WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: Alere™ HIV/Syphilis Duo
Number: PQDx 0179-012-00

Abstract

Alere™ HIV/Syphilis Duo with product codes **06FK30** and **06FK35**, manufactured by Standard Diagnostics, Inc., **Rest-of-World regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 28 October 2015. This public report was amended on 15 June 2017.

Alere™ HIV/Syphilis Duo is a solid phase immunochromatographic assay for the qualitative detection of antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1/2 and/or *Treponema pallidum* (TP) simultaneously in human serum, plasma or whole blood.

Alere™ HIV/Syphilis Duo contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigen (gp41), recombinant HIV-2 capture antigen (gp36) and Recombinant HIV-sub O antigen on test band 1 region and recombinant *Treponema pallidum* antigens (17KDa) on test band 2 region, respectively.

The recombinant HIV-1/2 antigen (gp41, gp36) - colloid gold conjugate, recombinant *Treponema pallidum* antigens colloid gold conjugate (17KDa), the specimen sample and sample diluent move along the membrane chromatographically to the test region (T) and form a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity.

This test device has a letter of HIV, SYP and C as Test Line HIV (HIV-1/2), Test Line SYP (Syphilis) and Control Line on the surface of the device. Both the Test Lines and Control Line in result window are not visible before applying any sample. The Control Line is used as a procedural control. The Control Line should always appear if the test procedure is performed properly and the test reagents of the Control Line are working.

The test kit contains:

	1x25 tests/kit Product code 06FK30	1x25 tests/kit Product code 06FK35
Test devices Individually foil pouched with a desiccant	25	25
Assay diluent Plastic bottle	1 vial of 4mL	1 vial of 4mL
Specimen transfer devices (capillary pipettes) Disposable, 20µl	N/A	25 units
Lancets Disposable, sterilized	N/A	25 units
Alcohol swabs Disposable	N/A	25 units
Instructions for Use	1 unit	1 unit

Storage:
The test kit should be stored at 1 - 30 °C.

Shelf-life:
24 months.

Summary of prequalification status for Alere™ HIV/Syphilis Duo

	Initial acceptance	
	Date	**Outcome**
PQ status amended	15 June 2017	listed
Status on PQ list	28 October 2015	listed
Dossier assessment	14 July 2015	MR
Inspection status	24 July 2015	MR
Laboratory evaluation	16 September 2015	MR

MR: Meets Requirements
NA: Not Applicable

Alere™ HIV/Syphilis Duo was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

Background information

Standard Diagnostics, Inc. submitted an application for prequalification of Alere™ HIV/Syphilis Duo. Based on the established prioritization criteria, Alere™ HIV/Syphilis Duo was given priority for prequalification.

Product dossier assessment

Standard Diagnostics, Inc. submitted a product dossier for Alere™ HIV/Syphilis Duo 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) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for Alere™ HIV/Syphilis Duo for prequalification.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (Production: 65, Borahagal-ro, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea 446-930 and Warehouse: 19-22, Dongtansandan 3-gil, Dongtan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea) of Alere™ HIV/Syphilis Duo in May 2015 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1). The 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 24 July 2015.

Laboratory evaluation

Alere™ HIV/Syphilis Duo was evaluated by WHO in the last quarter of 2014/first quarter of 2015 using serum/plasma specimens. From this evaluation, we drew the following conclusions:

Alere™ HIV/Syphilis Duo is an immunochromatographic assay for the detection of HIV-1/2 and syphilis antibodies in human serum/plasma, fingerstick/venous whole blood specimens. A volume of 10 µL of serum/plasma is needed 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 is done visually i.e. subjectively read.

In this limited evaluation a panel of 400 clinically-derived specimens was used. For antibodies to HIV, we observed an initial sensitivity (95% CI) of 100% (98.2% - 100%) and

an initial specificity (95% CI) of 99.5% (97.2% - 100%) compared to the reference assays. The final sensitivity (95% CI) was 100% (98.2% - 100%) and the final specificity (95% CI) was 99.5% (97.2% - 100%) compared to the reference assays. Lot to lot variation was acceptable.

For the case of antibodies to Treponema pallidum, we observed an initial sensitivity (95% CI) of 86.5% (81.0% - 90.9%) and an initial specificity (95% CI) of 99.5% (97.2% - 100%) compared to the reference assays. The final sensitivity (95% CI) was 87.0% (81.5% - 91.3%) and the final specificity (95% CI) was 99.5% (97.2% - 100%) compared to the reference assays. Lot to lot variation was acceptable.

For HIV eight seroconversion panels were evaluated. Alere™ HIV/Syphilis Duo detected on average 0.125 specimens earlier than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics) [EIA].

For syphilis one seroconversion panel was evaluated. Alere™ HIV/Syphilis Duo detected on average 2 specimens earlier than the benchmark assay; Vitros Syphilis (Ortho Clinical Diagnostics) [treponemal EIA] .

For the HIV mixed titer panel, Alere™ HIV/Syphilis Duo correctly identified all specimens compared to the reference result.

For the syphilis mixed titer panel, Alere™ HIV/Syphilis Duo correctly identified all specimens compared to the reference result.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210] Alere™ HIV/Syphilis Duo gave an indeterminate result for the HIV-1 group O specimen. Alere™ HIV/Syphilis Duo correctly identified all HIV-1 group M subtypes and HIV-2 specimens.

For the 1st International Reference Panel for anti-treponemal [NIBSC code 05/132], Alere™ HIV/Syphilis Duo correctly identified all specimens.

In this study, 2.5% of the results for syphilis were recorded as indeterminate, 0% for HIV. Results were interpreted independently by three technicians; the inter-reader variability was 0% for HIV and 4% for syphilis. The invalid rate was 0%.

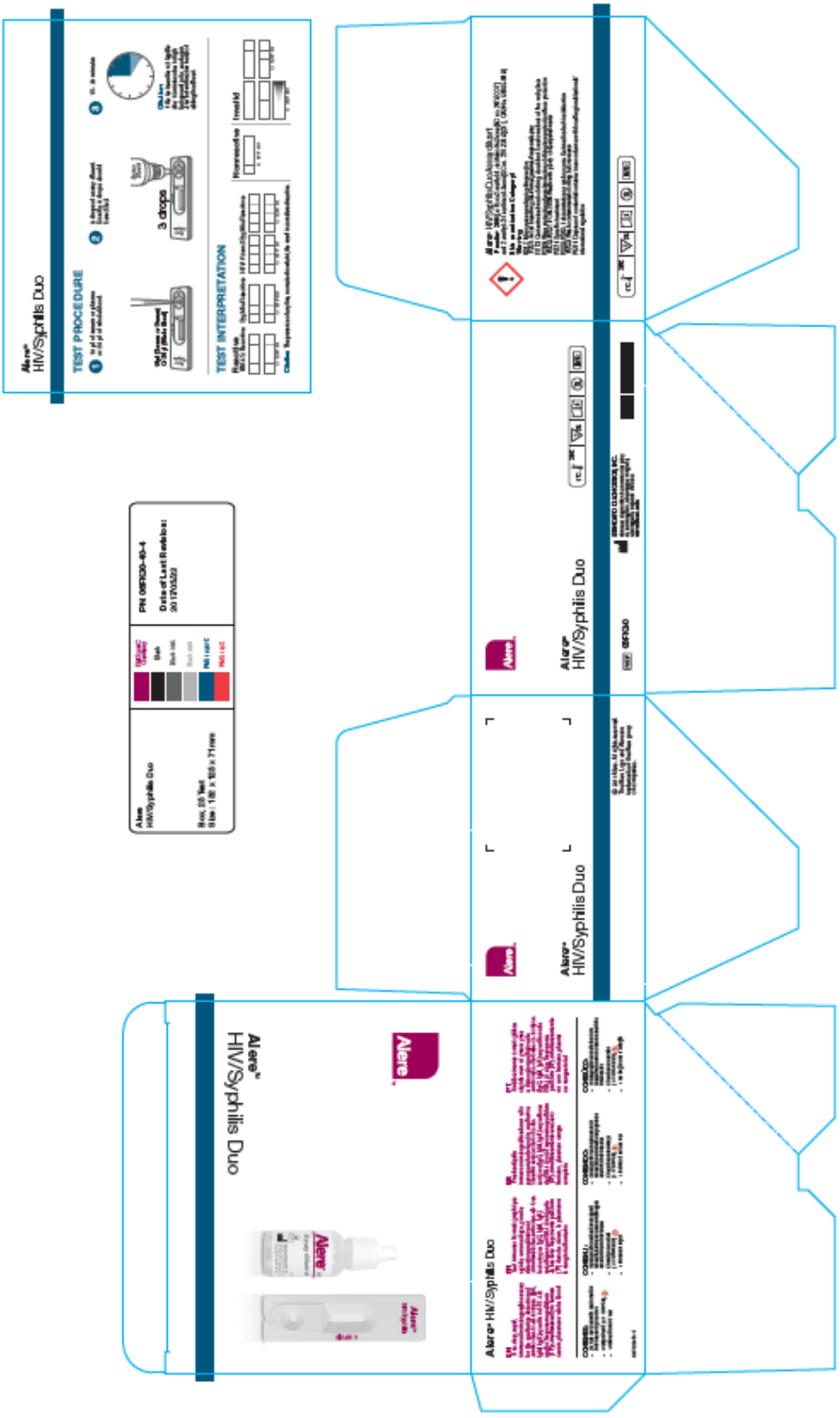
Change notification

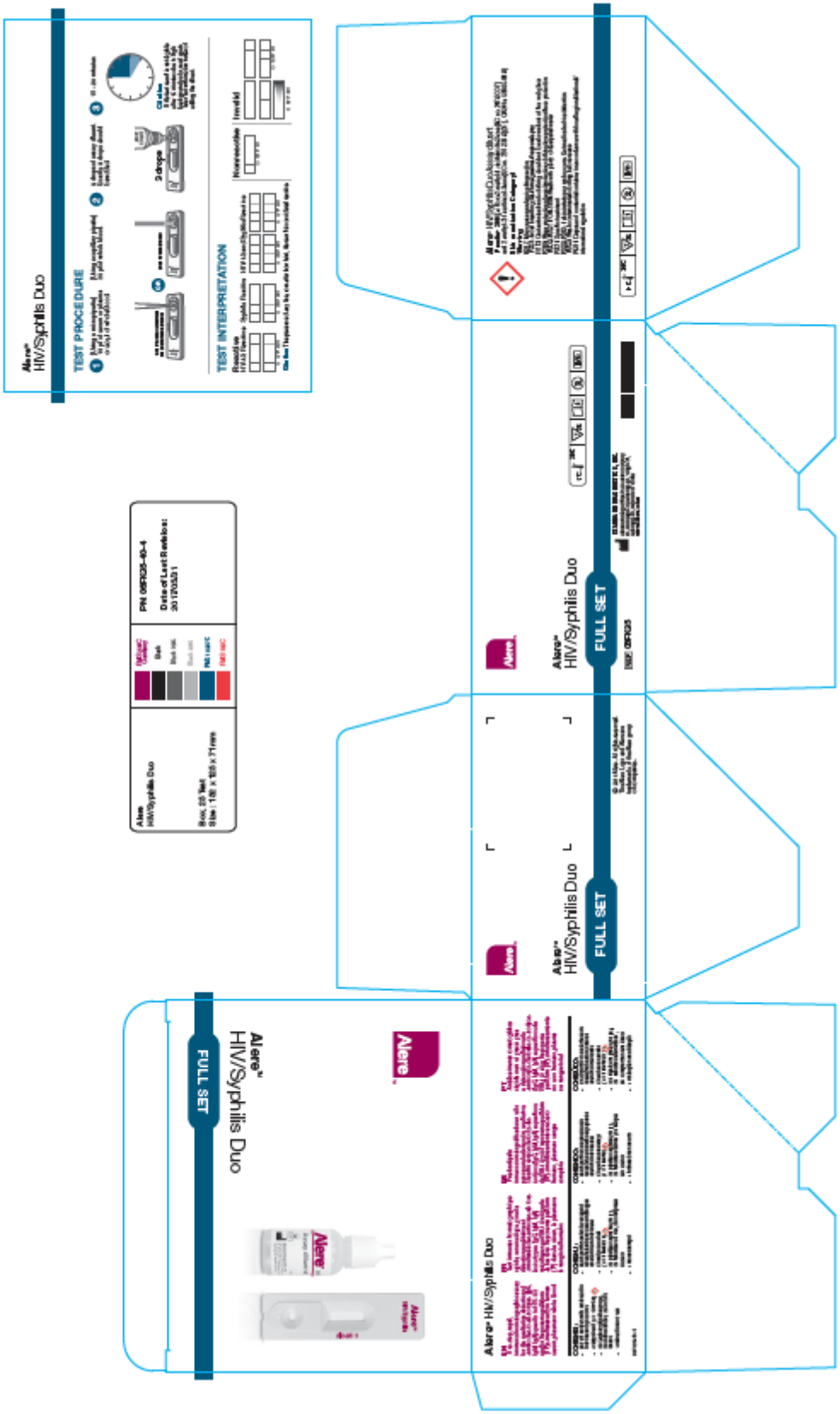
In 2017, Standard Diagnostics, Inc., submitted a change notification related to change of product name. This change notification was assessed and the product was found to meet WHO prequalification requirements.

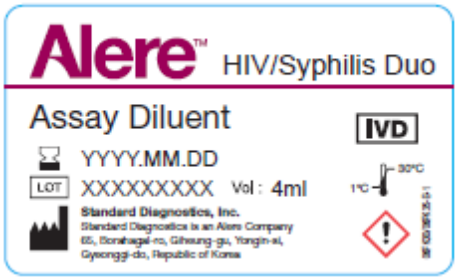
Labelling

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

1. Labels


Alere
HIV/Syphilis Duo
TEST PROCEDURE
3 drops
TEST INTERPRETATION
Alere
HIV/Syphilis Duo
Alere
HIV/Syphilis Duo
Alere
HIV/Syphilis Duo
Alere
HIV/Syphilis Duo


Alere™
HIV/Syphilis Duo
TEST PROCEDURE
TEST INTERPRETATION
FULL SET
Alere™
HIV/Syphilis Duo



2. Instructions for use

Alere™

Alere™ HIV/Syphilis Duo

REF 06FK30, 06FK35

Instructions for use / Mode d'emploi / Instrucciones de uso / Instruções de utilização

INTENDED USE

The Alere™ HIV/Syphilis Duo test is a rapid, qualitative test for the detection of antibodies to all isotypes(IgG, IgM, IgA) specific to HIV-1 including subtype-O, HIV-2 and Syphilis (*Treponema pallidum*) in human serum, plasma or whole blood. The Alere™ HIV/Syphilis Duo is intended only for professional use, for an initial screening test and for *in vitro* diagnostic use. Reactive specimens should be confirmed by a supplemental assay such as ELISA or Western Blot for antibodies to HIV-1/HIV-2 and the treponemal test (e.g. TPPA, TPHA) and non-treponemal test (e.g. RPR and VDRL) for antibodies to *T. pallidum*.

INTRODUCTION

HIV (Human Immunodeficiency Virus) is recognized as the etiologic agent of Aquired Immune Deficiency Syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected blood, certain body fluids or tissues, and from mother to fetus or child during the perinatal period. HIV-1 has been isolated from patients with AIDS and AIDS related complex, and from healthy persons with high potential risk of developing AIDS. Patients with HIV-2 are found primarily in parts of West Africa. Its course is marked by increasing levels of viral replication and the emergence of more virulent viral strains. HIV-1 and HIV-2 are similar in their morphology, cell tropism, host interaction and generic structure. Serological studies have determined that HIV-1 and HIV-2 have multiple common epitopes in core antigens but much less so in the envelope antigens. This clinical diagnosis of HIV may include the detection of antibodies to HIV-1/2 in human plasma or serum by immunoassay. The presence of HIV can be identified by detection of antibodies to HIV 1/2 in human serum, plasma and whole blood by immunoassay. This advanced assay utilizes recombinant antigens targeted against immunogenic proteins. The major immunoreactive antigens of these proteins are HIV-1 gp41, p24 and HIV-2 gp36.

The spirochetal bacillus *Treponema pallidum* (TP) is the causative agent of the venereal disease syphilis. The presence of TP can be identified by detection of antibodies to TP in human blood by immunoassay. This advanced assay utilizes recombinant antigens targeted against immunogenic TP membrane proteins.[7]

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TEST PRINCIPLE

The Alere™ HIV/Syphilis Duo test is a solid phase immunochromatographic assay for the qualitative, simultaneous detection of antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1/2 and/or *Treponema pallidum* in human serum, plasma or whole blood. The Alere™ HIV/Syphilis Duo contains a nitrocellulose membrane strip with two pre-coated test line regions. Test line region HIV is pre-coated with recombinant HIV-1 capture antigen (gp41), recombinant HIV-2 capture

antigen (gp36) and recombinant HIV-sub O antigen; test line region SYP is pre-coated with recombinant *Treponema pallidum* antigens (17KDa). When specimen and assay diluent is added to the specimen pad, it moves across the antigen-colloid gold conjugate pad. This mixture continues to migrate through the solid phase to the immobilized antigen on the membrane and forms a visible line. This antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to the antigen-colloid gold and to the immobilized recombinant antigens, forming visible HIV and C lines in the window. If antibodies to syphilis are present in the specimen, the antibodies bind to the antigen-colloid gold and to the immobilized recombinant antigens, forming visible SYP and C lines in the window. If antibodies to HIV-1 and/or HIV-2 and TP are undetectable, the antigen-colloid gold flows past the window and one control line appears in the window.

MATERIALS PROVIDED AND ACTIVE INGREDIENTS OF MAIN COMPONENTS

1. The Alere™ HIV/Syphilis Duo test kit contains the following items to perform the assay:

Catalog No.	06FK30	06FK35
Contents	1. 25 Test devices with desiccant in individual foil pouches 2. Assay diluent (1 x 4 ml/vial) ⚠ 3. 1 Instructions for use	1. 25 Test devices with desiccant in individual foil pouches 2. Assay diluent (1 x 4 ml/vial) ⚠ 3. 25 Capillary pipettes (20 µl), 25 Sterile lancets, 25 Alcohol swabs 4. 1 Instructions for use

2. Active ingredients of main components:

1 test strip includes:	Assay diluent includes:
- Gold conjugates: Recombinant HIV-1 antigen – gold colloid (0.029±0.006 µg), Recombinant HIV-2 antigen – gold colloid (0.043±0.009 µg), Recombinant Treponema pallidum antigen – gold colloid (0.017±0.003 µg), Chicken IgY– gold colloid (0.038±0.008 µg) - Test line HIV: Recombinant HIV-1 antigen (gp41) (0.384±0.077 µg), Recombinant HIV-2 antigen (gp36) (0.640±0.128 µg), Recombinant HIV - sub O antigen (0.224±0.045 µg) - Test line SYP: Recombinant Treponema pallidum antigen (17KDa) (0.480±0.096 µg) - Control line: Mouse monoclonal anti-chicken IgY (0.640±0.128 µg)	- 20 mM Tris-HCl buffer (100 mM) - Tricine (100 mM) - Casein (0.2 w/v%) - Proclin™ 300 (0.05 w/v%) - Sucrose (5 w/v%) - Sodium azide (0.01 w/v%)

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※ Classification according to Regulation (EC) No 1272/2008:

Product identifier	Trade name	Alere™ HIV/Syphilis Duo Assay diluent		
	Hazardous substance	Proclin™ 300 (5-chloro-2-methyl-4- isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1), CAS No. 55965-84-9)		
Classification		Skin sensitization Category 1	Signal Word	Warning
Hazard Pictogram			Hazard Statement	H317: May cause an allergic skin reaction
Precautionary statements				
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapours/spray P272: Contaminated work clothing should not be allowed out of the workplace P280: Wear protective gloves/protective clothing/eye protection/face protection			
Response	P302+P352: IF ON SKIN: Wash with plenty of soap and water P321: Specific treatment P333+P313: If skin irritation or rash occurs: Get medical advice/attention P363: Wash contaminated clothing before reuse			
Disposal	P501: Dispose of contents/container in accordance with local/regional/national/international regulation			

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipette, Protective gloves, Timer, Biohazard container

KIT STORAGE AND STABILITY

1. The test kit should be stored at a temperature between 1 °C and 30 °C. Do not freeze the kit or its components. Assay diluent cap should be kept firmly sealed between each use.
2. Assay diluent may be opened and resealed for each assay. It is stable until expiration date if kept at 1 - 30 °C.
3. The test device is sensitive to both heat and humidity. Check the desiccant for color change of the humidity indicator. Do not use the test device if the desiccant is green.

ENGLISH

– 3 –

4. Perform the test immediately after removing the test device from the foil pouch.
5. Do not use the test kit beyond its expiration date. The shelf life of the kit is as indicated on the outer package.
6. Do not use the test kit if the pouch is damaged or the seal is broken.

WARNINGS

1. The test devices are for *in vitro* diagnostic use only. Do not reuse the test device.
2. The instructions must be followed exactly to achieve accurate results. Any individual performing an assay with this product must be trained in its use and must be proficient.
3. Do not use the pipette by mouth, smoke, drink, eat, apply cosmetics, or handle contact lenses in areas where specimens or kit components are being handled.
4. Wear protective gloves while handling specimens and wash hands thoroughly afterwards.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials in a biohazard container as if they were infectious waste.
7. Do not mix or interchange different specimens.
8. Do not eat the desiccant from the foil pouch.
9. Avoid splashing or aerosol formation of specimen and assay diluent.
10. Do not mix or interchange components among different lots or those for other products.
11. Do not drink assay diluent.
12. Care should be taken to avoid contamination of the bottle nozzle when dropping assay diluent into the specimen well.
13. The assay diluent contains a proprietary antimicrobial agent, sodium azide, which presents no hazard to the user if normal laboratory safety precautions are followed. If contact with assay diluent to the eyes and/or skin, wash affected area with soap and water immediately. If irritation or signs of toxicity occur, seek medical attention.
14. The assay diluent contains sodium azide, which may react with lead or copper plumbing to form highly explosive metal azide compounds. When disposing of these reagents through plumbing fixtures, flush with a large volume of water to prevent azide build-up in drains.
15. Safety data sheet available for professional user on request.

ENGLISH

SPECIMEN COLLECTION AND HANDLING

1. **Whole blood**

[Collection by venipuncture]

- Using venipuncture, draw whole blood into the collection tube (containing anticoagulants including heparin, EDTA and sodium citrate).
- If the blood specimen is not immediately tested, it must be refrigerated at 2 - 8 °C.
- If stored at 2 - 8 °C, the blood specimen must be tested within 3 days of refrigeration.
- Do not use a blood specimen stored for more than 3 days; it can cause a nonspecific reaction.
- Bring blood specimens to room temperature (15 - 30 °C) prior to use.

[Collection using a lancet]

- Clean the area to be lanced with an alcohol swab.
- Squeeze the fingertip then prick the lateral side of the finger with the sterile lancet provided. Wipe away the first blood drop. Then, safely dispose of the lancet immediately after.
- Immerse the open end of a new 20 µl capillary pipette in the next blood drop and release the pressure to draw blood into the capillary pipette up to the black line.

2. **Plasma or serum**

- If plasma or serum specimens are not tested immediately, they must be refrigerated at 2 - 8 °C. For storage period longer than 2 weeks, freezing (below -20 °C) is required. Bring plasma or serum specimens to room temperature (15 - 30 °C) prior to use.
- Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

[Plasma]

- Using venipuncture, draw whole blood into the collection tube (containing anticoagulants including heparin, EDTA and sodium citrate) then centrifuge the tube to generate a plasma specimen.

[Serum]

- Using venipuncture, draw whole blood into the collection tube (NOT containing anticoagulants) then leave for 30 minutes to allow blood coagulation to occur. Centrifuge the tube to generate a serum specimen.

*Anticoagulants including heparin, EDTA and sodium citrate do not affect the test result. Use of other anticoagulants has not been validated. Their use may affect the test result.

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TEST PROCEDURE (REFER TO FIGURE)

1. Allow all kit components and specimens to reach a temperature between 15 °C and 30 °C prior to testing.
2. Remove the test device from foil pouch and place it on a flat, dry surface. Label the test device with a patient identifier.
3. [Using a micropipette]
   Dispense 10 µl of plasma or serum specimen or dispense 20 µl of whole blood specimen into the specimen well marked "S".
   Or,
   [Using a capillary pipette]
   Dispense 20 µl of drawn whole blood specimen into the specimen well marked "S".
   Caution: Lightly touch the capillary pipette to the specimen pad while dispensing.
4. Dispense 3 drops (approximately 100 µl) of assay diluent into the specimen well marked "S".
   Caution: Do not let bottle nozzle touch device in order to avoid cross-contamination. Hold bottle vertically while dispensing. If you do not hold the bottle vertically, it can lead to inaccurate results. Exactly, 3 drops should be added. Adding more than 3 drops may result in reddish color background or an invalid result.
5. As the test begins to work, you will see purple color move across the result window in the center of the test device.
6. Interpret test results 15 - 20 minutes after adding assay diluent. Do not read after 20 minutes.
   Caution: If the test result is not legible after 15 minutes due to high background color, read again later but within 20 minutes of adding the diluent. Reading outside of this time frame (before 15 min or after 20 min) may provide false results.

TEST INTERPRETATION (REFER TO FIGURE)

The characters "HIV", "SYP" and "C" are printed on the test device. They correspond to the positions of the HIV, Syphilis and control lines in the test window.

ENGLISH

- **Nonreactive result:**
  The presence of only one purple colored band at the control line (C) position indicates a nonreactive result.
- **Reactive result:**
  Caution: The presence of any line, no matter how faint, the result is considered reactive.
  1) HIV-1/2 reactive: The presence of both test line HIV and the control line (C) indicates a reactive result for anit-HIV-1/2.
  2) Syphilis reactive: The presence of both test line SYP and the control line (C) indicates a reactive result for anti-*Treponema pallidum*.

  3) HIV-1/2 and Syphilis reactive: The presence of test line HIV, test line SYP and the control line (C) indicates reactive result for both anti-HIV-1/2 and anti-*Treponema pallidum*.
- **Invalid result:**
  Absence of the control line (C) and/or presence of a pink/purple smear in the result window indicate an invalid result. Instructions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be retested using a new test device.

TEST LIMITATIONS

1. A reactive result with the Alere™ HIV/Syphilis Duo test indicates presence of antibodies to *Treponema pallidum*. A reactive result alone is insufficient for diagnosis of Syphilis infection. Results must be interpreted along with other clinical information available to the physician.
2. A reactive result may indicate infection with HIV-1/2. Immunochromatographic testing alone cannot be used to diagnose AIDS. An AIDS diagnosis can only be made in a clinical setting if an individual meets the case definition for AIDS established by the Centers for Disease Control (CDC). Reactive specimens should be confirmed by a supplemental assay such as ELISA or Western Blot test.
3. A nonreactive result does not eliminate the possibility of infection with HIV-1/2 and/or *Treponema pallidum*. The specimen may contain low levels of antibodies that cannot be detected by Alere™ HIV/Syphilis Duo. If a test result is nonreactive and clinical symptoms persist, additional testing using other clinical methods is recommended.
4. Some known HIV-infected persons taking antiretroviral medication have been shown to produce false negative results when tested by rapid diagnostic tests.[9, 10]
5. Where clinical presentation or other data would suggest an inconsistent test result then the individual should be tested by nucleic acid testing (NAT) technologies immediately and/or retested for antibodies to HIV after more than 21 days since the original testing.

INTERNAL QUALITY CONTROL

The Alere™ HIV/Syphilis Duo test device has test line HIV, test line SYP and the control line C on the surface of the device. The lines are not visible before applying a specimen. The control line is used for procedural control and shows that the diluent has been applied successfully and that the active ingredients of main components on the strip are functional, but is not a guarantee that the specimen has been properly applied and does not represent a reactive specimen control.

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PERFORMANCE CHARACTERISTICS

1. Diagnostic sensitivity
Clinical performance studies were performed by two external evaluation centres, the Institute of Tropical Medicine in Belgium and the German Red Cross in Germany.

1) Anti-HIV-1 and anti-HIV-2 reactive specimens
400 anti-HIV-1 reactive specimens, among which 40 non-B subtype and 100 anti-HIV-2 reactive specimens were tested in the Institute of Tropical Medicine, Belgium. The Diagnostic Sensitivity for anti-HIV antibody detection, calculated on 500 reactive specimens, is 99.8%.

	Alere™ HIV/Syphilis Duo		
	HIV nonreactive	HIV reactive	Sensitivity (95 % CI)
Anti-HIV-1 reactive	1	259	
Anti-HIV-1 reactive non-B subtype	0	40	
Anti-HIV-1 and anti-TP reactive	0	100	
Anti-HIV-2 reactive	0	100	
Total	1	499	99.8 % (98.9 - 100 %)

2) Anti-*Treponema pallidum* reactive specimens
150 anti-*Treponema pallidum* reactive specimens, among which 100 anti-HIV-1 reactive and 50 anti-HIV-1 nonreactive specimens were tested in the Institute of Tropical Medicine, Belgium. The Diagnostic Sensitivity for anti-*Treponema pallidum* antibody detection, calculated on 150 confirmed reactive specimens is 90.0%.

	Alere™ HIV/Syphilis Duo		
	Syphilis nonreactive	Syphilis reactive	Sensitivity (95 % CI)
Anti-HIV-1 reactive	13	87	
Anti-HIV-1 nonreactive	2	48	
Total	15	135	90.0 % (84.0 - 94.3 %)

2. Diagnostic specificity
In total 1,000 EDTA plasma and 500 whole blood specimens from blood donors were tested. The specimens originated from 2 collection sites in Germany; Frankfurt and Kassel.

ENGLISH

– 8 –

		HIV		Syphilis	
		Nonreactive	Reactive	Nonreactive	Reactive
Blood donors	EDTA Plasma	1,000	0	1,000	0
	Whole blood	500	0	499	1
Specificity (95 % CI)		100 % (99.7 - 100 %)		99.9 % (99.6 - 100 %)	

3. Laboratories in Ghana, Mexico, Laos, Togo, Kenya, and Myanmar participated in the evaluation during 2012–2013. Each site characterized sera using *T. pallidum* particle agglutination assay or *T. pallidum* hemagglutination assay and HIV enzyme immunoassay, Western Blot, and/or HIV antibody rapid tests.

1) Laboratory Performance for Detection of Anti-HIV

Country	Year	*n*	True Reactive	False Reactive	False Nonreactive	True Nonreactive	Sensitivity Estimate (95 % CI)	Specificity Estimate (95 % CI)
Ghana	2012	400	250	0	0	150	100 % (98.54 - 100 %)	100 % (97.57 - 100 %)
Togo	2013	310	203	0	0	107	100 % (98.20 - 100 %)	100 % (96.61 - 100 %)
Myanmar	2013	245	114	1	0	130	100 % (96.82 - 100 %)	99.24 % (95.82 - 99.98 %)
Kenya	2013	698	345	0	1	352	99.71 % (98.40 - 99.99 %)	100 % (98.96 - 100 %)
Mexico	2013	527	158	0	0	369	100 % (97.69 - 100 %)	100 % (99.01 - 100 %)
Laos	2013	156	53	3	0	100	100 % (93.28 - 100 %)	97.09 % (91.72 - 99.40 %)
Total	-	2,336	1,123	4	1	1,208	99.91 % (99.51 - 100%)	99.67 % (99.16 - 99.91 %)

2) Laboratory performance for Detection of Anti-*Treponema pallidum*

Country	Year	*n*	True Reactive	False Reactive	False Nonreactive	True Nonreactive	Sensitivity Estimate (95 % CI)	Specificity Estimate (95 % CI)
Ghana	2012	400	250	1	0	149	100 % (98.54 - 100 %)	99.33 % (96.34 - 99.98 %)
Togo	2013	241	88	1	0	152	100 % (95.89 - 100 %)	99.35 % (96.41 - 99.98 %)
Myanmar	2013	200	74	1	1	124	98.67 % (92.79 - 99.97 %)	99.20 % (95.62 - 99.98 %)
Kenya	2013	698	85	0	0	613	100 % (95.75 - 100 %)	100 % (99.40 - 100 %)
Mexico	2013	414	106	1	1	306	99.07 % (94.90 - 99.98 %)	99.67 % (98.20 - 99.99 %)
Laos	2013	106	6	0	0	100	100 % (54.07 - 100 %)	100 % (96.38 - 100 %)
Total	-	2,059	609	4	2	1,444	99.67 % (98.82 - 99.96 %)	99.72 % (99.29 - 99.92 %)

ENGLISH

– 9 –

4. Sensitivity on seroconversion panels
30 commercially available HIV seroconversion panels were tested showing how early detection can be obtained with the Alere™ HIV/Syphilis Duo test. 20 HIV Ab panels (pertaining 62 early seroconversion samples) were from the Institute of Tropical Medicine and 10 panels (pertaining 12 early seroconversion samples) were from the Paul-Ehrlich-Institute. The performance of the Alere™ HIV/Syphilis Duo test on seroconversion panels is comparable to other CE marked Anti-HIV-1/2 screening and rapid tests.

5. Analytical specificity

- Cross reactivity
The following 16 potential cross-reacting pathogens had no effect on test results of Alere™ HIV/Syphilis Duo.

Anti-HBs	EBV	HIV (to SYP line)	Syphilis (to HIV line)
Borrelia burgdorferi	HAV	HTLV	Toxoplasma
Chlamydia	HBsAg	Influenza	*Trypanosoma cruzi I / II*
CMV	HCV	*Plasmodium vivax*	*Trypanosoma gambiense*

- Interfering substances
The following 9 substances had no effect on test results of Alere™ HIV/Syphilis Duo.
: Pregnant women, High cholesterol (245 mg/dL), High bilirubin (1.4 mg/dL), Rheumatoid factor (28 IU/ml), Lipemic, Hemolyzed, Autoimmune, Alcoholic cirrhosis, Multiparous pregnancy
- Pharmaceutical substances
There was no significant interference with the following 25 drugs on Alere™ HIV/Syphilis Duo. All drugs were tested at concentrations of 250 µg/ml.

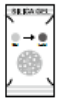
Abacavir	Cholecalciferol	Folic acid	L-ascorbic acid	Pantoprazole
Acetaminophen	Cyclobenzaprine	Hydrochlorothiazide	Magnesium sulfate	Pyrazinamide
Acetylsalicylic acid	Darunavir	Ibuprofen	Metformin	Rifampicin
Amoxicillin	Diclofenac	Iron Chloride	Naproxen	Ritonavir
Aspirin	Ergocalciferol	Isoniazid	Nevirapine	Salicylic acid

6. Reproducibility of the Alere™ HIV/Syphilis Duo has been demonstrated by within-run, between-run and batch-to-batch studies using in-house reference panels. All values were identical to reference panel acceptance criteria.

ENGLISH

PREPARATION

1 

2 
Yellow : Valid
Green : Invalid

Open the foil pouch and look for the following:
1. Test device
2. Desiccant

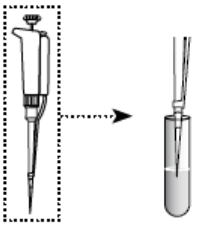
Then, label the device with the patient identifier.

***Caution:** If Yellow - OK, if Green – do not use test device, discard.

TEST PROCEDURE

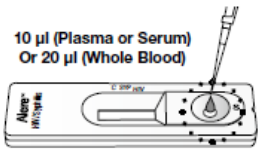
I. Blood (by venipuncture), Plasma or Serum specimen

1 Using a micropipette draw
plasma or serum: **10 µl** specimen
or
whole blood: **20 µl** specimen.

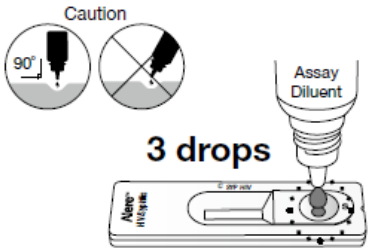


ENGLISH

2 Dispense
10 µl of plasma or serum specimen
or
20 µl of whole blood specimen
into the specimen well marked "S".



3 Dispense **3 drops** (approximately 100 µl) of assay diluent into the specimen well marked "S". Hold bottle vertically while dispensing. Do not let bottle nozzle touch device in order to avoid cross-contamination.

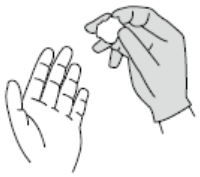


ENGLISH

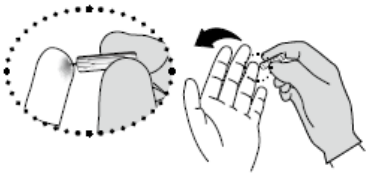
4 Interpret test results 15 - 20 minutes after adding assay diluent. Reading outside of this time frame (before 15 min or after 20 min) may provide false results.

II. Blood specimen (with a lancet)

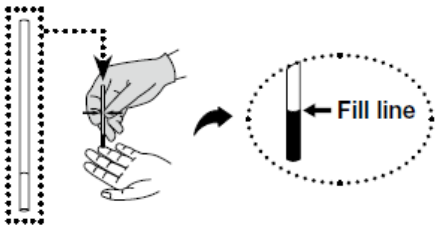
1 Clean the area to be lanced with an alcohol swab.



2 Squeeze the fingertip then prick the lateral side of the finger with the sterile lancet provided. Wipe away the first blood drop. **Then, safely dispose of the lancet immediately after.**



3 Immerse the open end of a new **20 µl** capillary pipette in the next blood drop and release the pressure to draw blood into the capillary pipette up to the black line.



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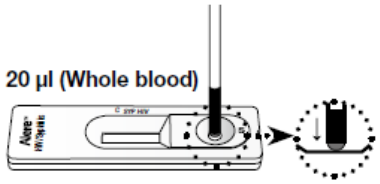
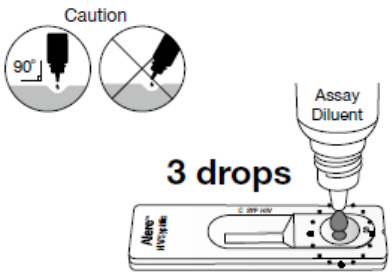
4 Dispense **20 µl** of drawn whole blood specimen in the specimen well marked "S". Lightly touch the capillary pipette to the specimen pad while dispensing.

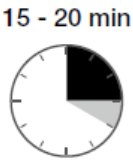


5 Dispense **3 drops** (approximately 100 µl) of assay diluent into the specimen well marked "S". Hold bottle vertically while dispensing. Do not let bottle nozzle touch device in order to avoid cross-contamination.



ENGLISH

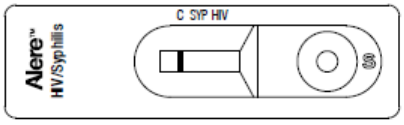
6 Interpret test results 15 - 20 minutes after adding assay diluent. Reading outside of this time frame (before 15 min or after 20 min) may provide false results.



INTERPRETATION

Nonreactive

The presence of only one purple colored line (C) within the result window indicates a nonreactive result.



Reactive

***Caution:** The presence of any line, no matter how faint, the result is considered reactive.

HIV-1/2 Reactive

Presence of both "C" and "HIV" lines

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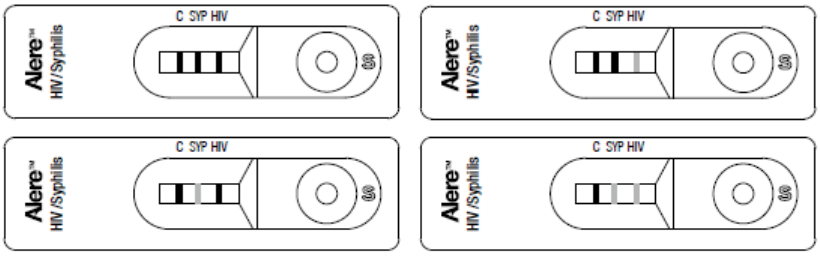
Syphilis Reactive

Presence of both "C" and "SYP" lines

HIV-1/2 and Syphilis Reactive

Presence of "C", "HIV" and "SYP" lines



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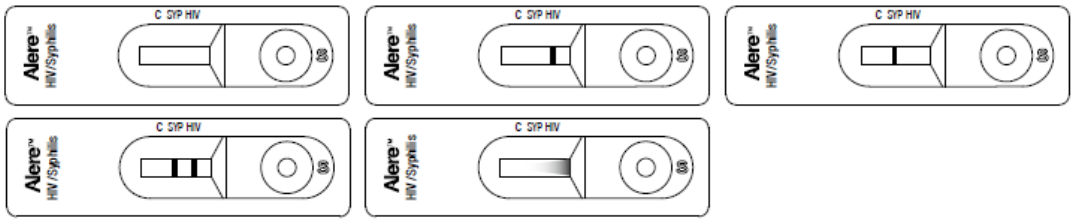
– 16 –

Invalid

Absence of the control line (C) and/or presence of a pink/purple smear in the result window indicate an invalid result. Instructions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be retested using a new test device.



Product. Disclaimer:
While every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the manufacturer and distributor and test results may accordingly be affected by environmental factors and/or user error. The subject of the diagnosis should consult a doctor for further confirmation of the test result.

Warning:
The manufacturers and distributors of this product shall not be liable for any direct, indirect, or consequential losses, liability, claims, costs or damages arising from or related to an incorrect reactive or non-reactive diagnosis using this product.

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– 17 –