# WHO Prequalification of In Vitro Diagnostics
# PUBLIC REPORT

## Product: TrinScreen HIV
## WHO reference number: PQDx 0473-052-00

**TrinScreen HIV,** product code **5551100**, manufactured by **Trinity Biotech Manufacturing Ltd** , **Rest of World regulatory version**, was accepted onto the WHO list of prequalified in vitro diagnostics and was listed on 9 February 2022.

## Summary of WHO prequalification assessment for TrinScreen HIV

| | Date | Outcome |
|---|---|---|
| **Prequalification listing** | 9 February 2022 | listed |
| **Dossier assessment** | 17 December 2021 | MR |
| **Site inspection(s) of quality management system** | 14 May 2019 | MR |
| **Product performance evaluation** | Quarter 4 2020 | MR |

MR: Meets Requirements

**Intended use**

According to the intended use from Trinity Biotech Manufacturing Ltd, *"TrinScreen HIV test is a single use, rapid immunoassay for the qualitative detection of antibodies to human immunodeficiency virus type 1 and 2 in serum, plasma, and whole blood (venipuncture and fingerstick). The test is for in vitro diagnostic use and intended as an aid to diagnosis of HIV in symptomatic, asymptomatic populations and persons at risk of HIV infection. The test is not intended for use on neonates or infants below two years. The TrinScreen HIV test is intended to be performed by a healthcare professional at the point-of-care (POC) as an aid in the diagnosis of HIV infection."*

**Assay description**

According to the assay description from Trinity Biotech Manufacturing Ltd, *" HIV is the causative agent of AIDS (Acquired Immunodeficiency Syndrome). AIDS is the end stage of a 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 infection is diagnosed by tests that assess whether an individual's immune system has produced a HIV-specific immune response (antibodies to HIV).*

*TrinScreen HIV is a single use rapid assay for the detection of antibodies to HIV-1 and 2 in serum, plasma, and whole blood. This manual assay works on the principle of immunochromatographic sandwich formation.*

*On each TrinScreen HIV test device, recombinant proteins representing the immunodominant region of HIV-1 gp120/gp41 and HIV-2 gp36 are immobilized in the test line region of the nitrocellulose membrane. A control line region above the test line is immobilized with antibodies against the control proteins. The HIV and control line proteins are linked to coloured latex microparticles that are impregnated onto a glass fiber pad located below the test line region of the device.*

*During the test procedure, one drop of serum, plasma, or whole blood is added to the sample port of the device followed by two drops of Wash Solution and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the latex-conjugated antigens. The antibody latex complex moves chromatographically along the membrane to the test and control line regions of the test device, where it forms a pink/red line at the test line region."*

**Test kit contents**

| **Component** | **100 tests (product code 5551100)** |
|---|---|
| Test devices, single use, individually pouched with one desiccant. Coated with HIV-1 and HIV-2 recombinant antigens and control antibodies. | 100 |
| Wash Solution | 4 dropper bottles x 2 mL |
| Disposable pipettes | 100 |
| Single use sterile lancets | 100 |
| Single use alcohol swabs | 100 |
| Instructions for use | 1 |

***Items required but not provided***

- Timer or stopwatch.
- Suitable pen to use for labelling samples and tests.
- Biohazard disposal waste container.
- Disposable gloves and/or protective clothing.

***Fingerstick Samples:***

- Sterile gauze pads.
- Adhesive bandages.

***Venipuncture Whole Blood, Serum or Plasma Samples:***

Blood collection devices needed for samples destined for testing of venipuncture whole blood, serum or plasma.

**Storage**

The test kit should be stored at 2-30°C.

**Shelf-life upon manufacture**

24 months.

**Warnings/Limitations**

Please refer to current version of manufacturer's IFU for Warning/Limitations.

**Prioritization for prequalification**

Based on the established eligibility criteria, TrinScreen HIV was given priority for WHO prequalification assessment.

## Dossier assessment

Trinity Biotech Manufacturing Ltd. submitted a product dossier for TrinScreen HIV as per the "*Instructions for compilation of a product dossier*" (PQDx_018 version 3). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 17 December 2021.

**Commitment for prequalification**

Commitment to Prequalification 1: Please agree to provide the interim and final reports for in-use and real time stability studies by 14 May 2022.

Based on the product dossier screening and assessment findings, the product dossier for TrinScreen HIV meets WHO prequalification requirements.

## Manufacturing site inspection

An inspection of Trinity Biotech Manufacturing Ltd located at 1 Southern Cross IDA Business Park in Bray, Ireland was conducted from 24 to 26 October 2018. TrinScreen HIV was not in scope of that inspection. However, TrinScreen HIV is manufactured under the same quality management system and at the same site as the product inspected. Therefore, on the basis of that inspection, it was considered that, at the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current inspection performed at a manufacturing site for *in vitro* diagnostic products and gives a summary of the inspection findings.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 14 May 2019.

## Product performance evaluation

TrinScreen HIV Test (Trinity Biotech) was evaluated by the Institute of Tropical Medicine, Belgium, on behalf of the WHO in the quarter 4 of 2020, according to protocol PQDx_030, version 11.

**Clinical performance evaluation**

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 1200 plasma/serum specimens was used. The specimens were characterized using the following reference algorithm: Vironostika HIV Ag/Ab (bioMérieux) and Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics), or Genscreen Ultra HIV Ag/Ab (BioRad) and Vidas HIV Duo Quick (bioMérieux); followed by INNO-LIA HIV I/II Score (Fujirebio).

| **Clinical performance characteristics in comparison with an agreed reference standard** | |
|---|---|
| Sensitivity %<br>(N=470) | 100 (99.2%-100%) |
| Specificity %<br>(N= 730) | 100 (99.5%-100%) |
| Invalid rate %<br>(N= 1200) | 0.3 |
| Inter-reader variability %<br>(N= 1200) | 0 |

**Analytical performance evaluation**

| Analytical performance characteristics | |
|---|---|
| Sensitivity during seroconversion on 5 seroconversion panels in comparison with a benchmark assay (Wantai AiD anti-HIV 1+2 ELISA) | Of a total of 34 specimens, 11 were detected by the assay under evaluation; versus 10 specimens detected by the benchmark assay. |
| Analytical sensitivity on a mixed titer panel (SeraCare, ref 0800-436) | 20 of 20 specimens were correctly classified. |
| Analytical sensitivity on WHO reference preparation panel(s) (NIBSC code 02/210) | 6 of 6 specimens were detected. |
| Lot to lot variation on a dilution panel | Lot to lot variation was within +/- 1 two-fold dilutions for 7 dilution series.<br>Lot to lot variation was 2 two-fold dilutions for 3 dilution series. |

**Operational characteristics and ease of use**

This assay does not require laboratory equipment and can be performed in laboratories with limited facilities or in non-laboratory settings.

The assay was found easy to use by the operators performing the evaluation.

The endpoint stability (read time window) is 2 minutes, requiring careful attention that testing is conducted so that tests are read only within 10 and 12 minutes after addition of the diluent.

| **Key operational characteristics** | |
|---|---|
| Specimen types and volume | 1 drop of serum, plasma (EDTA, ACD or heparin), fingerstick or venous whole blood |
| Number of steps* | 2 steps in total<br>No step with precision pipetting (1 drop of specimen is added using the pipette supplied with the kit) |
| Time to result | 10 minutes |
| Endpoint stability (read time window) | 2 minutes (the test must be read between 10 and 12 minutes after addition of diluent) |
| Internal QC | Yes, reagent addition control |

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch, and reading), e.g. for RDTs: add specimen, add buffer (2 steps).*

Based on these results, the performance evaluation for the TrinScreen HIV Test meets the WHO prequalification requirements.

## Labelling

1. **Labels**
2. **Instructions for use**

## 1. Labels

### 1.1 Kit box artwork

277

Trinity Biotech

2°C – 30°C IVD 100 2

Manufactured by
Trinity Biotech Plc, IDA Business Park, Bray, Co. Wicklow, Ireland
Tel: 353 1 276 9800, Fax: 353 1 276 9888,
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TRIN SCREEN HIV

067-001A 07/18

Trinity Biotech

TRIN SCREEN HIV

Trinity Biotech

TrinScreen™ HIV test is a single use rapid immunoassay for the qualitative detection of antibodies to HIV-1 and HIV-2 in serum, plasma or whole blood (venipuncture and fingerstick).

TRIN SCREEN HIV

Trinity Biotech

**Contents**
1 Instructions for Use
100 Test Devices
4 Bottles Wash Solution
100 Transfer Pipettes
100 Sterile Lancets
100 Alcohol Swabs

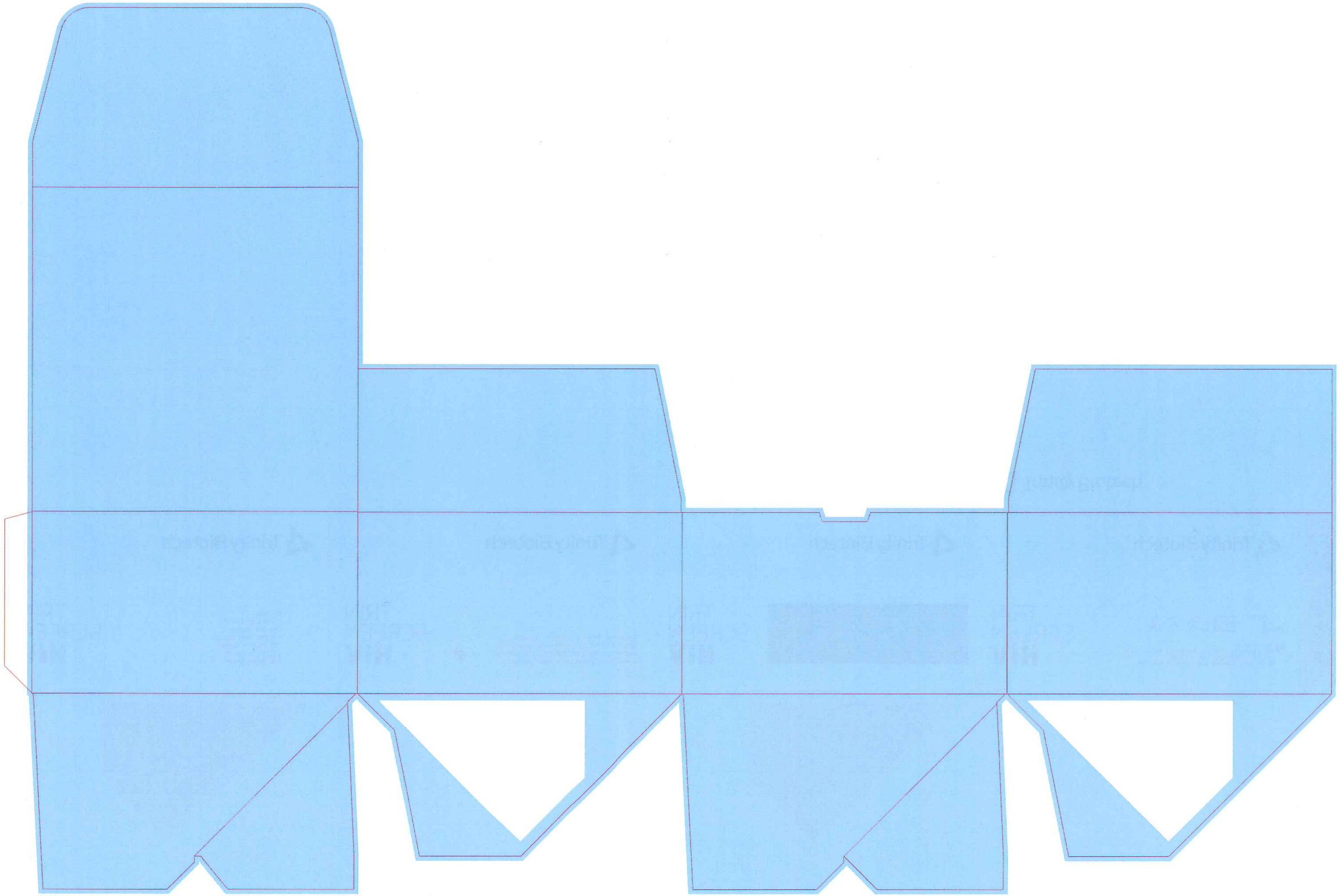
TRIN SCREEN HIV

Proof No: 1

| | | | |
|---|---|---|---|
| Job No: AO277 | | Part No:067-001A 07/18 | No. Cols: 1 |
| Job Ref: Trin Screen Kit Box | | | |
| Die No: 523U | Dimensions: 215x215x120mm | Pharma Code: | |
| G Code: 277 | Braille Ref: | Foil Ref: | |
| Emboss Ref: | Cyrel Ref: | Varnish Ref: | |
| Material: | | | |
| Approved by: | | Date:04/07/2019 | |

PANTONE 288 C | Die Line | Varnish | YFA

ESSENTRA



| | | | | |
|---|---|---|---|---|
| Proof No: 1 | Job No: AO277 | | Part No:067-001A 07/18 | No. Cols: 1 |
| | Job Ref: Trin Screen Kit Box | | | |
| | Die No: 523U | Dimensions: 215x215x120mm | Pharma Code: | |
| | G Code: 277 | Braille Ref: | Foil Ref: | |
| | Emboss Ref: | Cyrel Ref: | Varnish Ref: | |
| | Material: | | | |
| | Approved by: | | Date:04/07/2019 | |

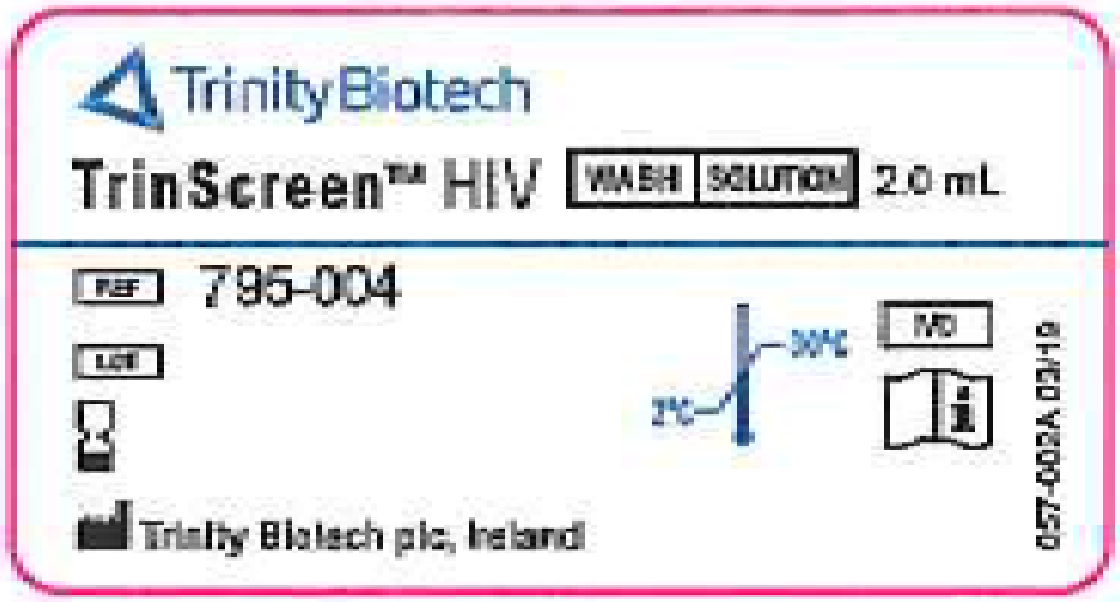
PANTONE 288 C | Die Line | Varnish | YFA

ESSENTRA

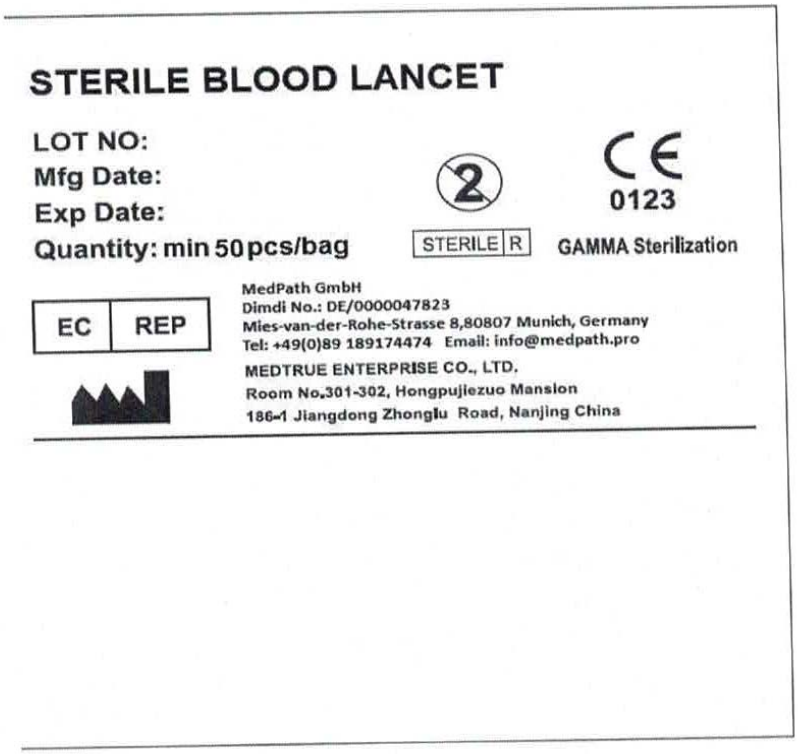
### 1.2 Kit box laser etch


### 1.3 Pouch artwork

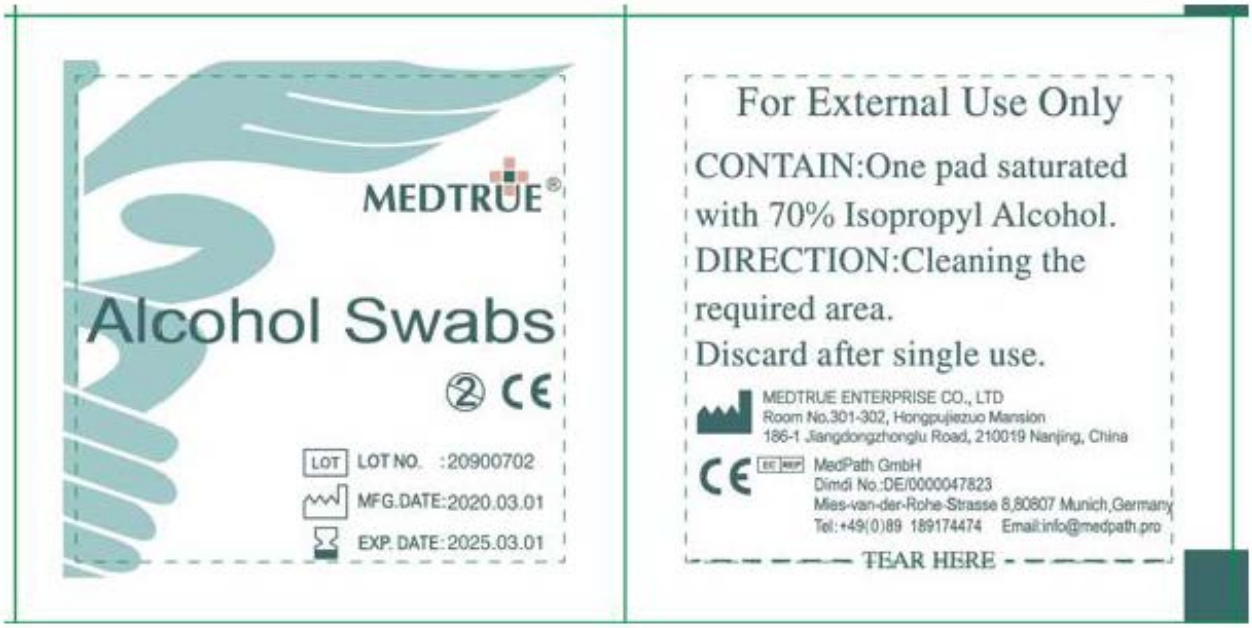
### 1.4 Wash solution label




### 1.5 Sterile lancet label



### 1.6 Alcohol swab label




## 2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

# TrinScreen™ HIV

REF 5551100

*Read these instructions for use completely before using the product. Follow the directions carefully. Not doing so may result in incorrect test results.*

## INTENDED USE

TrinScreen™ HIV test is a single use, rapid immunoassay for the qualitative detection of antibodies to human immunodeficiency virus type 1 and 2 in serum, plasma, and whole blood (venipuncture and fingerstick). The test is for *in vitro* diagnostic use and intended as an aid to diagnosis of HIV in symptomatic, asymptomatic populations and persons at risk of HIV infection. The test is not intended for use on neonates or infants below two years. The TrinScreen™ HIV test is intended to be performed by a healthcare professional at the point-of-care (POC) as an aid in the diagnosis of HIV infection.

## SUMMARY AND PRINCIPLES OF THE PROCEDURE

HIV is the causative agent of AIDS (Acquired Immunodeficiency Syndrome). AIDS is the end stage of a 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 infection is diagnosed by tests that assess whether an individual's immune system has produced a HIV-specific immune response (antibodies to HIV)[1].

TrinScreen™ HIV is a single use rapid assay for the detection of antibodies to HIV-1 and 2 in serum, plasma, and whole blood. This manual assay works on the principle of immunochromatographic sandwich formation.

On each TrinScreen™ HIV test device, recombinant proteins representing the immunodominant region of HIV-1 gp120/gp41 and HIV-2 gp36 are immobilized in the test line region of the nitrocellulose membrane. A control line region above the test line is immobilized with antibodies against the control proteins. The HIV and control line proteins are linked to coloured latex microparticles that are impregnated onto a glass fiber pad located below the test line region of the device.

During the test procedure, one drop of serum, plasma, or whole blood is added to the sample port of the device followed by two drops of Wash Solution and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the latex-conjugated antigens. The antibody latex complex moves chromatographically along the membrane to the test and control line regions of the test device, where it forms a pink/red line at the test line region.

## MATERIALS PROVIDED

Each kit contains:

- 100 test devices, single use, individually pouched with one desiccant. Coated with HIV-1 and HIV-2 recombinant antigens and control antibodies.
- 4 dropper bottles of Wash Solution (approx. 2.0 mL each), multiple use. Preservatives: Sodium Azide (0.09 % (w/v).
- 100 single use, disposable pipettes for use with serum, plasma or whole blood (fingerstick and venipuncture).
- 100 sterile lancets, single use.
- 100 alcohol swabs, single use.
- 1 instructions for use (IFU).

## MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or stopwatch.
- Suitable pen to use for labelling samples and tests
- Biohazard disposal waste container.
- Disposable gloves and/or protective clothing.

**Fingerstick Samples:**

- Sterile gauze pads.
- Adhesive bandages.

**Venipuncture Whole Blood, Serum or Plasma Samples:**

- Blood collection devices needed for samples destined for testing of venipuncture whole blood, serum or plasma.

## WARNINGS

1. TrinScreen™ HIV is intended for *in vitro* diagnostic use only and is not to be used for testing donors of blood, plasma, cells or tissues.
2. TrinScreen™ HIV is for professional use only.
3. Read the IFU completely before using the product. The instructions must be followed carefully as not doing so may result in inaccurate results.
4. Results should be considered presumptive until confirmatory assays have been performed according to local practice or WHO guidelines.
5. It is recommended that spare test devices are available at all times.
6. Perform the test at room temperature i.e. between 15 to 30°C (storage between 2 to 30°C).
7. The specimen and Wash Solution must be added to the test device in the correct order.
8. Use a new disposable pipette for each specimen to be tested.

## PRECAUTIONS

**Safety Precautions**

1. Observe standard precautions for handling infectious agents when using this kit.
2. Wear protective clothing such as lab coat, safety glasses and disposable gloves when handling specimens and assay reagents.
3. Wash hands thoroughly after use.
4. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

**Biosafety Precautions**

Use appropriate biosafety practices 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 in which specimens are handled.
2. Dispose of all specimens, used devices, pipettes, lancets and swabs 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. Materials for disposal 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"[2].
3. If disposing of wash buffer containing sodium azide down a sink or other plumbing system, use copious amounts of water to flush the system.
4. Wipe all spills thoroughly using a suitable disinfectant such as a solution of 10% bleach.
5. Use a separate disposable pipette and device for each specimen tested.

**Handling Precautions**

1. Do not use if a new kit box safety seal is absent, damaged or broken.
2. Each device is for single use only. If the device or device packaging is damaged (the pouch has been perforated or the desiccant is missing), do not use the device.
3. Each lancet is for single use only. If a lancet is damaged, do not use the lancet.
4. Each swab is for single use only.
5. Do not mix Wash Solution and test devices from different kit lots.
6. Do not use the kit past the expiration date. This is printed on the kit box and Certificate of Analysis.
7. Adequate lighting is required to read the test results.
8. Read the result immediately at the end of the 10 minute incubation time following the addition of Wash Solution. Do not read results beyond 12 minutes.
9. Place used lancets in a puncture resistant container prior to disposal.

## STORAGE INSTRUCTIONS

1. Store the TrinScreen™ HIV test device and Wash Solution at 2-30°C.
2. Kit components are stable until the expiration date printed on the outer label, when stored as directed. The kit expiry date is determined by whichever of the components has the shortest expiry date. The kit expiry date is not impacted once the Wash Solution has been opened. Do not use kit components beyond overall kit expiry date.
3. If stored refrigerated, ensure all components are brought to room temperature before use.
4. Do not freeze the kit.

## SPECIMEN COLLECTION AND STORAGE

**Whole Blood Venipuncture and Plasma:**

- Use either EDTA, acid citrate dextran (ACD), sodium citrate or heparin as the anticoagulant.
- Other anticoagulants have not been fully tested and may give incorrect results.
- Do not use grossly hemolysed or lipemic samples.

**Whole blood: Fingerstick**

Add each whole blood sample collected by fingerstick immediately to the TrinScreen™ HIV device.

**Collection: Whole Blood Venipuncture**

- Use standard phlebotomy procedures to collect a venipuncture whole blood specimen.
- Use a blood collection tube with either EDTA, acid citrate dextran (ACD) or heparin.
- The whole blood sample can be tested immediately with a device or stored at 2-8°C for up to 5 days. Do not freeze whole blood.
- Before testing, gently mix the whole blood sample to ensure a homogenous sample.
- Preferably, when testing is delayed, centrifuge the samples and retain the plasma for further testing.

**Collection: Serum and Plasma**

Serum: If a whole blood sample is collected without anticoagulant and has started to clot, do not remix before testing, in such instances, pipette the clear serum off the clotted specimen into a secondary tube and use for analysis.

Plasma: Use standard phlebotomy procedures to collect a venipuncture whole blood specimen using a blood collection tube. When collecting plasma, use a blood collection tube containing either EDTA, acid citrate dextran (ACD) or heparin. Plasma must be generated within 8 hours of blood draw. After collection, 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. Then without disturbing the cell pellet, carefully transfer the plasma to a secondary tube.

Specimens may be tested immediately or stored between 2 to 8°C for up to 5 days where testing is delayed. Specimens must be stored at -20°C or below if storage extends beyond 5 days. Do not use grossly hemolysed or lipemic samples. Avoid multiple (maximum 3) freeze thaw cycles.

## TEST PROCEDURE FOR WHOLE BLOOD FINGERSTICK

1. Kits stored at room temperature may be used immediately (Figure 1). Allow kits (unopened devices and Wash Solution) stored in a refrigerator to reach room temperature. Once at room temperature remove the required number of TrinScreen™ HIV devices from their pouches (Figure 2). Devices must be used within 20 minutes of opening the foil pouch.

Figure 1. TrinScreen™ HIV kit and components.


Figure 2. Test device pouch.

2. Perform one test at a time.
3. Lay the device on a clean flat surface.
4. Label the device with the appropriate patient information / identification number.
5. Use an alcohol swab to clean the finger to be sampled. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad.
6. Using a sterile lancet provided, puncture the skin just off the centre of the finger pad. Hold the finger downward (Figure 3).
7. 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 (Figure 4). 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.
8. Never apply blood droplets directly from the fingertip onto the device as the drop size may vary.
9. Collect the blood into the disposable pipette:
    - Gently press the pipette bulb
    - Hold the pipette vertically to the sample (Figure 5). This is important as the specimen may not be adequate if the pipette is held in a horizontal position.
    - Slowly release pressure on the bulb to draw up the sample.


Figure 3. Position lancet.


Figure 4. Blood drop formation.


Figure 5. Draw sample into pipette

10. Hold the pipette vertically above the sample port, squeeze the bulb and discharge one (1) drop of whole blood onto the sample pad (Figure 6). Allow the sample to fully absorb.
    - The blood drop must not have bubbles as this may deliver insufficient volume to the device.
    - Ensure there are no air bubbles in the sample port.
    - Failure to hold the pipette in a vertical position may lead to erroneous test results.
    - Do not touch the sample pad with the disposable pipette.
    - Dispose of the pipette into biohazard waste.
11. Hold the Wash Solution dropper bottle vertically over the sample port; gently press the bottle and add two (2) drops of Wash Solution to the sample port (Figure 7). Time the assay from this point. Ensure no air bubbles are introduced into the sample port. Failure to hold the bottle in a vertical position may lead to erroneous test results. Do not touch the sample pad with the dropper bottle tip.


Figure 6. Add blood vertically into device.


Figure 7. Add Wash Solution vertically.

12. Read test results after 10 minutes incubation time but no later than 12 minutes.
13. To read and interpret results, refer to the Interpretation for whole blood, serum and plasma samples section.

## TEST PROCEDURE FOR VENIPUNCTURE WHOLE BLOOD, SERUM AND PLASMA

1. Allow the kit (unopened devices and Wash Solution) to reach room temperature if previously stored in the refrigerator. Once at room temperature, remove the required number of TrinScreen™ HIV devices from their pouches. Devices must be used within 20 minutes of opening the foil pouch.
2. 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 / identification number.
5. Fill a disposable pipette provided with sample. Ensure there are no air bubbles. Use only the pipette included in the kit and do not reuse.
6. Hold the pipette vertically over the sample port, squeeze the bulb and discharge one (1) drop of plasma/serum/whole blood onto the sample pad (Figure 8). Allow the sample to fully absorb. Ensure air bubbles are not introduced into the sample port. Do not touch the sample pad with the disposable pipette. Failure to hold the pipette in a vertical position may lead to erroneous test results.
7. Dispose of the pipette in biohazard waste.
8. Hold the dropper bottle of Wash Solution in a vertical position above the sample port, add two (2) drops of Wash Solution to the sample port (Figure 9). Time the assay from this point. Ensure no air bubbles are introduced into the sample port. Failure to hold the bottle in a vertical position may lead to erroneous test results. Do not touch the sample pad with the dropper bottle tip.


Figure 8. Add sample vertically into device.


Figure 9. Add Wash Solution vertically.

9. Read test results after 10 minutes incubation time but no later than 12 minutes.
10. To read and interpret results, refer to the interpretation for whole blood, serum and plasma samples section.

## INTERPRETATION FOR WHOLE BLOOD, SERUM AND PLASMA SAMPLES

Reactive Result: Two pink/red lines of any intensity in the device window, the first within the region surrounding the embossed letter "T" (test) and the second within the region surrounding the embossed letter "C" (control). This indicates a Reactive result that is interpreted as Preliminary Positive for antibodies to HIV.

Non-Reactive Result: A pink/red line of any intensity within the region of the embossed letter "C" (control), but no pink/red line within the region of the embossed letter "T" (test). This indicates a Non-Reactive result that is interpreted as Negative for antibodies to HIV.

Invalid Result: No pink/red line appears in the device window within the region of the embossed letter "C" (control), irrespective of whether or not a pink/red line in the device window within the region of the embossed letter "T" (test). This is an Invalid result that cannot be interpreted. An invalid result must be repeated.

***Disclaimer**: The diagrams above are for illustration purposes only.*

**Further Interpretation**

Broken Lines

*Test Line*: Where a specimen produces a broken test line with TrinScreen™ HIV, it is deemed initially reactive (conditional on the presence of a control line) but the sample must be retested in duplicate. When the duplicate results are either a broken or complete test line in one or both replicates, then the sample is interpreted as preliminary positive. If both replicates give no line at "T" (test) then the result is referred to as negative.

*Control Line*: A broken control line does not affect the validity of the test.

Whole Blood Migration

Whole blood sample may migrate into the device window (whole blood visible at the bottom). The test is valid and can be interpreted if there is no obstruction in the test line region at 10 to 12 minutes. If the sample infringes on the test line region, the test is invalid and must be repeated.

## QUALITY CONTROL

TrinScreen™ HIV has a built in control that demonstrates assay validity. A pink/red line appearing within the region surrounding the embossed letter "C" (control) indicates that the test is running correctly.

When using whole blood samples, a red color in the sample port validates the addition of the sample. The pink/red control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to the interpretation section).

Good Laboratory Practice necessitates the use of control specimens to ensure proper device performance at least once daily and when kit lots are changed.

NOTE: Commercial HIV controls may not perform properly with the Trinity Biotech TrinScreen™ HIV kit. For further information please contact Trinity Biotech at HIV@trinitybiotech.com.

## LIMITATIONS

1. TrinScreen™ HIV is limited to the detection of antibodies to HIV-1 (including group O) and HIV-2.
2. TrinScreen™ HIV is designed to detect antibodies to HIV-1 and HIV-2 in serum, plasma and whole blood. TrinScreen™ HIV has not been validated for use with other body fluids and must not be performed with such fluids as results derived may not be accurate results.
3. TrinScreen™ HIV is intended for testing undiluted samples only. Do not dilute samples before testing.
4. For whole blood fingerstick sample collection; during the procedure patients may experience a short-lived pain when a sterile lancet is used to puncture the skin.
5. For venipuncture whole blood and plasma, use either EDTA (K2 or K3), acid citrate dextran (ACD-A or ACD-B), sodium citrate or lithium heparin as the anticoagulant. Other anticoagulants have not been fully tested and may give incorrect results..
6. This test has not been validated at extreme temperatures and humidity. Do not perform the test at an extreme temperature/humidity (55°C/RH 80%) as this may give erroneous results for negative samples.
7. Ensure the blood drop added to the sample port does not have bubbles, as an insufficient blood volume may be added to the device.
8. The control line only indicates flow of diluent but does not specifically indicate that specimen has been successfully applied to the TrinScreen™ HIV device.
9. The intensity of a pink/red line at the "T" (test) region does not necessarily correlate to the antibody levels in the specimen.
10. A test or control line of inconsistent intensity does not affect the validity of the assay result.
11. Do not read test results earlier than 10 minutes or later than 12 minutes as this may give incorrect results.
12. A reactive result by TrinScreen™ HIV suggests the presence of anti-HIV antibodies in the specimen. TrinScreen™ HIV is intended as an aid in the diagnosis of infection by HIV-1 or HIV-2. AIDS and AIDS related conditions are clinical symptoms and their diagnosis can only be established clinically. Clinical diagnosis should be made after confirmatory testing and an overall clinical evaluation has been made.
13. Confirm reactive results using another method and then results should be evaluated as part of an overall clinical evaluation before a diagnosis is made.
14. A negative result with TrinScreen™ HIV does not exclude the possibility of infection with HIV. A false negative result can occur in the following circumstances:
    - Recent infection or low levels of antibody (e.g. early seroconversion specimens) below the detection limit of the test. Antibody response to a recent exposure may take several months to reach detectable levels. For negative results, repeat testing after 6 months is recommended to confirm negative status.
    - The test procedure has not been correctly followed.
    - Antibodies to a variant strain of HIV in a patient that do not react with specific antigens utilized in the assay configuration.
    - Improper specimen handling.
    - Failure to add sample.
    - Failure to allow kits to come to room temperature prior to use.
15. Immunosuppressed or immunocompromised individuals infected with HIV-1 or HIV-2 may produce extremely low antibody levels to the virus. In this situation, the use of any diagnostic test designed to detect antibodies may give negative results and so is not a reliable test method for such patients.
16. The test has not been validated for testing samples from neonates or infants below two years.
17. The interference of Human African Trypanosomoasis (HAT) infection with HIV diagnosis is acknowledged[3]. As a result, it has been suggested HIV diagnosis using classical algorithms should be avoided for untreated HAT patients, unless the tests have been validated for interference with HAT[3]. TrinScreen™ HIV has not yet been validated for this purpose.
18. TrinScreen™ HIV is a qualitative test based on interpretation of a coloured (pink/red) line and therefore, may not be suitable for interpretation by individuals with colour blindness.

## PERFORMANCE CHARACTERISTICS

**Overall Sensitivity and Specificity**
TrinScreen™ HIV has been evaluated in the following study (Tables 1).

Clinical Evaluation (2020): This study was based on the testing of 622 fingerstick, 1,840 whole blood and 1,188 serum samples. The study was predominantly prospective except for 97 retrospective serum samples from HIV positive patients. The data for fingerstick, whole blood (venipuncture) and serum are presented separately in Table 1.

Table 1: Clinical Evaluation - Estimation of Sensitivity and Specificity

| Evaluation Performed | Sample Matrix | Estimated Sensitivity | 95% Confidence Interval | Estimated Specificity | 95% Confidence Interval |
|---|---|---|---|---|---|
| Prospective Clinical Evaluation (vs Venous Whole Blood) | Fingerstick (n=178 positives, n= 439 negatives) | 100.0% | 97.9%-100.0% | 100.0% | 99.2%-100.0% |
| Prospective Clinical Evaluation (vs Reference Method) | Whole Blood (n=483 positives, n= 1,357 negatives) | 99.0% | 97.6% - 99.7% | 99.8% | 99.4%-100.0% |
| | Serum (n=387 positives, n= 801 negatives) | 99.2% | 97.8% - 99.8% | 99.6% | 98.9% - 99.9% |
| | Fingerstick (n=181 positives, n= 441 negatives) | 98.3% | 95.2% - 99.7% | 100.0% | 99.2%-100.0% |

**HIV-2 Sensitivity**
The sensitivity of TrinScreen™ HIV in detecting HIV-2 positive samples was assessed using 117 samples positive for HIV-2 antibodies only. All HIV-2 samples were from the Ivory Coast. Of these, 20 samples were prospective and 97 samples retrospective samples. TrinScreen™ HIV sensitivity was 99.1%.

**HIV-1 Group M Non-B Subtypes**
To ensure optimal device sensitivity, an evaluation was carried out on various HIV-1 subtype specimens. A total of 67 samples of HIV-1 Group M Non-B subtypes were tested. All samples were reactive ("R") with TrinScreen™ HIV (Table 2).

Table 2: TrinScreen™ HIV Group M Non-B subtype evaluation

| Subtype | Number of samples tested | TrinScreen™ HIV result | % Reactive |
|---|---|---|---|
| A | 13 | R | 100% |
| C | 11 | R | 100% |
| D | 1 | R | 100% |
| F | 1 | R | 100% |
| G | 10 | R | 100% |
| H | 3 | R | 100% |
| K | 1 | R | 100% |
| CRF01_AE | 12 | R | 100% |
| CRF02_AG | 10 | R | 100% |
| CRF03_AB | 2 | R | 100% |
| CRF37_cpx | 3 | R | 100% |

Confirmed HIV-1 (Group B and O) and HIV-2 specimens were also detected by TrinScreen™ HIV.

**Seroconversion panels**
Twenty-nine (29) commercially available anti-HIV-1 seroconversion panels were evaluated in comparison to confirmatory western blot. Each panel consisted of sequential specimens obtained from a single individual during seroconversion. The twenty-nine (29) seroconversion panels consisted of two-hundred and nine (209) specimens. TrinScreen™ HIV detected HIV-1 antibodies at the same bleed or at an earlier bleed than western blot in twenty-nine (29) out of twenty-nine (29) panels.

**Interference studies**
To further evaluate the specificity of TrinScreen™ HIV, the product was challenged for antibody cross-reactivity with specimens from individuals with other disease states, non-HIV viral infections, other unrelated medical conditions and potentially interfering substances. The potentially cross-reacting and interfering substances did not affect the specificity of TrinScreen™ HIV (Table 3 and Table 4).

The sensitivity performance of TrinScreen™ HIV was further evaluated by spiking samples (listed in Table 3 and Table 4) with HIV-1 and HIV-2 antibody positive plasma. The potentially cross-reacting and interfering substances, as listed in Table 3 and 4, did not affect the sensitivity of TrinScreen™ HIV except for HIV positive/HAT positive samples (3/5 interfered). In addition, five HIV-positive donors with Tuberculosis or on anti-Tuberculosis, anti-parasitic or anti-retroviral drugs were tested and were shown not to interfere with the TrinScreen™ HIV result. All positive donors were found to give a reactive result on the TrinScreen™ HIV test as expected.
Table 3. Results from testing with specimens obtained from individuals with other disease states, non-HIV viral infections, or other unrelated medical conditions.

| Disease State or Health Condition | No. of samples tested | No. correctly identified non-reactive | % Non-reactive |
|---|---|---|---|
| Breast Cancer | 3 | 3 | 100% |
| Cirrhosis | 5 | 5 | 100% |
| Cytomegalovirus (Antibody) | 5 | 5 | 100% |
| Colon Cancer | 5 | 5 | 100% |
| Epstein-Barr virus | 5 | 5 | 100% |
| Hepatitis A (Antibody) | 5 | 5 | 100% |
| Hepatitis B (Antibody) | 5 | 5 | 100% |
| Hepatitis B (Antigen) | 5 | 5 | 100% |
| Hepatitis C (Antibody) | 5 | 5 | 100% |
| Human-T-lymphotropic virus 1 (HTLV-1) | 5 | 5 | 100% |
| Human-T-lymphotropic virus 2 (HTLV-2) | 5 | 5 | 100% |
| Icterus (Jaundice) | 4 | 4 | 100% |
| Influenza A | 5 | 5 | 100% |
| Influenza B | 5 | 5 | 100% |
| Leishmaniasis | 5 | 5 | 100% |
| Plasmodium falciparum | 5 | 5 | 100% |
| Schistosomiasis | 4 | 4 | 100% |
| Sickle cell disease | 5 | 5 | 100% |
| Syphilis | 5 | 5 | 100% |
| Systemic lupus erythematosus ( SLE ) | 5 | 5 | 100% |
| Varicella Zoster Virus (VZV) | 5 | 5 | 100% |
| Tuberculosis (TB) | 3 | 3 | 100% |
| Human African Trypanosomiasis (HAT) | 5 | 2 | 40% |

Table 4. Results from testing with specimens with potentially interfering substances

| Interfering Substance | No. of samples tested | No. correctly identified as non-reactive | % Non-reactive |
|---|---|---|---|
| ***Endogenous*** | | | |
| Anti-nuclear antibodies (ANA) | 5 | 5 | 100% |
| Bilirubin (2.40 – 4.90 mg/dL) | 4 | 4 | 100% |
| Haemoglobin | 5 | 5 | 100% |
| Human anti-mouse antibody (HAMA) | 5 | 5 | 100% |
| Human serum protein (8.4 – 10.7 g/dL) | 3 | 3 | 100% |
| Immunoglobulin G (elevated; >160 AU/mL) | 4 | 4 | 100% |
| Immunoglobulin M (elevated; > 160 AU/mL) | 3 | 3 | 100% |
| Rheumatoid Factor | 5 | 5 | 100% |
| Triglycerides (high; 475-520 mg/dL) | 5 | 5 | 100% |
| ***Exogenous*** | | | |
| Anti-malarial drugs | 5 | 5 | 100% |
| Anti-parasitic drugs | 5 | 5 | 100% |
| Anti-tuberculosis drugs | 5 | 5 | 100% |
| Aspirin (81 – 325 mg) | 5 | 5 | 100% |
| Caffeine (30.8 – 308 µmol/L) | 10 | 10 | 100% |
| Ethanol (21.7 – 43.4 mmol/L) | 10 | 10 | 100% |
| Ibuprofen (200 – 800 mg) | 3 | 3 | 100% |
| Paracetamol (30 – 300 mg/L) | 10 | 10 | 100% |
| ***Other*** | | | |
| Pregnant women | 5 | 5 | 100% |
| Multiparous pregnant women | 5 | 5 | 100% |
| Recipients of influenza vaccination | 4 | 4 | 100% |
| Recipients of multiple blood transfusions | 5 | 5 | 100% |
| *E. coli* (Antibodies) | 3 | 3 | 100% |

**Repeatability**
TrinScreen™ HIV was consistent and stable when tested over 20 days. Repeatability studies were performed twice daily, over a 20-day period, on 1 kit lot of TrinScreen™ HIV. One operator tested 7 samples, including HIV-1, HIV-2 positive plasma and serum, HIV negative plasma and serum, and whole blood samples (560/560 tests). One operator representative of end-users also tested 3 samples, including HIV-1, HIV-2 positive plasma and HIV negative serum (240/240 tests). The overall repeatability of the device was found to be 100% (800/800 tests).

**Reproducibility**
Four lots of TrinScreen™ HIV devices were tested by 3 operators, at 3 separate sites, testing 7 coded and blinded samples, in replicates of 5, once a day, over 5 days. There were 2,100 tests run (700 per site) with a total of 100 tests per sample, per site. The overall reproducibility of the device was found to be 99.8% (2,095/2,100 tests). Reproducibility was also not shown to be impacted by alternating testing between morning and afternoon testing.

## TECHNICAL ENQUIRIES

For any enquiries including technical support related to this product, please contact Trinity Biotech through one of the contact addresses below:
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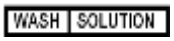
## REFERENCE


1. Schupbach *et al.*, *Clinical Virology Manual*, 3rd Edition 2000; 37: 513 – 541.
2. 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.
3. Lejon V, Ngoyi DM, Ilunga M, Beelaert G, Maes I, Büscher P, Fransen K. Low specificities of HIV diagnostic tests caused by Trypanosoma brucei gambiense sleeping sickness. J Clin Microbiol. 2010 Aug;48(8):2836-9. doi: 10.1128/JCM.00456-10. Epub 2010 Jun 23. PMID: 20573878; PMCID: PMC2916589.

## GUIDE TO SYMBOLS

| | |
|---|---|
|  Consult instructions for use |  Temperature limit |
|  Catalogue number |  Do not re-use |
|  Manufacturer |  Batch Code |
|  Use–by date |  Wash Solution |
|  Contains sufficient for <n> tests |  *In vitro* diagnostic medical device |

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