read but Non-Reactive but then changed the result to invalid. A second operator retested the specimen and a Reactive result was obtained.

Table 1F. Results for Venipuncture Whole Blood Specimens from Known HIV-1 Positive Individuals and Individuals at Risk of HIV Infection

		Reference Test Result		
Reveal G4 Test Result (Venipuncture)	**Reactivity**	True Positive	Negative	TOTAL
	Reactive	550	0	550
	Non-Reactive	3[1]	536	539
	Invalid	2	0	2
	TOTAL	555	536	1091

[1] Of the 3 false Non-Reactive specimens on Reveal G4, two were from known HIV-positive individuals who reported concomitant multi-therapy antiretrovirals. The third false Non-Reactive case may have been an instance of operator error; the operator initially scored the result as difficult to read but Non-Reactive, and subsequently changed the result to Invalid. A second operator re-tested the specimen and a Reactive result was observed.

The overall sensitivity of the Reveal G4 Rapid HIV-1 Antibody Test for the detection of anti-HIV-1 antibodies in venipuncture whole blood specimens for the confirmed HIV-1 positives from the combined High Risk and Known HIV-1 Positive populations was calculated to be 550/553 = 99.46% (95% CI = 98.42 – 99.89).

Sensitivity of the Reveal Rapid HIV-1 Antibody Test in the Detection of HIV-1 from Various Geographic Regions

The sensitivity of the Reveal Rapid HIV-1 Antibody Test for the detection of antibodies to HIV-1 Group M subtypes (A,B,C,D,E,F,G) from various geographic regions was assessed by testing 1026 confirmed HIV-1 antibody positive serum and plasma specimens obtained from various parts of the world. Of these 1026 specimens, 1024 were Reactive using the Reveal Rapid HIV-1 Antibody Test. Two confirmed HIV-1 antibody positive specimens from Canada were Non-Reactive using the Reveal Rapid HIV-1 Antibody Test.

Reactivity with Seroconversion Panels

Seven seroconversion panels were tested in comparison to a licensed anti-HIV-1/2 EIA. Each panel consisted of a series of sequential specimens obtained from a single individual undergoing seroconversion. Five of the seven panels were obtained from a commercial source, while the remaining 2 were from clinical settings. The seven seroconversion panels consisted of 36 specimens. In this study, the Reveal Rapid HIV-1 Antibody Test detected seroconversion similarly to the FDA-licensed HIV-1/2 EIA (Table 2).

Table 2. Performance of the Reveal Rapid HIV-1 Antibody Test with Seroconversion Panels

Specimen Information		Reveal Test	Licensed anti-HIV-1,2 EIA
Panel	Relative Day of Bleed		
AF	1	NR	NR
	3	NR	NR
	8	NR	NR
	10	NR	NR
	16	R	NR
	29	R	RR
	34	R	RR
	36	R	RR
	43	R	RR
D	1	NR	NR
	22	NR	NR
	50	R	NR
	93	R	RR
	100	R	RR
H	1	NR	NR
	8	NR	NR
	13	NR	NR
	20	NR	NR
	27	NR	NR
	29	R	RR
M	1	NR	NR
	23	R	RR
E	1	NR	NR
	8	NR	NR
	22	NR	NR
	36	NR	NR
	43	NR	NR
	50	NR	NR
	64	NR	NR
	85	NR	NR
	92	NR	RR
	127	R	RR
TORONTO PANEL 1	1	NR	RR
	22	R	RR
TORONTO PANEL 6	1	R	NR
	72	R	RR

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

Reactivity with Low Titer HIV-1 Antibody Performance Panel

A low titer HIV-1 antibody panel consisting of 15 specimens, obtained from a commercial source, was tested in comparison with FDA licensed anti-HIV-1/2 EIA tests. The results of the study are shown in Table 3. The Reveal Rapid HIV-1 Antibody Test was capable of detecting antibodies to HIV-1 similarly to the FDA licensed anti-HIV EIA-1/2 tests.

Table 3: Comparison of the Reveal Rapid HIV-1 Antibody Test and Licensed Anti-HIV EIA Tests Using a Low Titer HIV-1 Antibody Performance Panel

Panel Member	Reveal Test		Licensed Anti-HIV EIA Tests			
		EIA #1	EIA #2	EIA #3	EIA #4	EIA #5
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

Interfering Substances and Unrelated Medical Conditions

The effects of seromarkers associated with unrelated medical conditions was assessed using a panel of specimens testing positive for C-reactive protein (CRP; n=32), antistreptolysin O titer (ASOT; n=8), rheumatoid factor (RF; n=36), infectious mononucleosis (n=14), *Helicobacter pylori* IgG/IgM antibodies (n=16), hepatitis B virus seromarkers (HBsAg, anti-HBc IgG/IgM, and anti-HBs; n=68), hepatitis C virus (anti-HCV antibody / HCV RNA; n=51), hepatitis A virus IgM (anti-HAV; n=7), parvovirus IgG/IgM (n=18), herpes simplex virus IgG (HSV; n=11), cytomegalovirus IgG/IgM (n=9), mycoplasma IgM (n=1), mumps IgG (n=2), measles virus IgM (n=8), rubella IgM (n=5), Epstein-Barr virus (n=6), varicella (n=4), individuals vaccinated against smallpox who possess anti-vaccinia antibodies (n=103). The test panel was comprised of 374 specimens spiked with HIV-1 antibody positive specimens. Of the 374 specimens, 350 contained one of the seromarkers while 24 contained various combinations.

The effect of abnormal blood chemistry on the outcome of the Reveal Rapid HIV-1 Antibody Test was assessed using a panel comprised of 124 specimens with abnormal blood chemistry that were spiked with HIV-1 antibody positive specimens. Six specimens included in the study contained abnormal levels of alkaline phosphatase (n=1; range of elevated level tested, 299 U/l; reference range, 40-117 U/l), urea (n=2; 1.7-1.8 mmol/l; reference, 7.5-41.6 mmol/l), creatinine kinase (n=2; 142-1143 U/l; reference, 9-139 U/l), and glucose (n=1; 10 mmol/l; reference, 3.7-6 mmol/l). The remaining 118 specimens contained various combinations of the following analytes: markers related to kidney function: urea (n=38; range of elevated levels tested, 7.5-41.6 mmol/l; reference, 2.5-7.5 mmol/l), creatinine (n=31; 132-1239 μmol/l; reference, 62-115 μmol/l), sodium (n=1; 146 mmol/l; reference, 135-145 mmol/l), potassium (n=11; 5.2-6.4 mmol/l; reference, 3.5-5.3 mmol/l), chloride (n=11; 111-118 mmol/l; reference, 98-110 mmol/l); markers related to liver function: alkaline phosphatase (ALP)(n=34; 121-1248 u/l; reference, 40-117 u/l), alanine amino transferase (ALT)(n=33; 56-634 u/l; reference, 10-55 u/l), gamma glutamyl transferase (GGT)(n=24; 51-940.5 u/l; reference, 0-51 u/l), total protein (n=1; 83 g/l; reference, 63-80 g/l), aspartate aminotransferase (AST)(n=25; 50-1764 u/l; reference, 15-50 u/l), lactate

dehydrogenase (LD)(n=17; 211-676 u/l; reference, 15-50 u/l), total bilirubin (n=17; 28-343 μmol/l; reference, 4-24 μmol/l); markers related to lipid profile: cholesterol (n=25; 5.25-9.56 mmol/l; reference, 4-5.2 mmol/l), triglyceride (n=16; 2.02-6.38 mmol/l; reference, 0.4-1.9 mmol/l), HDL cholesterol (n=5; 2.02-2.41 mmol/l; reference, 0.9-2.0 mmol/l), LDL cholesterol (n=14; 3.5-6.7 mmol/l; reference, 1.6-3.4 mmol/l), CHL-LDL ratio (n=22; 4.03-7.4; reference, 0-4), plus others including: glucose (n=32; 3.4-22.9; reference, 3.7-6 mmol/l), amylase (n=4; 29.2-192 U/l; reference, 18-98 U/l) uric acid (n=9; 386-626 μmol/l; reference, 180-440μmol/l), creatinine kinase (CK)(n=12; 142-11344 U/l; reference, 9-139 U/l).

The effect of anticoagulants in plasma specimens, EDTA (n=73), heparin (n=89), and sodium citrate (n=20), was also determined.

The results of the studies indicated that none of the above conditions interfered with the sensitivity of the Reveal Rapid HIV-1 Antibody Test.

SPECIFICITY

Serum Specimens

Specificity studies were performed using repository serum specimens obtained from 850 previously screened HIV-1 antibody negative individuals from a high-risk population and 2914 freshly collected serum specimens from previously unscreened individuals from high-risk populations. Specimens were collected from three clinical sites. Of the 850 repository HIV-1 antibody negative serum specimens, 845 gave Non-Reactive results with the Reveal Rapid HIV-1 Antibody Test. Of the 2914 freshly collected serum specimens, 2763 gave Non-Reactive results using the Reveal Rapid HIV-1 Antibody Test. Five of the specimens were found to be Western blot indeterminate. The results of these studies are in Table 4A.

Table 4A. Performance of the Reveal Rapid HIV-1 Antibody Test on Serum Specimens Presumed to be Negative for Antibodies to HIV-1

Test Group	Total Samples	Reveal Non-Reactive	Licensed EIA Non-Reactive	True Negative*
Known HIV-1 Antibody Negative	850	845	850	850
High-Risk Population	2914	2763	2789	2789
Total	3764	3608	3639	3639

* True Negative status was based on negative or indeterminate test results using a licensed Western blot.

The overall specificity of the Reveal Rapid HIV-1 Antibody Test for serum specimens in these studies was calculated to be 3608/3639 = 99.1% (95% Confidence Interval; 98.8-99.4%), combining the number of Reveal Rapid HIV-1 Antibody Test Non-Reactive results obtained from the study of previously screened HIV-1 antibody negative serum specimens with the number of Reveal Rapid HIV-1 Antibody Test Non-Reactive results obtained from the studies of high-risk populations.

Plasma Specimens

Specificity studies were performed using plasma specimens that had been frozen once, obtained from 1000 previously screened HIV-1antibody negative individuals, and 2011 freshly collected plasma specimens from previously unscreened individuals from low risk-populations. All plasma specimens were collected from the same clinical site. Of the 1000 pre-screened HIV-1 antibody negative plasma specimens, 992 gave Non-Reactive results with the Reveal Rapid HIV-1 Antibody Test. Of the 2011

freshly collected HIV-1 antibody negative plasma specimens, 1978 gave Non-Reactive results using the Reveal Rapid HIV-1 Antibody Test. Four of the specimens were found to be Immunofluorescence Assay (IFA) indeterminate. The results of these studies are shown in Table 4B.

Table 4B. Performance of the Reveal™ Rapid HIV-1 Antibody Test on Plasma Specimens Presumed to be Negative for Antibodies to HIV-1

Test Group	Total Samples	Reveal Non-Reactive	Licensed EIA Non-Reactive	True Negative*
Known HIV-1 Antibody Negative	1000	992	1000	1000
Low-Risk Population	2011	1978	2011	2011
Total	3011	2970	3011	3011

* True Negative status was based on negative or indeterminate test results using a licensed Western blot or IFA.

The overall specificity of the Reveal Rapid HIV-1 Antibody Test for plasma specimens in these studies was calculated to be 2970/3011 = 98.6% (95% Confidence Interval; 98.4-98.8%), combining the number of Reveal Rapid HIV-1 Antibody Test Non-Reactive results obtained from the study of previously screened HIV-1 antibody negative plasma specimens with the number of Reveal Non-Reactive results obtained from the studies of low-risk populations.

Whole Blood Specimens

A specificity study was performed at three US clinical trial sites using freshly obtained matching fingerstick whole blood, K_2-EDTA whole blood, and K_2-EDTA plasma specimens collected from 557 individuals at high risk for HIV-1 infection and 528 individuals at low or unknown risk of HIV infection. The mean age of study participants was 41.85 ± 12.42 years; 69.11% of participants were male and 30.89% were female. From ethnicity and race data collected at enrollment, 39.55% of subjects were Hispanic or Latino (60.45% were Non-Hispanic); 31.16% were African American, 27.97% were Caucasian, 7.58% reported being of mixed race, and the remaining 33.29% reported other race categories. Reveal G4 specificity was assessed in fingerstick and venipuncture K2 EDTA whole blood specimens, while the matched K_2-EDTA plasma specimens were used to complete the FDA approved anti-HIV-1 reference testing algorithm.

Fingerstick Whole Blood Specimens

Specificity studies were performed using fingerstick whole blood specimens collected from 528 individuals at low or unknown risk of HIV infection and 556 individuals at high risk for HIV-1 infection. Of the 1051 fingerstick specimens collected from the confirmed HIV-1 negative individuals, 1043 gave a Non-Reactive result with Reveal G4 and five were invalid, leaving a total of 1046 results available for analysis. Within the high risk group, 27 specimens were confirmed anti-HIV-1 positive by Western blot and of those, 27 were Reactive with Reveal G4. Within the low or unknown risk group, six specimens were confirmed anti-HIV-positive by Western blot and of those, five were reactive with Reveal G4, with one false Non-Reactive result observed. A total three Reveal G4 false Reactive results (one from the high risk group and two from the low or unknown risk group) were obtained from the 1046 specimens from HIV-negative individuals that produced valid Reveal G4 fingerstick results. The results of these studies are shown in Tables 4C and 4D.

Table 4C. Performance of Reveal G4 on Fingerstick Whole Blood Specimens Presumed to be Negative for Antibodies to HIV-1

Test Group	Total Specimens	Reveal G4 Result (Fingerstick)			Reference Test Result		
		Non-Reactive	Reactive	Invalid[1]	Non-Reactive[2]	Reactive	Negative[2]
Low- or Unknown-Risk of HIV Infection	528	518[3]	7	3	522	6	522
At-Risk of HIV Infection	556	526	28	2	529	27	529
Total	1084	1044	35[4]	5	1051	33	1051

[1] Invalid results were not included in the calculation of specificity. The 5 specimens which gave invalid results on the Reveal G4 device were Non-Reactive on the reference test.

[2] Reference test Reactive results were confirmed by licensed HIV-1 Western Blot and excluded from the calculation of specificity.

[3] In addition to 518 true Non-Reactive results, there was a false Non-Reactive result in an individual from the low or unknown HIV risk group.

[4] There were three specimens that gave a false Reactive result; one was from an individual at risk for HIV infection, and the remaining two were from individuals at low or unknown risk of HIV infection. In addition, there was a false Non-Reactive result in an individual from the low or unknown HIV risk group.

Table 4D. Results for Fingerstick Whole Blood Specimens from Individuals at Risk of HIV Infection and Individuals at Low or Unknown Risk of HIV Infection

		Reference Test Result		
Reveal G4 Test Result (Fingerstick)	**Reactivity**	Negative	Positive	TOTAL
	Non-Reactive	1043	1[1]	1044
	Reactive	3[2]	32	35
	Invalid[3]	5	0	5
	TOTAL	1051	33	1084

[1] One false Non-Reactive result was observed in an individual of low or unknown risk for HIV infection.

[2] Of the three specimens that gave false Reactive results, one was from an individual at risk for HIV infection, and the remaining two were from individuals at low or unknown risk of HIV infection.

[3] Invalid results were not included in the calculation of specificity. All five invalid results were Non-Reactive on the reference HIV test; two were from the at risk population and three were from the low or unknown risk population.

The overall specificity of the Reveal G4 Rapid HIV-1 Antibody Test in fingerstick whole blood specimens from the combined high risk and low or unknown risk populations was calculated to be 1043/1046 = 99.71% (95% CI = 99.16 – 99.94).

Venipuncture Whole Blood Specimens

Specificity studies were performed using venipuncture whole blood specimens collected from 529 individuals at low or unknown risk of HIV infection and 557 individuals at high risk for HIV-1 infection. Of the 1086 venipuncutre specimens collected from individual at risk of HIV infection or a low or unknown risk of HIV infection, 1077 gave valid results with Reveal G4. Thus, of the 1053 results that were HIV-1 negative by the HIV reference method, 1044 were included in the analysis. Out of these 1044 specimens, 1043 were Non-Reactive with Reveal G4. Within the high risk group, 27 specimens were confirmed anti-HIV-1 positive by Western blot and of those, 26 were Reactive with Reveal G4. Within the low or unknown risk group, six specimens were confirmed anti-HIV-positive by Western blot, all of which were reactive with Reveal G4. One Reveal G4 false Reactive result from a low or unknown risk specimen was

obtained from the 1044 specimens from HIV-negative individuals that produced valid Reveal G4 venipuncture whole blood results. The results of these studies are shown in Tables 4E and 4F.

Table 4E. Performance of Reveal G4 on Venipuncture Whole Blood Specimens Presumed to be Negative for Antibodies to HIV-1 from Individuals at High Risk of HIV Infection and at Low or Unknown Risk of HIV Infection

Test Group	Total Specimens	Reveal G4 Result (Venipuncture)			Reference Test Result		
		Non-Reactive	Reactive	Invalid[1]	Non-Reactive[2]	Reactive	Negative[2]
Low- or Unknown-Risk of HIV Infection	529	519	7[3]	3	523	6	523
At-Risk of HIV Infection	557	525	26[4]	6	530	27	530
Total	1086	1044	33	9	1053	33	1053

[1] Although there were nine specimens which initially gave invalid results on the Reveal G4 device, only one remained invalid when repeat testing was performed per protocol. Eight of the initially invalid results produced Non-Reactive results when repeat testing was performed. One repeat invalid result remained, which was Non-Reactive on the reference test.

[2] Reference test Reactive results were confirmed by licensed HIV-1 Western Blot and excluded from the calculation of specificity.

[3] One false Reactive result was observed in an individual at low or unknown risk of HIV infection.

[4] One false Non-Reactive result was observed in a subject at risk of HIV infection, as noted previously in footnote 1 of Table 1F.

Table 4F. Results for Venipuncture Whole Blood Specimens from Individuals at Risk of HIV Infection and Individuals at Low or Unknown Risk for HIV Infection

		Reference Test Result		
Reveal G4 Test Result (Venipuncture)	**Reactivity**	Negative	Positive	TOTAL
	Non-Reactive	1043	1[2]	1044
	Reactive	1[1]	32	33
	Invalid	1	0	1
	TOTAL	1045	33	1078

[1] The one specimen that gave a false Reactive result on Reveal G4 was from an individual at low or unknown risk of HIV infection

[2] One false Non-Reactive result was observed in a subject at risk of HIV infection, as noted previously in footnote 1 of Table 1F

The overall specificity of the Reveal G4 Rapid HIV HIV-1 Antibody Test in venipuncture whole blood specimens from the combined high risk and low or unknown risk populations was calculated to be 1043/1044 = 99.90% (95% CI = 99.47 – 100.00).

Interfering Substances and Unrelated Medical Conditions

The effect of seromarkers associated with unrelated medical conditions on the specificity of the Reveal Rapid HIV-1 Antibody Test was assessed using a panel of specimens. The seromarkers studied were; C-reactive protein (CRP; n=41), antistreptolysin O titer (ASOT; n=19), rheumatoid factor (RF; n=33), infectious mononucleosis (n=17), *Helicobacter pylori* IgG/IgM antibodies (n=8), hepatitis B virus seromarkers (HBsAg, anti-HBc IgG/IgM, and anti-HBs; n=68), hepatitis C virus (anti-HCV antibody / HCV RNA; n=48), hepatitis A virus IgM (anti-HAV; n=5), parvovirus IgG/IgM (n=20), herpes simplex virus IgG (HSV; n=13), cytomegalovirus IgG/IgM (n=9), mycoplasma IgM (n=11), mumps IgG (n=1), EBV (n=1), human T- Lymphotrophic virus (n=10), measles virus IgM (n=13), rubella IgM (n=13), syphilis reagin (RPR/TPPA; n=16), varicella (n=11), individuals vaccinated against smallpox who possess anti-vaccinia

antibodies (n=103). The test panel was comprised of 447 HIV-1 antibody negative specimens. Of the 447 specimens, 422 contained one of the seromarkers while 25 contained various combinations.

The effect of abnormal blood chemistry on the outcome of the Reveal Rapid HIV-1 Antibody Test was assessed using a panel comprised of 105 HIV-1 antibody negative specimens. Six specimens included in the study contained abnormal levels of uric acid (n=1; range of elevated level tested, 532 μmol/l; reference range, 180-440 μmol/l) glucose (n=1; 7.3, reference, 3.7-6 mmol/l), total bilirubin (n=1; 261.9 μmol/l; reference, 4-24 μmol/l), amalyase (n=1; 2231U/l; reference, 18-98 U/l), creatinine kinase (n=1; 2598 U/l; reference, 9-139U/l), gamma glutamyl transferase (n=1; 1212 U/l; reference, 61-1037U/l). The remaining 99 contained various combinations of the following analytes: markers related to kidney function: urea (n=36; range of elevated levels tested, 7.7-46.1 mmol/l; reference, 2.5-7.5 mmol/l), creatinine (n=38; 121-1214 μmol/l; reference, 62-115 μmol/l), sodium (n=6; 146-152 mmol/l; reference, 135-145 mmol/l), potassium (n=11; 5.3-6.5 mmol/l; reference, 3.5-5.3 mmol/l), chloride (n=6; 111-125 mmol/l; reference, 98-110 mmol/l); markers related to liver function: alkaline phosphatase (ALP)(n=28; 123-1694 u/l; reference, 40-117 u/l), alanine amino transferase (ALT)(n=33; 57-2296 u/l; reference, 10-55 u/l), gamma glutamyl transferase (GGT)(n=21; 61-1037 U/l; reference, 0-51 u/l), total protein (n=1; 125 g/l; reference, 63-80 g/l), aspartate aminotransferase (AST)(n=22; 51-1764 u/l; reference, 15-50 u/l), lactate dehydrogenase (LD)(n=15; 229.5-733 u/l; reference, 15-50 u/l), total bilirubin (n=20; 27-334 μmol/l; reference, 4-24 μmol/l); marker related to lipid profile: cholesterol (n=27; 5.3-32.5 mmol/l; reference, 4-5.2 mmol/l), triglyceride (n=27; 1.91-51.65 mmol/l; reference, 0.4-1.9 mmol/l), HDL cholesterol (n=1; 2.13 mmol/l; reference, 0.9-2.0 mmol/l), LDL cholesterol (n=10; 3..6-27.1 mmol/l; reference, 1.6-3.4 mmol/l), CHL-LDL ratio (n=19; 4.24-10.18; reference, 0-4), plus others including: glucose (n=22; 6.2-15.7; reference, 3.7-6 mmol/l), amylase (n=3; 134-2231 U/l; reference, 18-98 U/l) uric acid (n=6; 252-940 μmol/l; reference, 180-440 μmol/l), creatinine kinase (CK)(n=4; 239-2598 U/l; reference, 9-139 U/l).

The effect of anticoagulants in plasma specimens; EDTA (n=79), heparin (n=20), and sodium citrate (n=26), on the Reveal Rapid HIV-1 Antibody Test was also determined.

The results of the studies indicated that none of the above conditions interfered with the specificity of the Reveal Rapid HIV-1 Antibody Test.

REPRODUCIBILITY

The reproducibility of the Reveal Rapid HIV-1 Antibody Test was studied at three sites using three lots of the device on three different days by three operators per site. Coded panels of 15 samples were used in triplicate for this study. Each panel consisted of strongly and weakly Reactive HIV-1 antibody serum/plasma samples, as well as HIV-1 antibody negative specimens. In addition, three lots of MedMira Positive and Negative Test Controls were included in the panel. A total of 810 tests were performed (270 per site) with a total of 54 tests performed per panel member. The overall reproducibility of the Reveal Rapid HIV-1 Antibody Test was found to be 810/810 = 100%.

PRODUCT WARRANTY

MedMira Laboratories Inc. guarantees the quality of this product if stored and used as instructed. Any component of the test found to be defective shall be replaced free of charge upon return of the defective product. MedMira Laboratories Inc. disclaims any implied warranty of merchantability or

fitness for a particular purpose, and in no event shall MedMira Laboratories Inc. be liable for consequent damage.

MedMira places high value on input and feedback from its customers. Please visit www.medmira.com to provide us with your comments.

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Explanation of Symbols

l	Temperature Limit	D	Do not reuse
M	Manufacturer	H	Use by date
h	Catalog number	V	For *In Vitro* Diagnostic Use
g	Lot number	i	Consult instructions for use

M **Manufacturer:**

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