WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device
Number: PQDx 0141-051-00

Abstract

ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device with product code IHI-T402W[1], manufactured by **ABON Biopharm Hangzhou Co., Ltd.**, **rest of world regulatory version**, was accepted for the WHO list of prequalified diagnostics and was listed on 25 August 2014. This public report was amended on 6 April 2017 to reflect addition of one new packaging configuration with code **IHI-T402WG** and the change of the existing code IHI-T402W to **IHI-T0402A**.

Intended use:
HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device is an in vitro diagnostic rapid immunochromatographic assay for the qualitative detection of antibodies to HIV-1, including subtype O, and HIV-2 in venous and capillary whole blood, serum and plasma specimens. The product may be used as an aid in the diagnosis of HIV infection. A reactive result should be confirmed by supplemental testing as part of a validated HIV testing algorithm. This product has not been evaluated on paediatric and neonatal specimens.

Test principle:
The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device test strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad which contains a mixture of HIV-1 envelope and core antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies react and bind to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If

[1] Product code at time of WHO prequalification was IHI-T402. It was subsequently changed to IHI-T402W and this report amended 16 September 2014

the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of coloured lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A colored line will appear in the control line region if the migration of liquid has been successful, and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition.

If used as a first line (screening) assay, any reactive specimens should be referred for additional testing using another method to confirm reactivity. Depending on the prevalence of disease, this may require one or two additional reactive results on at least two other assays.

Component	**40 tests (product code IHI-T402WA)**	**40 tests (product code IHI-T402WG)**
Pouched test devices, with desiccant	40	40
Sterile safety lancets, single-use	N/A	40
Capillary tubes, heparinized for 50µl	N/A	40
Alcohol swabs, 70% ethanol	N/A	40
Specimen droppers	40	40
Buffer	2 bottles (3m/vial)	2 bottles (3m/vial)
Instructions for use	1	1

Items required but not provided:

Item
Consumables: Biosafety waste containers, sharps and non-sharps Specimen collection equipment for venous whole blood, if serum/plasma/venipuncture whole blood
Equipment: Timer Centrifuge, if serum/plasma

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

Shelf-life upon manufacture:
24 months[2]

[2] Shelf-life stated at time of original prequalification public report was 24 months.

Warnings/limitations:

Warnings

- For in vitro diagnostic use only.
- Read these instructions carefully before performing the test.
- Apply standard biosafety precautions when handling and disposing of potentially infectious material.
- Handle all specimens as potentially infectious.
- Wear protective clothing such as gloves, laboratory coats, and eye protection when specimens are being tested.
- The test device and accessory should be disposed in a proper biohazard waste container after testing
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Avoid splashes and clean up spills immediately with appropriate disinfectant.
- The buffer contains 0.02% sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Do not use the test kit beyond the expiration date.
- Do not use if the packaging is damaged.
- Do not use the heparinized capillary tube, dispensing bulb, specimen dropper (for fingerstick whole blood), single-use lancet and alcohol pad if it is already damaged.
- Dispose the heparinized capillary tube, specimen dropper (for fingerstick whole blood), single-use lancet in the sharps container if it is already damaged before use.
- Do not set the lancet down before discarding it.
- Do not use the lancet on more than one person.
- In case of Post-exposure prophylaxis for HIV, operators should familiarize themselves with PEP policy prior to conducting the testing.
- Humidity and temperature can adversely affect results.
- The optimal number of specimens to be tested at one time is ten.
- Do not use any other specimen than those specified. For plasma/venipuncture whole blood, EDTA-K2/sodium heparin can be used as anticoagulant. Other anticoagulants have not been tested and may give incorrect results.

Limitations

1. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) is for *in vitro diagnostic use* only. This test should be used for the detection of antibodies to HIV-1/2 in human whole blood, serum or plasma. The concentration of antibodies to HIV-1/2 can be determined by this qualitative test.
2. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) will only indicate the presence of antibodies to HIV-1/2 in the specimen and should not be used as the sole criteria for the diagnosis of HIV-1, HIV-2, and/or HIV-1 subtype O infection.

3. For confirmation of reactive test results, specimens should undergo further testing using different assays, such as rapid diagnostic tests, EIA and/or Western blotting in accordance with a validated HIV testing algorithm.
4. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5. Results should not be used to determine the serotype of HIV infections.
6. Due to possible antibody cross reactivity, the appearance of lines in both T1 and T2 does not necessarily indicate co-infection from HIV-1 and HIV-2.
7. False reactive results may arise due to rheumatoid factors, antinuclear antibodies, other viral infections (i.e. hepatitis B or hepatitis C), parasitic infections (i.e. schistosomiasis and trypanosomiasis), damage to test components by heat or humidity, when other test kit components (e.g. buffer or droppers) are substituted between test kits.
8. False non-reactive results may arise when titers of antibodies to HIV1/2 are very low, titers of antibodies to HIV1/2 are very high (high-hook effect), insufficient specimen volume added, excess of buffer added, damage to test components by heat or humidity.
9. False-negative results may be observed in individuals who are receiving effective antiretroviral therapy.

Summary of prequalification status for ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device

	Initial acceptance	
	Date	**Outcome**
PQ status amended	6 April 2017	listed
Status on PQ list	25 August 2014	listed
Dossier review	14 July 2014	MR
Site inspection(s) of quality management system	13 August 2014	MR
Laboratory evaluation of performance and operational characteristics	7 November 2013	MR

MR: Meets Requirements
NA: Not Applicable

ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device was accepted for the WHO list of prequalified diagnostics on the basis of data submitted and publicly available information.

Background information

Prioritization for prequalification
Based on the established criteria, ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device was given priority for WHO prequalification.

Product dossier assessment
ABON Biopharm Hangzhou Co., Ltd. submitted a product dossier for ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device as per the "Instructions for compilation of a product dossier" (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors).
The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 14 July 2014.

Manufacturing site inspection
Two comprehensive inspections were performed at the site of manufacture (Hangzhou, China) of the ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device in September 2012 and in November 2013 as per the "Information for manufacturers on

prequalification inspection procedures for the sites of manufacture of diagnostics" (PQDx_014 v1). The second inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality. The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 16 April 2014.

Laboratory evaluation

ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device was evaluated by WHO at the Institute of Tropical Medicine, Antwerp, Belgium - a WHO Collaborating Centre for HIV/AIDS Diagnostics and Laboratory Support. The laboratory evaluation was conducted according to the "WHO protocol for the laboratory evaluation of HIV serology assays" (PQDx_030 v1.0), and drew the following conclusions:

ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device is an immunochromatographic rapid diagnostic test for the discriminatory detection of HIV-1 and HIV-2 antibodies in human serum, plasma, capillary and venous whole blood. A volume of 25µl of serum/plasma or 50µl of whole blood is required to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities and non-laboratory settings. Reading of the results can be done visually.

In this limited evaluation on a panel of 1118 serum/plasma specimens, we observed an initial sensitivity (95% CI) of 100% (99.2% - 100%) and an initial specificity (95% CI) of 99.7% (98.9% - 100%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.2 % - 100%) and the final specificity (95% CI) was 99.7% (98.9% - 100%) compared to the reference assays. In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the overall inter-reader variability was 3.9% (0.1% for the HIV-1 band, 3.8% for the HIV-2 band). The invalid rate was 0.9%. Lot to lot variation was acceptable for all but one dilution series (WHO3-0690). False reactivity was observed for the HIV-2 test line for the first 4 dilutions when tested with lot HIV 2090089. No false reactivity was observed for the HIV-2 line when the same dilution series was tested with lot HIV 2090023.

ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device was unable to discriminate between HIV-1 and HIV-2 infection for 150 of the 1118 specimens (2 HIV-2 positives and 148 HIV-1 positives). This is a notable limitation of the product.

For eight seroconversion panels, ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device detected on average 0.5 (95% CI -0.3 – 1.0) specimens later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device correctly classified all specimens. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device correctly classified all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

Change notification

In 2017, ABON Biopharm Hangzhou Co., Ltd., notified WHO of a change related to addition of one new product code and change of existing product code related to changes in labelling and configuration to now include all accessories and reagents required to conduct the test procedure. This notification of changes was assessed and found to meet WHO prequalification requirements.

Labelling

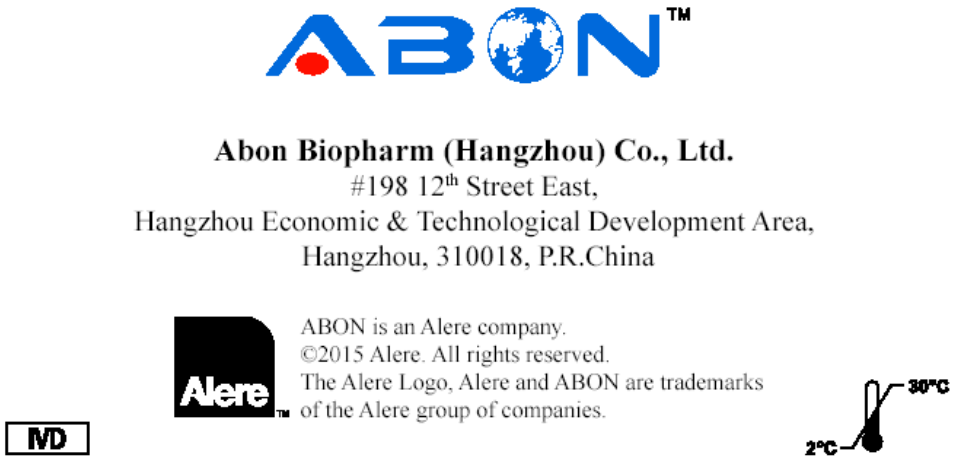
1. **Labels**
2. **Instructions for use**

1. Labels

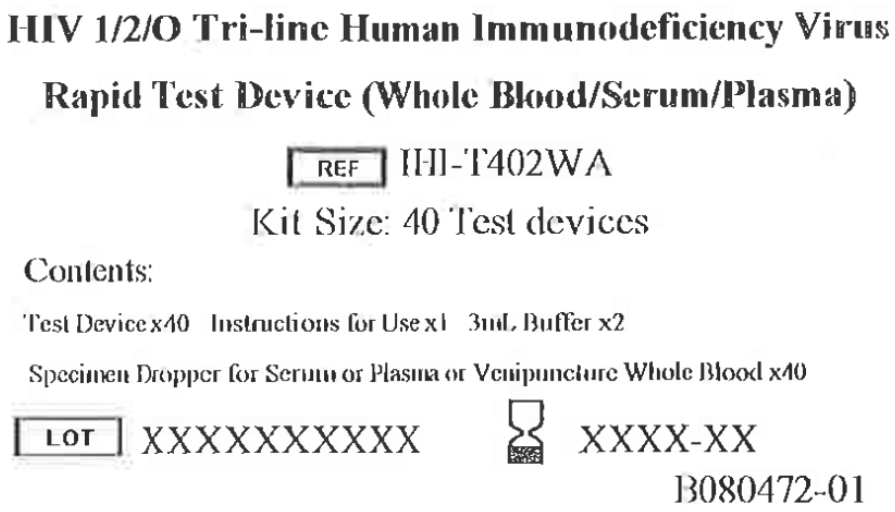
Ⅰ - BOX

- **IHI-T402WA**

1-Test printed on the box



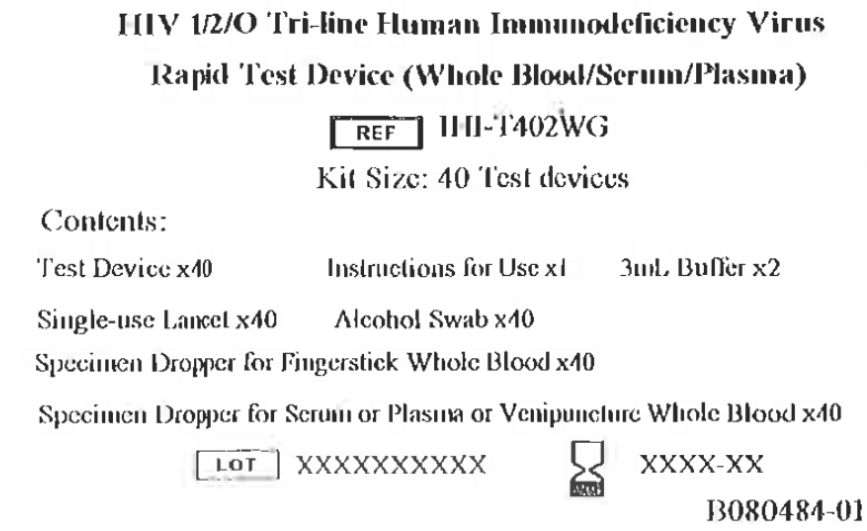
2- Box labels



- **IHI-T402WG**

1-Test printed on the box

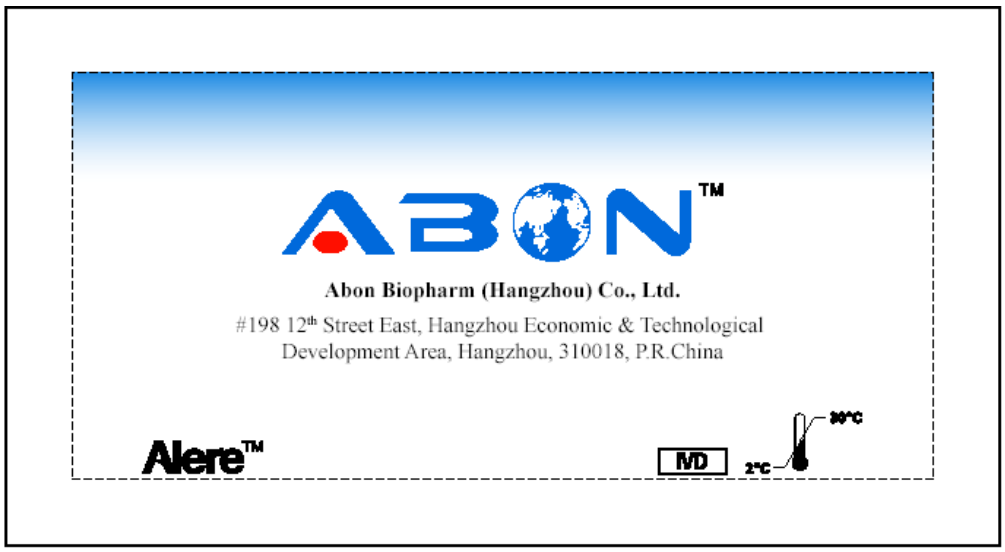
2- Box labels



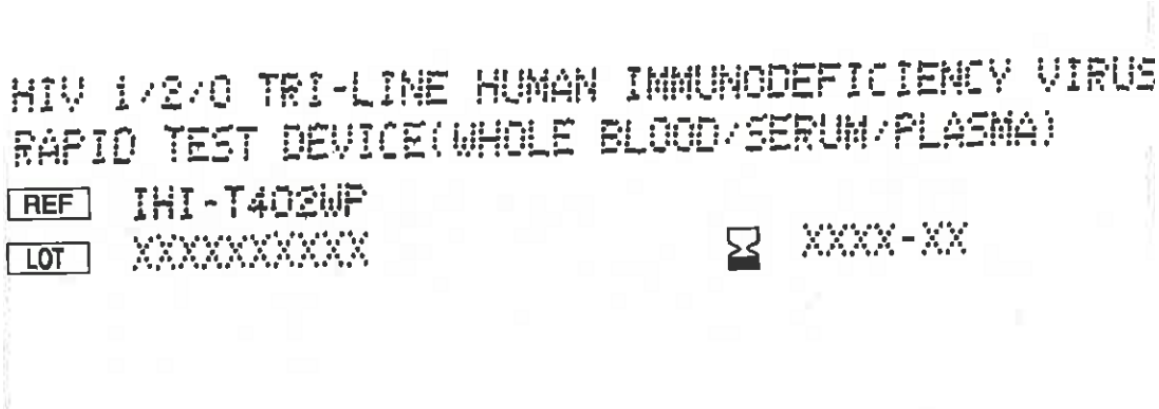
Ⅱ-Buffer labels

Ⅲ-Pouch

1- Test printed on the pouch

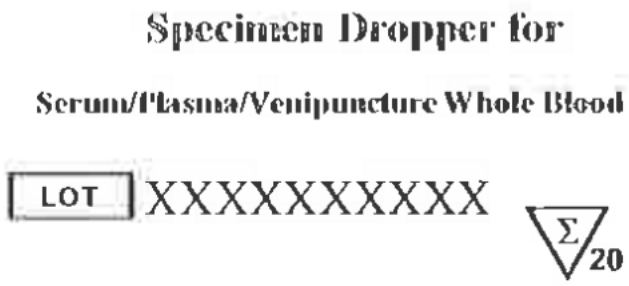


2-pouch ink jetting

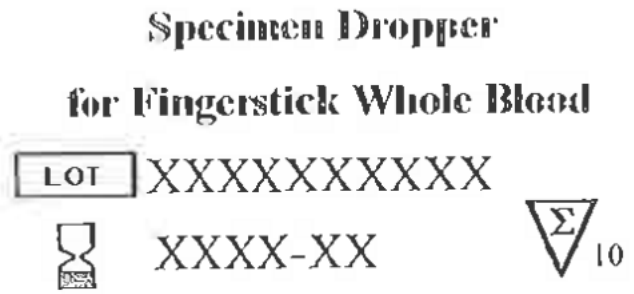


Ⅳ. Accessories

1. Specimen Dropper for Serum or Plasma or Venipuncture Whole Blood label ink printing



2. Specimen Dropper for for Fingerstick Whole Blood label ink printing

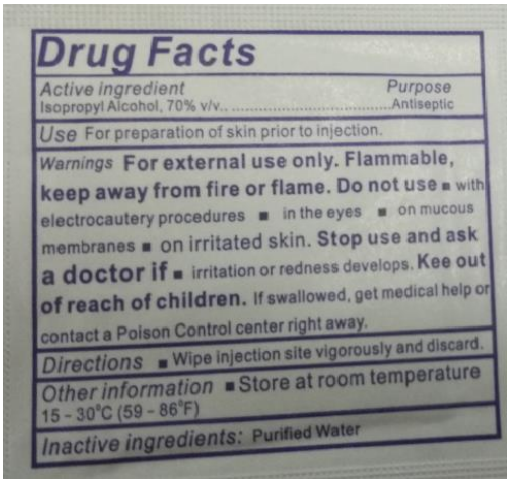


3. Single-Use Lancet label ink printing

4. Alcohol swab

Alcohol swab side1

Alcohol swab side2



2. Instructions for use

Ⅰ - IHI-T402WA

Effective date: 2017-02-22
IFU version 03

1156100903

ABON™ **HIV 1/2/O**

Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma)
Instructions for Use
English
(Catalogue number: IHI-T402WA)

A rapid diagnostic test for the qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) type 1, including subtype O, and type 2 in whole blood, serum or plasma.
For professional use only. IVD

INTENDED USE

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) is an *in vitro* diagnostic rapid immunochromatographic assay for the qualitative detection of antibodies to HIV-1, including subtype O, and HIV-2 in venous and capillary whole blood, serum and plasma specimens. The product may be used as an aid in the diagnosis of HIV infection. A reactive result should be confirmed by supplemental testing as part of a validated HIV testing algorithm. This product has not been evaluated on paediatric and neonatal specimens.

PRINCIPLE

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) test strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad which contains a mixture of HIV-1 envelope and core antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies react and bind to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of colored lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A colored line will appear in the control line region if the migration of liquid has been successful, and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C (storage in refrigerator is permitted). **Do not store in the freezer**. Protect the test kit from humidly. The test device is stable until the expiration date printed on the test kit and/or sealed test device pouch. Do not use beyond the expiration date. The test device must remain in the sealed pouch until use.

WARNINGS AND PRECAUTIONS

- For *In vitro* diagnostic use only.
- Read these instructions carefully before performing the test.
- Apply standard biosafety precautions when handling and disposing of potentially infectious material.
- Handle all specimens as potentially infectious.
- Wear protective clothing such as gloves, laboratory coats, and eye protection when specimens are being tested.
- The test device and accessory should be disposed in a proper biohazard waste container after testing
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Avoid splashes and clean up spills immediately with appropriate disinfectant.
- The buffer contains 0.02% sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Do not use the test kit beyond the expiration date.
- Do not use if the packaging is damaged.
- Do not use the heparinized capillary tube, dispensing bulb, single-use lancet and alcohol pad if it is already damaged.
- Dispose the heparinized capillary tube, single-use lancet in the sharps container if it is already damaged before use.
- Do not set the lancet down before discarding it.
- Do not use the lancet on more than one person.
- In case of Post-exposure prophylaxis for HIV, operators should familiarize themselves with PEP policy prior to conducting the testing.
- Humidity and temperature can adversely affect results.
- The optimal number of specimens to be tested at one time is 10.
- Do not use any other specimen than those specified. For plasma/venipuncture whole blood, EDTA-K2/sodium heparin can be used as anticoagulant. Other anticoagulants have not been tested and may give incorrect results.

SPECIMEN COLLECTION AND PREPARATION

- HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect **fingerstick whole blood** specimens:
  - Wear gloves.
  - Clean entire fingertip (preferable 3rd or 4th finger from non-dominant hand)with alcohol swab. Allow to dry (30 seconds).
  - Puncture the side of the finger with a new lancet each time. Disposed the lancet in sharps container immediately after using it. Do not use the lancet if the cap is already pulled off. Wipe away the first blood drop with a sterile gauze pad or cotton ball.
- To collect a fingerstick whole blood specimen by using a capillary tube:
  - Immerse the open end of the capillary tube into the blood drop and allow for the blood to draw into the capillary tube up to marked line. Avoid air bubbles.
  - After collecting the sample, place a gauze pad or cotton ball on the finger until the bleeding stops.
  - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense all whole blood on the specimen well (S) of the test device for testing.
  - Dispose the capillary tube in sharps container after testing.
- To collect **serum or plasma or venipuncture whole blood** specimens:
  - Collect according to safe phlebotomy procedures, using vacuum technique into tubes for serum or plasma or venipuncture whole blood preparation.
  - Prepare serum or plasma from whole blood as soon as possible to avoid hemolysis. Don't use turbid or haemolysed specimens.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature (15-30°C) for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be stored at -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing.
- No qualitative performance difference were observed between experimental controls and 20 nonreactive or 20 reactive specimens subjected to 6 freeze/thaw cycles; however, multiple freeze/ thaw cycles should be avoided.

MATERIALS

Materials Provided

Components	IHI-T402WA
Test Device	x40
3mL Buffer	x2
Specimen Dropper (For Serum/plasma/Venipuncture Whole Blood)	x40
Instructions for Use	x1

Materials Required But Not Provided

- Specimen collection equipment and containers
- Single-use lancets, alcohol swabs, cotton wool or gauze pad (for fingerstick whole blood only)
- Centrifuge
- Timer
- Heparinized capillary tubes with 50 µL marked line and dispensing bulb (for fingerstick whole blood only)
- Biohazard waste containers for sharps and non sharps

TEST PROCEDURE

Allow the test device, buffer and specimen to reach room temperature (15-30°C) prior to testing.

1. Remove the test device from the foil pouch and use it as soon as possible (within one hour).
2. Place the test device on a clean and level surface. Label with specimen ID.
   For **serum or plasma** specimens: Hold the dropper vertically and **transfer 1 drop of serum or plasma** (approximately 25 µL) to the specimen well (S) of the test device, then **add 1 drop of buffer** (approximately 40 µL) and start the timer.
   For **venipuncture whole blood** specimens: Hold the dropper vertically and **transfer 2 drops of whole blood** (approximately 50 µL) to the specimen well (S) of the test device, then **add 2 drops of buffer** (approximately 80 µL) and start the timer.
   For **fingerstick whole blood** specimens: Take whole blood specimen with a 50 µL capillary tube until marked line. And **add drawn specimen** (about 50 µL) on the specimen well (S) of the test device, then **add 2 drops of buffer** (approximately 80 µL) and start the timer.
3. Wait for the colored line(s) to appear. **Read results at 10 minutes. Do not read results after 20 minutes.**

INTERPRETATION OF RESULTS

(Please refer to the illustration above)
REACTIVE:* Two or three distinct colored lines appear. One line should always appear in the control line region (C), and another one or two apparent colored line(s) should appear in the test line region(s) (T1 and/or T2).
***NOTE 1:** The intensity of the color in the test line region (T1 and/or T2) will vary but any shade of color in the test line region (T1 and/or T2) should be considered reactive.
***NOTE 2:** Dual infection of HIV-1 and HIV-2 is quite rare. Dual reactivity observed in Abon HIV 1/2/O Tri-line HIV Rapid Test Device, i.e. HIV-1 line and HIV-2 line both reactive, is more likely to be caused by antibody cross-reactivity. Any specimen with dual reactivity should be referred for specific HIV-2 confirmatory testing, if a discretionary result is required.
NON-REACTIVE: One colored line appears in the control region (C). No apparent colored lines appear in the test line regions (T1 and/or T2).
INVALID: No line appears in the control line region (C). If this occurs, read the test procedure again and repeat the test with a new test device. If the result is still invalid, stop using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A control line is included in the test as an internal control. The test must absorb liquid and allow it to migrate along the membrane for the control line to appear. The control line does not control for the addition of adequate volume of specimen.
Quality control specimens are not supplied with this kit; however, it is recommended that quality control specimens be tested as a good laboratory practice.

LIMITATION

1. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) is for *in vitro* diagnostic use only. This test should be used for the detection of antibodies to HIV-1/2 in human whole blood, serum or plasma. The concentration of antibodies to HIV-1/2 can be determined by this qualitative test.
2. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) will only indicate the presence of antibodies to HIV-1/2 in the specimen and should not be used as the sole criteria for the diagnosis of HIV-1, HIV-2, and/or HIV-1 subtype O infection.
3. For confirmation of reactive test results, specimens should undergo further testing using different assays, such as rapid diagnostic tests, EIA and/or Western blotting in accordance with a validated HIV testing algorithm.
4. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5. Results should not be used to determine the serotype of HIV infections.
6. Due to possible antibody cross reactivity, the appearance of lines in both T1 and T2 does not necessarily indicate co-infection from HIV-1 and HIV-2.
7. False reactive results may arise due to rheumatoid factors, antinuclear antibodies, other viral infections (i.e. hepatitis B or hepatitis C), parasitic infections (i.e. schistosomiasis and trypanosomiasis), damage to test components by heat or humidity, when other test kit components (e.g. buffer or droppers) are substituted between test kits.
8. False non-reactive results may arise when titers of antibodies to HIV1/2 are very low, titers of antibodies to HIV1/2 are very high (high-hook effect), insufficient specimen volume added, excess of buffer added, damage to test components by heat or humidity.
9. False-negative results may be observed in individuals who are receiving effective antiretroviral therapy. [1,2,3]

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) was evaluated with 1,640 specimens from different countries in an unpublished multi-center field study, 1,000 specimens from a blood donation center and 3430 specimens from an in-house clinical study. Of the 6070 total specimens (which included whole blood, serum and plasma specimens), 1602 were found HIV seropositive and 4468 specimens were found HIV seronegative by a characterization testing algorithm comprising of EIA and/or Western blot. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) showed 99.9% relative sensitivity, and 99.8% relative specificity compared to EIA and/or Western blot.

HIV 1/2/O Tri-line Rapid Test Device vs. EIA and/or Western blot

		EIA and/or Western blot		Total Results
	Results	Positive	Negative	
HIV 1/2/O Tri-line Rapid Test Device	Reactive	1601	10	1611
	Non-reactive	1	4458	4459
Total Results		1602	4468	6070

Relative Sensitivity: 99.9% (99.7-100.0%)*
Relative Specificity: 99.8% (99.6-99.9%)*
Relative Accuracy: 99.8% (99.7-100.0%)*
* 95% Confidence Interval

Specimen Types Consistency

50 HIV seropositive whole blood and paired plasma specimens, 26 HIV seropositive whole blood, paired plasma and serum specimens, 50 negative whole blood, paired plasma and serum specimens were tested with HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma).

EIA and/or Western blot	Specimen type	No. tested	HIV 1/2/O Tri-line Rapid Test Device	
			No. tested	Reactive
Negative	Plasma	50	50	0
	Serum	50	50	0
	Whole blood	50	50	0
Positive	Serum	26	0	26
	Plasma	76	0	76
	Whole blood	76	0	76

Paired whole blood, plasma, serum specimens show the consistent results with HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma).

Precision

Intra-Assay (same lot)

Within-run precision has been determined by using 10 replicates of five specimens: a negative, a low titer HIV-1 positive, a low titer HIV-1 **(subtype O)** positive, a medium titer HIV-1 positive and a HIV-2 positive. All above values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same five specimens: a negative, a low titer HIV-1 positive, a low titer HIV-1 (subtype O) positive, a medium titer HIV-1 positive and a HIV-2 positive. Three different lots of the HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) have been tested using above specimens. The specimens were correctly identified >99% of the time.

BIBLIOGRAPHY

1. Delaney KP, Branson BM, Uniyal A, et al. *Evaluation of the Performance Characteristics of 6 Rapid HIV Antibody Tests.* Clinical Infectious Diseases. 2011; 52(2): 257-263.
2. O'Connell RJ, Merritt TM, Malia JA, et al. *Performance of the OraQuick Rapid Antibody Test for Diagnosis of Human Immunodeficiency Virus Type 1 Infection in Patients with Various Levels of Exposure to Highly Active Antiretroviral Therapy.* Journal of Clinical Microbiology. 2003; 41(5):2153-2155
3. O'Connell RJ, Agan BK, Anderson SA, et al. *Sensitivity of the Multispot HIV-1/HIV-2 Rapid Test Using Samples from Human Immunodeficiency Virus Type 1-Positive Individuals with Various Levels of Exposure to Highly Active Antiretroviral Therapy.* Journal of Clinical Microbiology. 2006; 44(5): 1831-1833

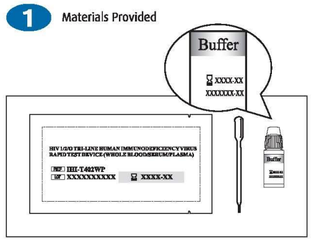
Index of Symbols

IVD	For *in vitro* diagnostic use only	LOT	Lot Number
2°C 30°C	Store between 2-30°C		Use by
REF	Catalogue number		Tests per kit

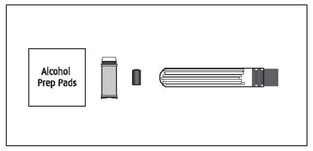
ABON™
Manufacturer
Abon Biopharm (Hangzhou) Co., Ltd.
#198 12th Street East,
Hangzhou Economic & Technological Development Area,
Hangzhou, 310018, P.R.China
Tel: +86-571-81638384
E-mail: abon.tech@alere.com

Preparation

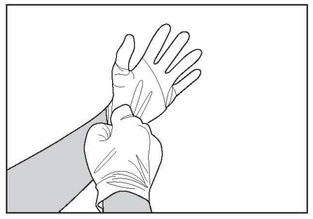
1 Materials Provided



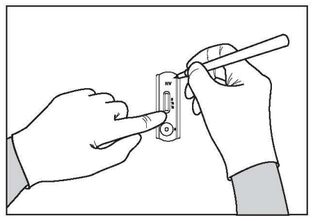
Materials Required But Not Provided



2 Wear gloves

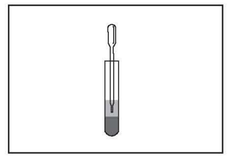


3 Open the pouch, Label with specimen ID. Use it as soon as possible (within one hour).

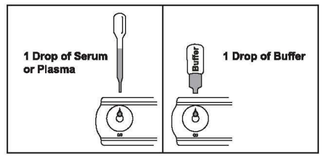


Serum or plasma specimens

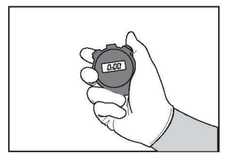
4 Draw the specimen from the specimen tube with a dropper.



5 Transfer 1 drop of serum or plasma (approximately 25 µL), then add 1 drop of buffer (approximately 40 µL).

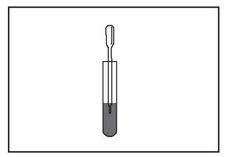


6 Start the timer.

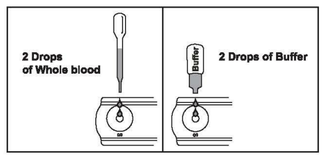


Venipuncture whole blood specimens

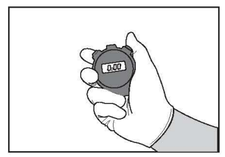
4 Draw the specimen from the specimen tube with a dropper.



5 Transfer 2 drops of whole blood (approximately 50 µL), then add 2 drops of buffer (approximately 80 µL).

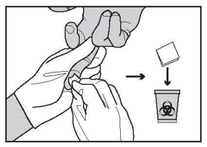


6 Start the timer.

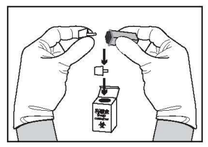


Fingerstick whole blood specimens

4 Clean entire fingertip (preferable 3rd or 4th finger from non-dominant hand) with alcohol swab. Dispose the alcohol swab.

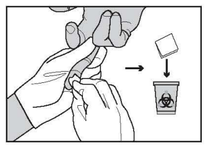


5 Take off the cap of the lancet and dispose the cap in sharps container.

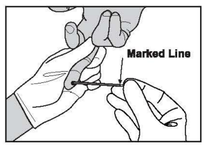


6 Puncture the side of the finger. Dispose the lancet in sharps container immediately after using it.

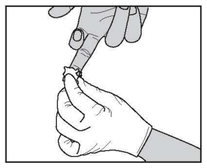
7 Wipe away the first blood drop with a sterile gauze pad or cotton wool.



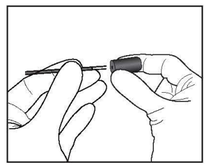
8 Immerse the open end of the capillary tube into the blood drop and allow for the blood to draw into the capillary tube up to marked line.



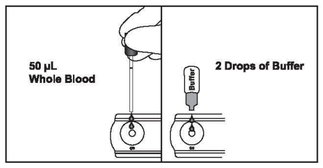
9 After collecting the sample, place a gauze pad or cotton wool on the finger until the bleeding stops.



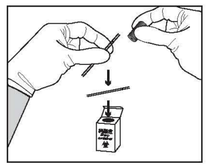
10 Place the bulb onto the top end of the capillary tube.



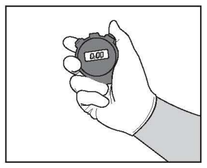
11 Squeeze the bulb to dispense all whole blood on the specimen well (approximately 50 µL) , then add 2 drops of buffer (approximately 80 µL).



12 Dispose the capillary tube in sharps container after testing.

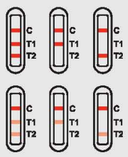


13 Start the timer.



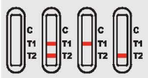
Read results

Wait for the colored line(s) to appear.
Read results at **10-20minutes**.



REACTIVE:* Two or three distinct colored lines appear. One line should always appear in the control line region (C), and another one or two apparent colored line(s) should appear in the test line region(s) (T1 and/or T2).
***NOTE 1:** The intensity of the color in the test line region (T1 and/or T2) will vary but any shade of color in the test line region (T1 and/or T2) should be considered reactive.
***NOTE 2:** Dual infection of HIV-1 and HIV-2 is quite rare. Dual reactivity observed in Abon HIV 1/2 /O Tri-line HIV Rapid Test Device, i.e. HIV-1 line and HIV-2 line both reactive, is more likely to be caused by antibody cross-reactivity. Any specimen with dual reactivity should be referred for specific HIV-2 confirmatory testing, if a discretionary result is required.

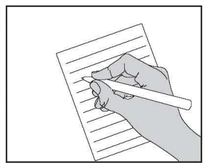
NON-REACTIVE: One colored line appears in the control region (C). No apparent colored lines appear in the test line regions (T1 and/or T2).



INVALID: No line appears in the control line region (C). If this occurs, read the test procedure again and repeat the test with a new test device. If the result is still invalid, stop using the test kit immediately and contact your local distributor.

Clear up/Record

Dispose devices, gloves in a proper biohazard waste container.



Record the test results.

Ⅱ - IHI-T402WG

Effective date: 2017-02-22
IFU version 01

1156117601

ABON™ **HIV 1/2/O**

Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma)
Instructions for Use
English
(Catalogue number: IHI-T402WG)

A rapid diagnostic test for the qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) type 1, including subtype O, and type 2 in whole blood, serum or plasma.
For professional use only. IVD

INTENDED USE

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) is an *in vitro* diagnostic rapid immunochromatographic assay for the qualitative detection of antibodies to HIV-1, including subtype O, and HIV-2 in venous and capillary whole blood, serum and plasma specimens. The product may be used as an aid in the diagnosis of HIV infection. A reactive result should be confirmed by supplemental testing as part of a validated HIV testing algorithm. This product has not been evaluated on paediatric and neonatal specimens.

PRINCIPLE

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) test strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad which contains a mixture of HIV-1 envelope and core antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies react and bind to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of colored lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A colored line will appear in the control line region if the migration of liquid has been successful, and must be present for the test to be valid. Its presence does not confirm sufficient specimen addition.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C (storage in refrigerator is permitted). **Do not store in the freezer**. Protect the test kit from humidity. The test device is stable until the expiration date printed on the test kit and/or sealed test device pouch. Do not use beyond the expiration date. The test device must remain in the sealed pouch until use.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Read these instructions carefully before performing the test.
- Apply standard biosafety precautions when handling and disposing of potentially infectious material.
- Handle all specimens as potentially infectious.
- Wear protective clothing such as gloves, laboratory coats, and eye protection when specimens are being tested.
- The test device and accessory should be disposed in a proper biohazard waste container after testing
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Avoid splashes and clean up spills immediately with appropriate disinfectant.
- The buffer contains 0.02% sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Do not use the test kit beyond the expiration date.
- Do not use if the packaging is damaged.
- Do not use the specimen dropper (for fingerstick whole blood), single-use lancet and alcohol pad if it is already damaged.
- Dispose the specimen dropper (for fingerstick whole blood), single-use lancet in the sharps container if it is already damaged before use.
- Do not set the lancet down before discarding it.
- Do not use the lancet on more than one person.
- In case of Post-exposure prophylaxis for HIV, operators should familiarize themselves with PEP policy prior to conducting the testing.
- Humidity and temperature can adversely affect results.
- The optimal number of specimens to be tested at one time is 10.
- Do not use any other specimen than those specified. For plasma/venipuncture whole blood, EDTA-K2/sodium heparin can be used as anticoagulant. Other anticoagulants have not been tested and may give incorrect results.

SPECIMEN COLLECTION AND PREPARATION

- HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect **fingerstick whole blood** specimens:
  - Wear gloves.
  - Clean entire fingertip (preferable 3rd or 4th finger from non-dominant hand)with alcohol swab. Allow to dry (30 seconds).
  - Puncture the side of the finger with a new lancet each time. Disposed the lancet in sharps container immediately after using it. Do not use the lancet if the cap is already pulled off. Wipe away the first blood drop with a sterile gauze pad or cotton ball.
- To collect a fingerstick whole blood specimen by using a specimen dropper (for fingerstick whole blood):
  - Hold the specimen dropper. DO NOT TOUCH OR SQUEEZE BULB.
  - Immerse the open end of the specimen dropper into the blood drop and allow for the blood to draw into the specimen dropper up to marked line. Avoid air bubbles.
  - Squeeze bulb by covering the 2 air holes in it to dispense all whole blood onto the specimen well (S) of the test device for testing. Keep pressure on bulb while moving dropper away (avoids back suction).Then add 2 drops of buffer into the specimen well (S) and start the timer.
- To collect **serum or plasma or venipuncture whole blood** specimens:
  - Collect according to safe phlebotomy procedures, using vacuum technique into tubes for serum or plasma or venipuncture whole blood preparation.
  - Prepare serum or plasma from whole blood as soon as possible to avoid hemolysis. Don't use turbid or haemolysed specimens.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature (15-30°C) for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be stored at -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing.
- No qualitative performance difference were observed between experimental controls and 20 nonreactive or 20 reactive specimens subjected to 6 freeze/thaw cycles; however, multiple freeze/ thaw cycles should be avoided.

MATERIALS

Materials Provided

Components	IHI-T402WG
1. Test Device	x40
2. Specimen Dropper (For Serum/Plasma/Venipuncture Whole blood)	x40
3. 3mL Buffer	x2
4. Alcohol Swab	x40
5. Single-use Lancet	x40
6. Specimen Dropper (For Fingerstick Whole Blood)	x40
7. Instructions for Use	x1

Materials Required But Not Provided

- Specimen collection equipment and containers
- Cotton wool or gauze pad (for fingerstick whole blood only)
- Centrifuge
- Timer
- Biohazard waste containers for sharps and non sharps

TEST PROCEDURE

Allow the test device, buffer and specimen to reach room temperature (15-30°C) prior to testing.

1. Remove the test device from the foil pouch and use it as soon as possible (within one hour).
2. Place the test device on a clean and level surface. Label with specimen ID.
   For **serum or plasma** specimens: Hold the specimen dropper (for serum/plasma/Venipuncture Whole blood) vertically and **transfer 1 drop of serum or plasma** (approximately 25 µL) to the specimen well (S) of the test device, then **add 1 drop of buffer** (approximately 40 µL) and start the timer.
   For **venipuncture whole blood** specimens: Hold the specimen dropper (for serum/plasma/Venipuncture Whole blood)vertically and **transfer 2 drops of whole blood** (approximately 50 µL) to the specimen well (S) of the test device, then **add 2 drops of buffer** (approximately 80 µL) and start the timer.
   For **fingerstick whole blood** specimens: Take whole blood specimen with a 50 µL specimen dropper (for fingerstick whole blood) until mark line. And **add drawn specimen** (about 50 µL) on the specimen well (S) of the test device, then **add 2 drops of buffer** (approximately 80 µL) and start the timer.
3. Wait for the colored line(s) to appear. **Read results at 10 minutes. Do not read results after 20 minutes.**

INTERPRETATION OF RESULTS

(Please refer to the illustration above)
REACTIVE:* Two or three distinct colored lines appear. One line should always appear in the control line region (C), and another one or two apparent colored line(s) should appear in the test line region(s) (T1 and/or T2).
***NOTE 1:** The intensity of the color in the test line region (T1 and/or T2) will vary but any shade of color in the test line region (T1 and/or T2) should be considered reactive.
***NOTE 2:** Dual infection of HIV-1 and HIV-2 is quite rare. Dual reactivity observed in Abon HIV 1/2/O Tri-line HIV Rapid Test Device, i.e. HIV-1 line and HIV-2 line both reactive, is more likely to be caused by antibody cross-reactivity. Any specimen with dual reactivity should be referred for specific HIV-2 confirmatory testing, if a discretionary result is required.
NON-REACTIVE: One colored line appears in the control region (C). No apparent colored lines appear in the test line regions (T1 and/or T2).
INVALID: No line appears in the control line region (C). If this occurs, read the test procedure again and repeat the test with a new test device. If the result is still invalid, stop using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A control line is included in the test as an internal control. The test must absorb liquid and allow it to migrate along the membrane for the control line to appear. The control line does not control for the addition of adequate volume of specimen.
Quality control specimens are not supplied with this kit; however, it is recommended that quality control specimens be tested as a good laboratory practice.

LIMITATION

1. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) is for *in vitro* diagnostic use only. This test should be used for the detection of antibodies to HIV-1/2 in human whole blood, serum or plasma. The concentration of antibodies to HIV-1/2 can be determined by this qualitative test.
2. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) will only indicate the presence of antibodies to HIV-1/2 in the specimen and should not be used as the sole criteria for the diagnosis of HIV-1, HIV-2, and/or HIV-1 subtype O infection.
3. For confirmation of reactive test results, specimens should undergo further testing using different assays, such as rapid diagnostic tests, EIA and/or Western blotting in accordance with a validated HIV testing algorithm.
4. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5. Results should not be used to determine the serotype of HIV infections.
6. Due to possible antibody cross reactivity, the appearance of lines in both T1 and T2 does not necessarily indicate co-infection from HIV-1 and HIV-2.
7. False reactive results may arise due to rheumatoid factors, antinuclear antibodies, other viral infections (i.e. hepatitis B or hepatitis C), parasitic infections (i.e. schistosomiasis and trypanosomiasis), damage to test components by heat or humidity, when other test kit components (e.g. buffer or droppers) are substituted between test kits.
8. False non-reactive results may arise when titers of antibodies to HIV1/2 are very low, titers of antibodies to HIV1/2 are very high (high-hook effect), insufficient specimen volume added, excess of buffer added, damage to test components by heat or humidity.
9. False-negative results may be observed in individuals who are receiving effective antiretroviral therapy. [1,2,3]

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) was evaluated with 1,640 specimens from different countries in an unpublished multi-center field study, 1,000 specimens from a blood donation center and 3430 specimens from an in-house clinical study. Of the 6070 total specimens (which included whole blood, serum and plasma specimens), 1602 were found HIV seropositive and 4468 specimens were found HIV seronegative by a characterization testing algorithm comprising of EIA and/or Western blot. HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) showed 99.9% relative sensitivity, and 99.8% relative specificity compared to EIA and/or Western blot.

HIV 1/2/O Tri-line Rapid Test Device vs. EIA and/or Western blot

		EIA and/or Western blot		Total Results
HIV 1/2/O Tri-line Rapid Test Device	Results	Positive	Negative	
	Reactive	1601	10	1611
	Non-reactive	1	4458	4459
Total Results		1602	4468	6070

Relative Sensitivity: 99.9% (99.7-100.0%)*
Relative Specificity: 99.8% (99.6-99.9%)*
Relative Accuracy: 99.8% (99.7-100.0%)*
* 95% Confidence Interval

Specimen Types Consistency

50 HIV seropositive whole blood and paired plasma specimens, 26 HIV seropositive whole blood, paired plasma and serum specimens, 50 negative whole blood, paired plasma and serum specimens were tested with HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma).

EIA and/or Western blot	Specimen type	No. tested	HIV 1/2/O Tri-line Rapid Test Device	
			No. tested	Reactive
Negative	Plasma	50	50	0
	Serum	50	50	0
	Whole blood	50	50	0
Positive	Serum	26	0	26
	Plasma	76	0	76
	Whole blood	76	0	76

Paired whole blood, plasma, serum specimens show the consistent results with HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma).

Precision

Intra-Assay (same lot)

Within-run precision has been determined by using 10 replicates of five specimens: a negative, a low titer HIV-1 positive, a low titer HIV-1 **(subtype O)** positive, a medium titer HIV-1 positive and a HIV-2 positive. All above values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same five specimens: a negative, a low titer HIV-1 positive, a low titer HIV-1 (subtype O) positive, a medium titer HIV-1 positive and a HIV-2 positive. Three different lots of the HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device (Whole Blood/Serum/Plasma) have been tested using above specimens. The specimens were correctly identified >99% of the time.

BIBLIOGRAPHY

1. Delaney KP, Branson BM, Uniyal A, et al. *Evaluation of the Performance Characteristics of 6 Rapid HIV Antibody Tests.* Clinical infectious Diseases. 2011; 52(2): 257-263.
2. O'Connell RJ, Merritt TM, Malia JA, et al. *Performance of the OraQuick Rapid Antibody Test for Diagnosis of Human Immunodeficiency Virus Type 1 Infection in Patients with Various Levels of Exposure to Highly Active Antiretroviral Therapy.* Journal of Clinical Microbiology. 2003; 41(5):2153-2155
3. O'Connell RJ, Agan BK, Anderson SA, et al. *Sensitivity of the Multispot HIV-1/HIV-2 Rapid Test Using Samples from Human Immunodeficiency Virus Type 1-Positive Individuals with Various Levels of Exposure to Highly Active Antiretroviral Therapy.* Journal of Clinical Microbiology. 2006; 44(5): 1831-1833

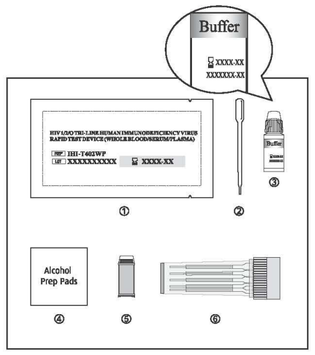
Index of Symbols

IVD	For *in vitro* diagnostic use only	LOT	Lot Number
	Store between 2-30°C		Use by
REF	Catalogue number		Tests per kit

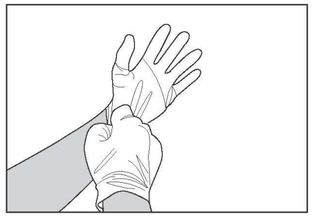
ABON™
Manufacturer
Abon Biopharm (Hangzhou) Co., Ltd.
#198 12th Street East,
Hangzhou Economic & Technological Development Area,
Hangzhou, 310018, P.R.China
Tel: +86-571-81638384
E-mail: abon.tech@alere.com

Preparation

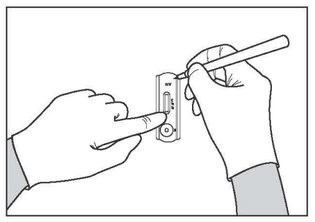
1 Open the package and check the content and the expiration date.



2 Wear gloves

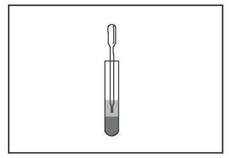


3 Open the pouch, Label with specimen ID. Use it as soon as possible (within one hour).

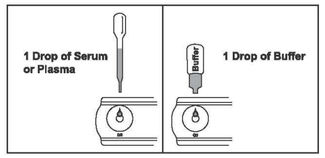


Serum or plasma specimens

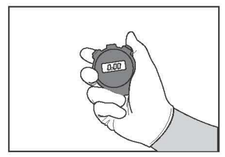
4 Draw the specimen from the specimen tube with a dropper (for serum/plasma/Venipuncture Whole blood).



5 Transfer 1 drop of serum or plasma (approximately 25 µL), then add 1 drop of buffer (approximately 40 µL).

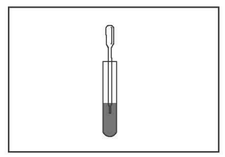


6 Start the timer.

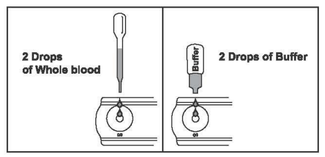


Venipuncture whole blood specimens

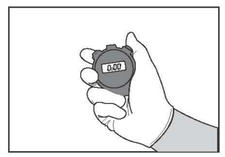
4 Draw the specimen from the specimen tube with a dropper (for serum/plasma/Venipuncture Whole blood).



5 Transfer 2 drops of whole blood (approximately 50 µL), then add 2 drops of buffer (approximately 80 µL).

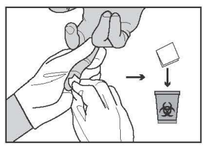


6 Start the timer.

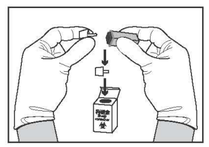


Fingerstick whole blood specimens

4 Clean entire fingertip (preferable 3rd or 4th finger from non-dominant hand) with alcohol swab. Dispose the alcohol swab.

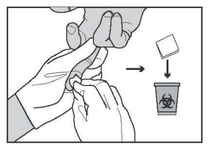


5 Take off the cap of the lancet and dispose the cap in sharps container.



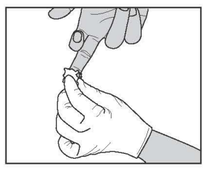
6 Puncture the side of the finger. Dispose the lancet in sharps container immediately after using it.

7 Wipe away the first blood drop with a sterile gauze pad or cotton wool.

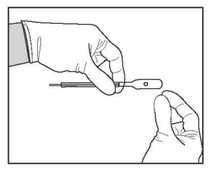


8 Hold the specimen dropper (for fingerstick whole blood). DO NOT TOUCH OR SQUEEZE BULB. Immerse the open end of the specimen dropper into the blood drop and allow for the blood to draw into the specimen dropper up to marked line.

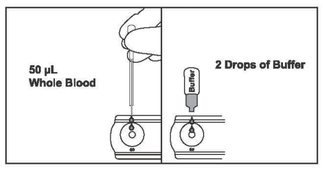
9 After collecting the sample, place a gauze pad or cotton wool on the finger until the bleeding stops.



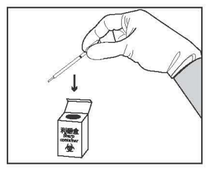
10 Covering the 2 air holes.



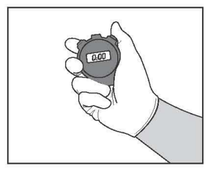
11 Squeeze bulb to dispense all whole blood onto the specimen well (approximately 50 µL). Keep pressure on bulb while moving dropper away (avoids back suction).Then add 2 drops of buffer into the specimen well (approximately 80 µL).



12 Dispose the specimen dropper (for fingerstick whole blood) in sharps container after testing.

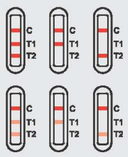


13 Start the timer.



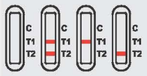
Read results

Wait for the colored line(s) to appear.
Read results at **10-20minutes**.



REACTIVE:* Two or three distinct colored lines appear. One line should always appear in the control line region (C), and another one or two apparent colored line(s) should appear in the test line region(s) (T1 and/or T2).
***NOTE 1:** The intensity of the color in the test line region (T1 and/or T2) will vary but any shade of color in the test line region (T1 and/or T2) should be considered reactive.
***NOTE 2:** Dual infection of HIV-1 and HIV-2 is quite rare. Dual reactivity observed in Abon HIV 1/2 /O Tri-line HIV Rapid Test Device, i.e. HIV-1 line and HIV-2 line both reactive, is more likely to be caused by antibody cross-reactivity. Any specimen with dual reactivity should be referred for specific HIV-2 confirmatory testing, if a discretionary result is required.

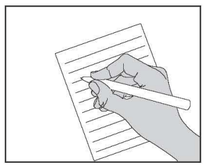
NON-REACTIVE: One colored line appears in the control region (C). No apparent colored lines appear in the test line regions (T1 and/or T2).



INVALID: No line appears in the control line region (C). If this occurs, read the test procedure again and repeat the test with a new test device. If the result is still invalid, stop using the test kit immediately and contact your local distributor.

Clear up/Record

Dispose devices, gloves in a proper biohazard waste container.



Record the test results.