



**SeroCard™**  
HIV

**TRINITY BIOTECH PLC**

IDA Business Park  
Bray  
Co. Wicklow  
Ireland

**Phone:** 353-1-276 9800  
**Fax:** 353-1-276 9888  
**E-mail:** info@trinitybiotech.ie

**TRINITY BIOTECH USA**

2823 Girts Road  
P.O. Box 1059  
Jamestown  
N.Y. 14702-1059  
USA

**Phone:** 1-716-483 3851  
**Fax:** 1-716-488 1990  
**Toll-free:** 888-864-4653  
**E-mail:** sales@trinityusa.com

**Web Site:** <http://www.trinitybiotech.com>

**FOR INFORMATION USE ONLY**  
**Not to be used for performing the assay.**  
**Refer to insert accompanying kit**

**1. Intended Use**

The Trinity Biotech SeroCard™ HIV is an *in vitro* qualitative assay for the detection of antibodies to Human Immunodeficiency Virus (HIV) types 1 and 2 in human serum, plasma or whole blood.

**2. Summary and Explanation of the Test**

Human Immunodeficiency Virus (HIV) has been recognised as the etiological agent of the acquired immunodeficiency syndrome (AIDS). HIV has been isolated from:

- (a) Numerous patients with AIDS and related disorders.
- (b) Asymptomatic members of groups at high risk from AIDS.
- (c) Patients without other high risk factors who have contracted AIDS after receiving blood transfusions.

Most patients with AIDS or AIDS Related Complexes (ARC) have antibodies to the HIV viral structural proteins. In addition a proportion of clinically healthy members of high risk groups such as male homosexuals, haemophiliacs and drug users are also seropositive. HIV infection is diagnosed by detecting antibodies specific to the virus. Currently two types of HIV are known, HIV-1 and HIV-2, with 40-60% amino acid homology between the two strains.

The Trinity Biotech SeroCard™ HIV assay is a rapid test based on the enzyme-linked immunosorbent assay technique.

**3. Principles of the Procedure**

A solid matrix is coated with synthetic HIV peptides. These peptides represent the envelope proteins of HIV-1 and HIV-2, gp41 and gp36 respectively. The coated peptides are at a site removed from, but connected to, the sample port.

The serum component of the patient sample migrates toward the reaction port. If the specimen contains antibodies to HIV, the antibodies will form a complex with HIV antigens at the reaction port. After washing the unreacted serum component from the reaction port, an anti-human IgG conjugate labelled with alkaline phosphatase is applied to the reaction port. The conjugate binds to the antigen antibody-complex on the reaction port surface. If there is no anti-HIV IgG antibody in the test specimen, the conjugate will not bind to the reaction port surface and is removed by the subsequent wash step. Addition of the substrate reagent at the reaction port will result in a blue coloured spot in the presence of the bound enzyme. The intensity of the colour is directly related to the titre of anti-HIV antibodies in the specimen.

- a) **Test Cards:** (40) (20 packages, each containing 2 cards). Each test card contains a zone, or port which is coated with synthetic HIV peptides.
- b) **Enzyme Conjugate Reagent:** (5 ml) (Green cap). Anti-human IgG labelled with alkaline phosphatase, stabilisers, BSA and 0.095% sodium azide.
- c) **BCIP Substrate Reagent:** (8 ml) (Blue cap). Contains substrate in a buffered solution and 0.095% sodium azide.
- d) **Wash Reagent:** (24 ml) (Purple cap). Imidazole buffer containing casein and 0.095% sodium azide.
- e) **Disposable Pipettes:** (40)

**Materials required but not provided.**

- \* Timer or stopwatch
- \* Blood collection devices.

**5. Storage and Stability**

The Trinity Biotech SeroCard™ HIV kit components should be stored between 2-8°C.

Kit components must be brought to room temperature prior to use.

The kit may be used up until the expiration date printed on the kit box.

If using only one Trinity Biotech SeroCard™ HIV device from a pouch, place the unused test card and desiccant back in the foil pouch and reseal.

**6. Precautions**

The Trinity Biotech SeroCard™ HIV test is intended for *in vitro* use.

Use a separate disposable pipette and card for each specimen tested.

Sodium azide is toxic and solutions containing same should be handled with care. If solutions containing sodium azide are disposed through the drain, they should be flushed thoroughly with water.

The blue-capped substrate reagent may be light sensitive and should not be exposed to excessive illumination.

Do not mix reagents from different kits.

Do not mix reagent caps.

Do not smoke, eat or drink in areas in which specimens are handled.

Dispose of all 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.

All spills should be wiped thoroughly using a suitable disinfectant such as a sodium hypochlorite solution.

Whole blood, serum or plasma may be used.

- a) **Whole Blood:** If fingerstick whole blood is used, drops of blood produced should be taken up from the finger-tip by the pipette supplied and dropped from the pipette onto the device. Blood droplets **should not** be dropped directly from the fingertip onto the device as their size may vary. Note that two drops of whole blood (approx. 80 µl) are required for this test and that this volume may not always be possible from a fingerstick.

Whole blood specimens should be used within ten minutes of collection for optimum performance.

- b) **Anti-Coagulated Whole Blood:** Heparin, EDTA and citrate may be used as anticoagulants. If an anticoagulant has been added, whole blood can be used directly on the card.

- c) **Serum or Plasma:** If a specimen has started to clot, do not remix before testing. In such instances, the clear serum should be pipetted off the clotted specimen and used for analysis.

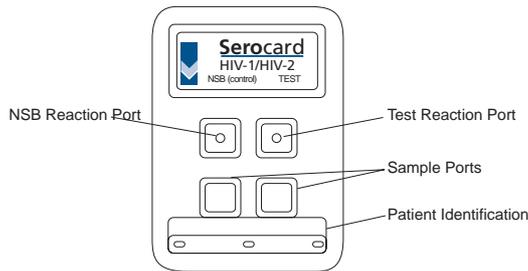
Serum or plasma may be kept for seven days at 2-8°C. Samples should be frozen for longer storage. Avoid repeated freezing and thawing of samples.

The Trinity Biotech SeroCard™ HIV assay incorporates a novel quality control system to screen out any false positive reactions that may occur due to the presence of antibodies non-specific to HIV. The NSB (non-specific binding) reaction port does not contain antigens specific to HIV. If any non-specific antibodies are present on the NSB or test reaction ports, the enzyme reagent will bind to these molecules resulting in identical colour intensity forming in both ports. The test reaction port will yield a darker intensity **only** when HIV specific antibodies are present.

## 9. Test Procedure

Once the assay has begun the procedure should be completed without interruption.

### (a) Steps performed at Sample Ports.



The following steps are performed at the **Sample Ports** located at the **bottom** of the test cards.

- (1) Dispense one drop (approx. 40 µl) of wash reagent (purple cap) onto each of the two sample ports. Allow to absorb completely.
- (2) Using the disposable pipette included in the kit, dispense one drop (approx. 40 µl) of patient (or control) specimen onto each of the two sample ports and allow to absorb.
- (3) Incubate card(s) at ambient temperature for 30 seconds.
- (4) Add one drop of wash reagent (purple cap) to each sample port. Allow to absorb completely.
- (5) Incubate for 30 seconds at ambient temperature.

### (b) Steps performed at Reaction Ports

The following steps are performed at the **Reaction Ports** located at the **top** of the test cards once the above procedure is completed.

- (1) Dispense one drop of wash reagent (purple cap) to each reaction port. Allow the drop to absorb completely.
- (2) Dispense one drop (approx. 50 µl) of enzyme reagent (green cap) to each reaction port. Allow the drop to absorb completely.
- (3) Incubate test card(s) for 1 minute at ambient temperature, after the enzyme reagent has absorbed into the small ports.
- (4) Add four drops of wash reagent (purple cap) to each reaction port. Allow each drop to absorb completely before adding a subsequent drop.
- (5) Dispense two drops of substrate reagent (blue cap) to each reaction port.

### (c) Incubation

Allow test card(s) to incubate at ambient temperature for 5 minutes ± 30 seconds before reading results.

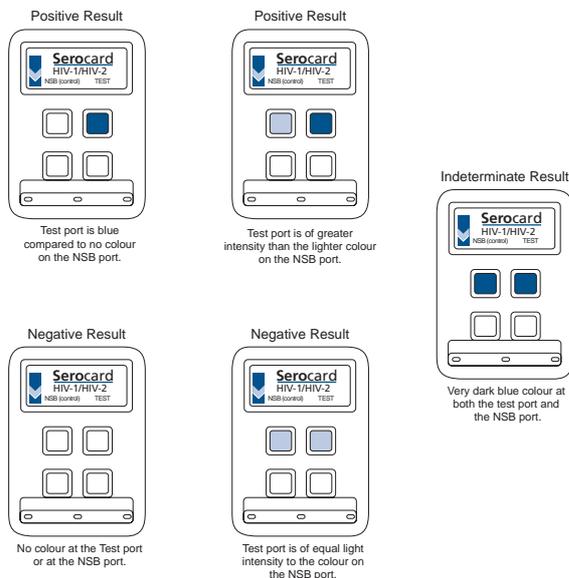
### (d) Reading

Test results should be interpreted at the end of the 5 minute incubation period.

### (e) Stopping the reaction (optional)

If required the Trinity Biotech Serocard™ HIV assay may be stopped as follows:

Add one drop (approx. 40 µl) of 3N hydrochloric acid solution (3N HCl) to each reaction port (top) after the incubation period (c) above. The test result will then be stable for up to 12 hours. This solution is not provided in the Trinity Biotech Serocard™ HIV kit.



**Test results are interpreted at the top reaction ports only.** Any colour development on the bottom sample ports should be ignored.

A **POSITIVE** result is indicated by a blue colour on the small right hand top port that is of darker intensity than its corresponding small port on the left.

As with all screening assays, any results should be considered presumptive until confirmatory assays have been performed according to local practice or WHO guidelines.

A **NEGATIVE** result yields no colour on the small top ports or pale colour of equal intensity on both small ports.

Should the small top left port develop a strong colour of equal or greater intensity to its corresponding right hand port, the test is invalid. The test should be repeated with a fresh device.

## 11. Limitations

Immunosuppressed or immunocompromised individuals infected with HIV-1 or HIV-2 may not produce antibodies to the virus. Testing with any kit designed to detect antibodies may give negative results and would not be a reliable test methodology for such patients.

Infants may receive antibodies from an infected mother or they may not produce antibodies in response to an infection. Therefore, it is necessary to exercise great care in interpreting their test results.

The Trinity Biotech Serocard™ HIV test is optimised to yield the stated sensitivity at room temperature or approximately 23°C. If room temperature is significantly lower than 23°C, the colour at the end of the 5 minute incubation period may be less intense. Specimens containing high anti-HIV titre may produce dark blue colour reactions in less than the stated 5 minute incubation period.

## 12. Performance Data

The specificity and sensitivity of the Trinity Biotech Serocard™ HIV test was determined by the analysis of 4,882 samples by several independent international laboratories (Switzerland (2), Ireland (4), England, Senegal (2), Tanzania, Italy, Mexico, Peru, USA, and Uganda). The patient samples used for this study included whole blood, fresh plasma, fresh serum, frozen plasma and frozen serum. All specimens had also been tested for the presence of HIV antibodies and results confirmed by numerous established market leading assays and/or Western Blot. For the purpose of sensitivity and specificity calculations 26 indeterminate samples were omitted from the final calculations. The results are summarised in the table below:

		Reference Test Result			
		HIV-1 +	HIV-2 +	HIV-1 & 2 +	HIV-1 & 2 -
Serocard™ HIV	+	1092	125	16	22
	-	2	0	0	3599

**Sensitivity HIV-1: >99%**  
**Sensitivity HIV-2: >99%**  
**Sensitivity HIV-1 & 2: >99%**

**Overall Sensitivity: >99%**  
**Overall Specificity: >99%**

The Trinity Biotech Serocard™ HIV was also evaluated against seven Boston Biomedica Seroconversion panels. All panel members which reference test positive or reference test negative were detected and confirmed as such by the Trinity Biotech Serocard™ HIV illustrating 100% sensitivity and specificity with these seven seroconversion panel members.

## 13. Cross Reactivity

The cross-reactivity of the Trinity Biotech Serocard™ HIV was also evaluated by assaying known HIV positive and negative samples against 238 potentially interfering samples as listed below:

### Potentially Interfering Substances

- Adenovirus antibodies
- *Borrelia burgdorferi* antibodies
- Chlamydia antibodies
- Cytomegalovirus IgG antibodies
- Cytomegalovirus IgM antibodies
- Epstein Barr Virus IgM antibodies
- HBsAg + Hepatitis E antibodies
- HBsAg antibodies
- Hepatitis A IgG antibodies
- Hepatitis A IgM antibodies
- Hepatitis B Surface Antigen
- Hepatitis C antibodies
- Herpes simplex antibodies
- Influenza antibodies
- Measles antibodies
- Mycoplasma antibodies
- Parvovirus IgG antibodies
- Parvovirus IgM antibodies
- Q Fever antibodies
- Respiratory Syncytial Virus antibodies
- Rubella IgG antibodies
- Rubella IgM antibodies
- Toxoplasma antibodies
- *Varicella zoster* antibodies

Of all 238 samples tested, those that were detected as either HIV negative or positive by the Trinity Biotech Serocard™ HIV were confirmed as such by the reference test.

### Feorino, P.M., Jaffe, H.W., Palmer, E., et al.

Transfusion-associated Acquired Immunodeficiency Syndrome: evidence for persistent infection in blood donors. *New Engl. J. Med* 312: 1293-6. 1985

### Alter, H.J., Leitman, S.F., Klein, H.G., et al.

Clinical significance of anti-HIV antibodies in asymptomatic blood donors. A Prospective Study. III. International AIDS Conference, Washington DC (abs) 74. 1987

### Butler, J.E.

In Enzyme-Immunoassay (Maggio et, ED. ) CRC Press, Boca Raton, FL, Chpt 2. 1980.