# WL Check

## *HIV 1+2*

**For the detection of anti-HIV-1, anti-HIV-1 group O and HIV-2 antibodies in serum, plasma and whole blood**

### SUMMARY

The human immunodeficiency viruses (HIV-1 and HIV-2) are the causative agent of the Acquired Immunodeficiency Syndrome (AIDS). These retroviruses are transmitted by exposure to certain infected body fluids, primarily blood or genital secretions and contaminated products derived from blood and placental transmission. In individuals infected with these viruses antibodies appear as a consequence of the immune system response to the viral invasion. The detection of these antibodies is used as a diagnostic tool.

The U.S. Centers for Disease Control and Prevention (CDC) in their «Advancing HIV Prevention» (AHP) emphasize the importance of the use of rapid tests for HIV screening to facilitate access to early diagnosis in high prevalence areas, high-risk individuals or areas where other diagnostic methods such as ELISA, Western Blot or nucleic acid amplification are not available. They are also useful at the time of delivery, especially for the diagnosis of women who have not been controlled during pregnancy.

Moreover, these rapid tests are a diagnostic tool that can play an important role in prevention campaigns and in the diagnosis in clinical and non-clinical cases, thereby facilitating early detection of infection.

**WL Check HIV 1+2** is a rapid test that jointly detects the presence of anti-HIV-1, anti-HIV-1 group O and anti-HIV-2 antibodies.

### PRINCIPLE

**WL Check HIV 1+2** is an "in vitro" immunochromatographic test, visually read, for qualitative detection of antibodies against HIV-1 and HIV-2 in serum, plasma and whole blood.

The test consists of a plastic cassette containing:

- A nitrocellulose membrane sensitized with recombinant HIV-1 and HIV-2 antigens in the «T» test zone.
- A patch impregnated with recombinant antigens specific for HIV-1 and HIV-2 conjugated to colloidal gold.

The sample and buffer are added into the «S» sample well, solubilizing and mixing with the conjugate of recombinant antigens. Then, this mixture migrates by capillary action through the nitrocellulose membrane. If the sample is reactive, the antibodies to HIV-1 and HIV-2 that are present form a complex with the antigen conjugated to colloidal gold. This complex will then bind to the immobilized antigens in the «T» test zone of the nitrocellulose membrane, forming a line of pink-purple red color. The absence of this line indicates a negative result. As a control procedure, the test includes a light-blue colored «C» control zone that changes to pink-purple red after the addition of the sample. The absence of this line invalidates the results.

### PROVIDED MATERIAL

**A. Reagent A**: plastic cassette composed of a nitrocellulose membrane sensitized with recombinant antigens specific for HIV-1 and HIV-2 and conjugate of recombinant antigens. Ready to use.

**B. Reagent B**: 15 mm sodium borate buffer, 0.95 g/L azide, surfactant, pH = 9.1. Ready to use.

### INSTRUCTIONS FOR USE

The provided material is ready to use.

### NON-PROVIDED REQUIRED MATERIAL

- Automatic micropipette for measuring stated volumes.
- 20 uL disposable device for capillary whole blood sample (only provided in some Package Sizes).
- Disposable tips.
- Stopwatch or timer.
- Material for sample collection.
- Disposable gloves, lab coats, eye protection.
- Container for disposal of biological waste.
- Sodium hypochlorite.

### WARNINGS

- Carefully read the instruction manual before testing, and follow the instructions.
- Avoid using the test if the package is damaged.
- Avoid using reagents beyond the expiration date stated on the packaging.
- The reagents are for "in vitro" diagnostic use.
- This test provides a qualitative and visual outcome. A good light source is required for reading the results.
- Avoid mixing reagents from different lots.
- Avoid using reagents from other sources.
- Avoid touching the nitrocellulose membrane with your fingers.
- Follow safe practices when using biological samples and reagents:
  . Handle all patient specimens as potentially infectious.
  . Wear gloves, lab coats and eye protection.
  . Do not pipette by mouth.
  . Do not eat, drink, smoke, use cosmetics or handle contact lenses in areas where these materials are used.
  . Clean and disinfect spills of specimens or reagents using sodium hypochlorite (5% final concentration) or other suitable disinfectant. To inactivate the material used autoclave for 1 hour at 121ºC.
- Avoid bubble formation in «S» sample well when adding the sample and the Reagent B. When dispensing Reagent B discard any drop with bubbles.

- When performing the test, place the cassette on a clean, flat surface without vibrations.
- Avoid shaking the cassette during the test.
- The cassette is disposable, not reusable. Discard into containers for biohazard risk material.
- Reagent B has low concentrations of sodium azide as a preservative.
- The reagents and samples must be discarded according to current regulations.

**STABILITY AND STORAGE INSTRUCTIONS**

The kit is stable between 2-30ºC until the expiration date stated on the box. Do not freeze. If stored refrigerated, ensure that the pouch reaches room temperature before use, otherwise content moistening will be favored.
The cassette should remain in its original sealed pouch. Do not open the package until use.

**SAMPLE**

Serum, plasma and whole blood obtained by venipuncture or capillary puncture

**a) Collection:** collect specimens aseptically and avoid hemolysis.
- Serum: collected from whole blood without anticoagulants. Separate the serum from the clot immediately to avoid hemolysis. Serum collected in tubes with accelerator and separator gel may be used.
- Plasma: use plasma collected with EDTA, citrate or heparin.
- Whole blood (venipuncture): use blood collected with EDTA, citrate or heparin.
- Whole blood (capillary puncture): use blood collected with disposable device (only provided in some Package Sizes). Perform the test immediately. If another device is used, the user is responsible for validating the device for capillary whole blood sample collection.

**b) Stability and storage:**
- Serum and plasma: store between 2-10ºC. If the test is not performed within 5 days, store the sample at -20ºC. Avoid performing multiple freezing and thawing cycles. This can lead to erroneous results. If using frozen samples, they must be homogenized and centrifuged before use.
- Blood (venipuncture): can be stored up to 3 days between 2-10ºC. Do not freeze.
- Blood (capillary): use immediately. Do not freeze.

**c) Interfering Substances**: in enriched sera and plasma samples, no interference was observed by:
. Hemolysis: up to 1.1 g/dL hemoglobin.
. Lipemia: up to the equivalent of 1500 mg/dL triglycerides, after enrichment with Intralipid®.
. Bilirubin: up to 30 mg/dL.
. Ascorbic acid: up to 50 mg/dL.

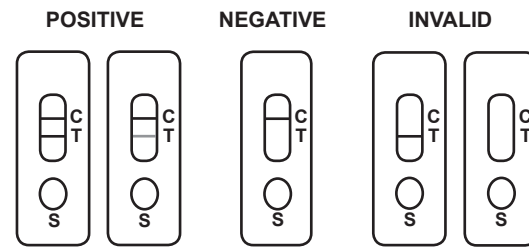
**d) Transportation**: if the samples must be transported, package according to legal specifications regarding the shipment of infectious material.

**PROCEDURE**

**1.** The reagents and samples must be at room temperature (18-30ºC) before use.
**2.** Remove the cassette from its sealed pouch immediately before use.
**3.** Place the cassette on a clean, flat surface without vibrations.
**4.** Place the sample on the absorbent surface of the «S» sample well.
**- For serum or plasma:**
. Place 10 uL with automatic micropipette.
. Wait for 10-15 seconds until the sample is absorbed.
. Add 3 drops (100 uL) of Reagent B in the «S» sample well.
. Start the timer.
**- For whole blood:**
. Place 20 uL with automatic micropipette*
. Wait for 10-15 seconds until the sample is absorbed.
. Add 3 drops (100 uL) of Reagent B in the «S» sample well.
. Start the timer.
NOTE: *If capillary whole blood is used employ the 20 uL disposable device (only provided in some Package Sizes).
**5.** In either case, read the results from 20 to 30 minutes. Do not read past the 30 minutes as erroneous results may be obtained. Some positive samples react immediately while others react more slowly within the specified reading time. Due to particular characteristics of some samples, the background color of the membrane may be slightly pink without affecting the interpretation of results.

**ASSAY VALIDATION CRITERIA**
- The cassette has a light blue line in the «C» control area that identifies the **WL Check HIV 1+2** test, indicating that the test components are present and active.
- When the control line changes from light blue to a pink-purple red color, it confirms that the appropriate sample volume was added, that its migration was appropriate and that the completion of the procedure was successful.
- It is the responsibility of the user to perform the Quality Control of the kit according to local regulations.

**RESULT INTERPRETATION**




**Positive Result (2 lines):** there are two pink-purple red colored lines, one in the «T» test zone and one in the «C» control area. The color intensity of the «T» line will depend on the sample under study. Any visible pink color, even very faint must be interpreted as positive. The result is positive although the intensity of the color of the «T» and «C» lines is different.

**Negative Result (1 line):** there is only one pink red-purple colored line in the «C» control area. No colored line appears in the «T» test zone.
**Invalid Result:** the absence of the pink-purple red colored line in the «C» control zone invalidates the result; whether or not the pink-purple red colored line is present in the «T» test zone. An invalid result usually indicates an error in performing the procedure or a problem with the sample. With certain samples difficulties like incomplete migration, very viscous sample or presence of fibrin may appear. In any case, review the procedure, centrifuge the sample again and repeat the test using a new cassette.

<table>
<tr><th colspan="3">Interpretation algorithm</th></tr>
<tr><td colspan="3">Original sample</td></tr>
<tr><td colspan="2">Positive Result by<br>WL Check HIV 1+2</td><td>Negative Result by<br>WL Check HIV 1+2</td></tr>
<tr><td colspan="2">Repeat by duplicate</td><td rowspan="4">NEGATIVE</td></tr>
<tr><td>One or both duplicates Positive by<br>WL Check HIV 1+2</td><td>Both duplicates Negative by<br>WL Check HIV 1+2</td></tr>
<tr><td>POSITIVE</td><td rowspan="2">NEGATIVE</td></tr>
<tr><td>Perform another test<br>(e.g.: ELISA) and confirm<br>by Western Blot</td></tr>
</table>

**NOTE:** take into account country-specific standards for the diagnosis of HIV infection.

**PROCEDURE LIMITATIONS**

- **WL Check HIV 1+2** is a complementary test in the diagnosis of HIV. Any result obtained with this test must be confronted with the clinical data before making a definitive diagnosis.
- A negative result does not exclude the possibility of HIV infection. A false negative result may be obtained in the following circumstances:
  § Low levels of anti-HIV antibodies in early stages of infection.
  § Infection with a virus strain that is not detected by this test.

For these reasons care should be taken when interpreting a negative result, especially in patients with clinical symptoms and risk factors. In this case it is recommended to test a new sample subsequently collected.

- Positive samples by **WL Check HIV 1+2** indicate the presence of anti-HIV-1 and anti-HIV-2 antibodies. These samples should be replicated using a further method (such as ELISA) and confirmed by Western Blot. The diagnosis can be established only after interpreting the results along with clinical data.
- For a positive result, the color intensity of the «T» line does not necessarily correlate with the concentration of specific anti-HIV antibodies in the sample.
- False positive results can occur in the following situations: autoimmune diseases, tuberculosis, lupus erythematosus, pregnancy, vaccination against hepatitis B and other immunizations, hemodialysis, liver disease and other diseases.
- **WL Check HIV 1+2** has been designed to detect antibodies to HIV-1 and HIV-2 in serum, plasma and human whole blood. Do not use other biological fluids such as saliva, cerebrospinal fluid or urine.
- Erroneous results can be obtained with serum or plasma with turbidity due to bacterial contamination or that have been subjected to several freezing and thawing cycles.
- The use of heat-inactivated samples may yield erroneous results.
- Do not use sample pools or diluted samples.
- See Known Interfering Substances under SAMPLE.

**PERFORMANCE**

**a) Sensitivity**

*Clinical Sensitivity on Performance Panels*

From a study on different international commercial panels, the following results were obtained:

- PRB 108 (Anti-HIV 1 Low Titer Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 13 out of 14 reactive samples were detected.
- PRB 109 (Anti-HIV 1 Low Titer Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 15 out of 19 reactive samples were detected.
- PRB 204 (Anti-HIV 1 Mixed Titer Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 23 out of 23 reactive samples were detected.
- WWRB303 (Worldwide HIV Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 13 out of 14 reactive samples for HIV-1 group M (subtypes A, B, C, D, F and G), HIV-1 group O and HIV-2 were detected.
- WWRB350 (Worldwide HIV Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 18 out of 18 reactive samples for HIV-1 group M (subtype A, CRF02_AG, B, C, D, CRF01_AE, F, G and H) were detected.
- PRB 601 (HIV-1 Incidence/Prevalence Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 15 out of 15 reactive samples for HIV-1 were detected, corresponding to recent and long term infections.
- PRZ 204 (Anti-HIV-1/2 Combo Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 14 out of 14 reactive samples for HIV-1 and HIV-2 were detected.
- PRZ 205 (Anti-HIV-1/2 Combo Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 14 out of 14

reactive samples for HIV-1 and HIV-2 were detected.
- PRZ 207 (Anti-HIV-1/2 Combo Performance Panel, Sera Care Life Sciences, USA - BBI Diagnostics): 14 out of 14 reactive samples for HIV-1 and HIV-2 were detected.
- PP 0508 (Performance Panel for HIV, Q Panel, Brazil): 16 out of 16 reactive samples were detected.
- PP 0409 (Performance Panel for HIV, Q Panel, Brazil): 16 out of 16 reactive samples were detected.

*Clinical Sensitivity on Seroconversion Panels*
A study on different international commercial seroconversion panels (Sera Care Life Sciences, USA - BBI Diagnostics), yielded the following results:

| Panel name | Days from first collection | Time (days) in wich sample yields reactive result | |
|---|---|---|---|
| | | WL Check HIV 1+2 | Western Blot (Sera Care data) |
| PRB 904-D | 0, 21, 49, 92, 99 | 92 | 92 |
| PRB 912-L | 0, 9, 14, 16, 28, 30 | 0 | 9 |
| PRB 916-P | 0, 4, 9, 15, 30, 35 | 30 | 30 |
| PRB 919-S | 0, 9, 11 | 9 | 9 |
| PRB 924-X | 0, 2, 8, 10, 26, 33, 35, 40 | 33 | 35 (Indet) |
| PRB 925-Y | 0, 10, 18, 22, 44, 49 | 44 | 44 (Indet) |
| PRB 929-AD | 0, 4, 14, 18, 21, 25, 28 | 25 | 25 (Indet) |
| PRB 930-AE | 0, 3, 7, 10 | 7 | 10 (Indet) |
| PRB 944-AT | 0, 2, 7, 9, 14, 16 | 14 | 14 (Indet) |
| PRB 947-AW | 0, 9, 11, 20 | 9 | 9 (Indet) |
| PRB 949-AY | 0, 6, 9, 18, 20 | 20 | 20 (Indet) |
| PRB 951-BA | 0, 2, 8, 11, 15, 19 | 19 | Negative up to 19 days |
| PRB 952-BB | 0, 7, 10, 14, 17, 21 | 17 | 14 (Indet) |
| PRB 953-BC | 0, 3, 7, 10 | 10 | Negative up to 10 days |
| PRB 966 | 0, 2, 20, 22, 30, 35, 37, 44, 48, 51 | 48 | Negative up to 51 days |

Indet: indeterminate

*Clinical Sensitivity on panels of reactive samples*
In a study of 68 reactive samples, including 4 HIV-1 group O samples, from a hospital, 68 samples were detected.
In another study, 30 whole blood samples were supplemented with 30 positive sera for HIV and the supplemented whole blood samples and their respective sera were tested in parallel. All the samples (whole blood and serum) were detected.
In a further study, 15 reactive serum and plasma samples obtained from the same patient were tested in parallel, and for 10 of these samples, whole blood obtained by capillary puncture was also tested. With all types of samples positive results were obtained.

**b) Specificity**
In a study of 1122 samples of serum, plasma and whole blood from 3 different health centers, a specificity of 99.91% was obtained with $CI_{95\%}$= 99.49% -99.98%.
In another study of 417 blood bank samples, the specificity obtained was 99.76% with $CI_{95\%}$= 98.65% -99.96%. For 100 of the 417 samples, serum and plasma samples from the same patient were tested in parallel and for 40 of these 100 samples, whole blood obtained by capillary puncture was also tested. With all types of samples negative results were obtained.
In another study of 393 serum and plasma samples from 3 different health centers, a specificity of 99.24% was obtained with $CI_{95\%}$= 97.78% -99.74%.
The possible occurrence of cross-reactivity was also studied testing 440 samples from individuals with different clinical conditions that may be causing nonspecific reactions with the ***WL Check HIV 1+2*** test. These conditions include pregnant women, patients on hemodialysis, patients with autoimmune diseases or infectious diseases other than HIV (Chagas, HTLV, Hepatitis C, Hepatitis B, Syphilis, etc.). For this population, the specificity was 98.18% with $CI_{95\%}$= 96.45% -99.08%.

**c) Precision**
The accuracy of the test was tested following the protocol EP5-A recommended by the CLSI (formerly NCCLS). The tests were conducted with 4 positive samples with different levels of reactivity and 1 negative sample. Two daily trials were conducted to evaluate each sample in duplicate during 20 days. The results were read after 20 minutes with an immunochromatographic reader and visually. The positive samples always yielded a reactive result and the negative sample always yielded a non-reactive result.

| | T Line | | | | | C Line | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Intra-assay | | Total | | Mean | Intra-assay | | Total | |
| | (OD) | S | CV | S | CV | (OD) | S | CV | S | CV |
| Positive sample 1 | 0,221 | 0,027 | 12,36% | 0,032 | 14,36% | 0,676 | 0,066 | 9,69% | 0,078 | 11,61% |
| Positive sample 2 | 0,085 | 0,018 | 20,88% | 0,021 | 24,23% | 0,686 | 0,077 | 11,22% | 0,087 | 12,61% |
| Positive sample 3 | 0,271 | 0,044 | 16,40% | 0,049 | 18,02% | 0,702 | 0,070 | 9,94% | 0,076 | 10,80% |
| Positive sample 4 (in dilution) | 0,049 | 0,017 | 35,79% | 0,017 | 35,36% | 0,679 | 0,075 | 11,11% | 0,085 | 12,59% |
| Negative sample | (-) | NA | NA | NA | NA | 0,591 | 0,073 | 12,34% | 0,104 | 17,61% |

N = 80; (-) = non-reactive by visual interpretation; NA = not applicable

**WIENER LAB PROVIDES**

- 25 tests (Cat. Nº 1690019)
- 25 tests with disposable devices (Cat. Nº 1690020)

**REFERENCES**


- Centro Nacional de referencia para el SIDA (http://www.cnrsida.org.ar)
- Centers for Disease Control and Prevention: Rapid HIV Testing (http://www.cdc.gov/hiv/topics/testing/rapid/index.htm)
- Greenwald JL, Burstein GR, Pincus J, Branson B. - A rapid review of rapid HIV antibody tests. - Curr. Infect. Dis. Rep. 8/2:125 (2006).
- Centers for Disease Control and Prevention: Advancing HIV Prevention: New Strategies for a Changing Epidemic, United States, 2003. MMWR 2003, 52:329–332. (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5215a1.htm)
- Centers for Disease Control and Prevention: Notice to Readers: Protocols for Confirmation of Reactive Rapid HIV Tests. MMWR 2004, 53:221-222 (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5310a7.htm)
- Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. EP5-A, Vol. 19, Nº 2, NCCLS.
- User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. EP12-A, Vol. 22, Nº 14, NCCLS.
- Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard, 5ª Edición. H4-A5, Vol. 24, Nº 21, CLSI (ex NCCLS).
- Evaluation of Stability of In Vitro Diagnostic Reagents. Approved Guideline. EP-25A, Vol. 29, Nº 20, CLSI (ex NCCLS).


**SYMBOLS EXPLANATION**

| Disp. | Desc. |
|---|---|

Disposable devices

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP Authorized representative in the European Community

IVD "In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. Contents

LOT Batch code

Manufactured by:

Xn Harmful

Corrosive / Caustic

Xi Irritant

Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL − Negative Control

REF Catalog number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