WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: First Response® HIV 1-2-0 Card Test
Number: PQDx 0018-010-00

Abstract

First Response® HIV 1-2-0 Card Test with product codes I05FRC100, I05FRC60, I05FRC30, and I05FRC05, manufactured by Premier Medical Corporation, rest of world (RoW) regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 14 July 2016.

Intended use:
First Response® HIV 1-2-0 Card Test is a qualitative in vitro diagnostic test (immunochromatographic rapid diagnostic test) for the detection of antibodies of all classes specific to HIV-1 (including Group O) and HIV-2 in human serum, plasma and venous or capillary whole blood. The test kit is not automated and does not require any additional instrument, it is intended for use by healthcare professionals. Reactive specimens should be confirmed by other methods.

Test principle:
First Response® HIV 1-2-0 Card test contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigens (gp41 including subtype O and p24) on test band “1” region and with recombinant HIV-2 capture antigen (gp36) on test band “2” region, respectively on the test device. The recombinant HIV-1 and 2 antigens (gp41, p24 and gp36) colloidal gold conjugate and specimen moves along the membrane chromatographically to the test region and forms a visible line (test line) as the antigen-antibody-antigen gold particle complex.

Test kit contents:

<table>
<thead>
<tr>
<th></th>
<th>5 tests Product code (I05FRC05)</th>
<th>30 tests Product code (I05FRC30)</th>
<th>60 tests Product code (I05FRC60)</th>
<th>100 tests Product code (I05FRC100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test devices</td>
<td>5</td>
<td>30</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Wrapped with 1 sample pipette and desiccant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffer</td>
<td>1 bottle of 0.5 ml</td>
<td>1 bottle of 2.5 ml</td>
<td>2 bottles of 2.5 ml</td>
<td>4 bottles of 2.5 ml</td>
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<tr>
<td>Carbonate buffered</td>
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</table>
saline containing proteins and preservative

<table>
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<tr>
<th>Lancets</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>100</th>
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</thead>
<tbody>
<tr>
<td>Sterile, single use</td>
<td></td>
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<table>
<thead>
<tr>
<th>Alcohol swabs</th>
<th>5</th>
<th>30</th>
<th>60</th>
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<tbody>
<tr>
<td>70% Isopropyl Alcohol</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Instructions for use</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
</tr>
</thead>
</table>

**Storage:**
The test kit should be stored at 4 °C to 30 °C.

**Shelf-life:**
24 months.

**Reading time:**
Interpret test results at 15 minutes. Do not interpret after 15 minutes.

### Summary of prequalification status for First Response® HIV 1-2-0 Card Test

<table>
<thead>
<tr>
<th>Initial acceptance</th>
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<tbody>
<tr>
<td><strong>Date</strong></td>
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<tr>
<td><strong>Outcome</strong></td>
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<td><strong>Status on PQ list</strong></td>
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<tr>
<td>14 July 2016</td>
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<td>listed</td>
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<tr>
<td><strong>Dossier assessment</strong></td>
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<tr>
<td>29 June 2016</td>
</tr>
<tr>
<td>MR</td>
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<tr>
<td><strong>Inspection status</strong></td>
</tr>
<tr>
<td>3 December 2014</td>
</tr>
<tr>
<td>MR</td>
</tr>
<tr>
<td><strong>Laboratory evaluation</strong></td>
</tr>
<tr>
<td>15 September 2014</td>
</tr>
<tr>
<td>MR</td>
</tr>
</tbody>
</table>

MR: Meets Requirements
NA: Not Applicable

First Response® HIV 1-2-0 Card Test was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

### Background information

Premier Medical Corporation submitted an application for prequalification of First Response® HIV 1-2-0 Card Test. Based on the established prioritization criteria, First Response® HIV 1-2-0 Card Test was given priority for prequalification.
Product dossier assessment

Premier Medical Corporation submitted a product dossier for First Response® HIV 1-2-0 Card Test 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 First Response® HIV 1-2-0 Card test for prequalification.

Commitments for prequalification:

Commitment 1: the manufacturer has agreed to carry out additional studies to demonstrate precision (repeatability and reproducibility) taking into consideration WHO recommendations.

Commitment 2: the manufacturer has agreed to carry out additional shipping stability study as per the agreed protocol and recommendations from WHO.

Manufacturing site inspection

A comprehensive inspection was performed at the sites of manufacture (Nani Daman: 32-35 Shri Ganesh Industrial Estate, Kachigam Nani Daman, 396215, India and Sarigam: A1-302, GIDC, Sarigam 396155 Dist Valsad, Gujarat, India) of the First Response® HIV 1-2-0 Card Test in July 2014 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 3 December 2014.

Laboratory evaluation

First Response® HIV 1-2-0 Card Test (Premier Medical Corporation Ltd) was evaluated by WHO in the first quarter of 2012 at the Institute of Tropical Medicine, 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:

First Response® HIV 1-2-0 Card Test (Premier Medical Corporation Ltd) is an immunochromatographic rapid diagnostic test for the discriminatory detection of HIV-1/HIV-2 antibodies in human serum, plasma, and venous and capillary whole blood. A volume of 10 µL of specimen 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 testing settings. Reading of the results can be done visually i.e. subjectively read.

In this limited evaluation on a panel of 1079 specimens, we found an initial sensitivity (95% CI) of 100% (99.1-100%) and an initial specificity (95% CI) of 98.94% (97.8-99.6%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1-100%) and the final specificity (95% CI) was 99.39% (98.5-99.8%) compared to the reference assays. Lot to lot variation observed was within the acceptance criteria for all but one dilution series.

For eight seroconversion panels, First Response® HIV 1-2-0 Card Test detected on average 0.25 specimens earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]).

For the mixed titer panel, First Response® HIV 1-2-0 Card Test correctly classified all specimens. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], First Response® HIV 1-2-0 Card Test detected all HIV-1 subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, and HIV-2) with the exception of HIV-1 type O.

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the overall inter-reader variability was 2.04% (0.83% for HIV-1 band, 1.21% for HIV-2 band). The invalid rate was 0%.
Labelling

1. Labels
2. Instructions for use
1. Labels

Outer box labels

First Response® HIV 1-2-0 Card Test kit box: 5 Tests

![Box of 5 Tests](image1)

First Response® HIV 1-2-0 Card Test kit box: 30 Tests

![Box of 30 Tests](image2)

First Response® HIV 1-2-0 Card Test kit box: 60 Tests

![Box of 60 Tests](image3)

First Response® HIV 1-2-0 Card Test kit box: 100 Tests

![Box of 100 Tests](image4)
Aluminium pouch label

OPEN POUCH SIZE : 208 mm (W) X 60 mm (H)

Assay buffer labels

Assay buffer label: 0.5 ml
Assay buffer label: 2.5 ml
2. Instructions for use
FIRST RESPONSE® HIV 1-2.O CARD TEST
Rapid Immunochromatographic Card Test for the detection of Antibodies to HIV-1 & 2 in Human Whole Blood/Serum/Plasma

Intended Use:
First Response® HIV 1-2.O Card Test is intended for use by healthcare professionals and is a qualitative, screening, in vitro diagnostic test for detection of antibodies specific to HIV-1 (including Group O) and HIV-2 in human serum, plasma or venous and capillary blood. The test kit is not automated and does not require any additional instrument. Reactive samples should be confirmed by supplemental testing.

Introduction:
HIV (Human Immunodeficiency Virus) is recognized as the etiologic agent of Acquired Immunodeficiency 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 prenatal period. The clinical diagnosis of HIV has been done by detection of HIV-1 and HIV-2 antibodies in human plasma, sera, or venous/capillary whole blood by immunobassay. Researchers have constructed HIV-1 and -2 genos for the expression of recombinant antigens in bacterium systems such as E. coli and focused on HIV-1 and -2 proteins, which are definitely immunogenic. The major immunoreactive antigens of these proteins have been reported to have HIV-1 gp41, p24, and HIV-2 gp36, based on western blot analysis.

First Response® HIV 1-2.O Card Test is a 3rd generation HIV immunobassay. The design of 3rd generation assays allows the detection of HIV specific IgG as well as IgM, which may occur early in infection.

Assay Principle:
The First Response® HIV 1-2.O Card Test is a 3rd generation lateral flow chromatographic immunobassay. The test cassette consists of: 1) a purple colored conjugate pad containing HIV-1 and -2 specific recombinant antigens (gp41 including Group O, gp36 and p24) and control protein conjugated with colloidal gold particles; and 2) a nitrocellulose membrane strip containing two test lines (1 and 2) and a control line (C). Test line '1' is pre-coated with HIV-1 recombinant antigens (gp41 including Group O and p24) for the detection of antibodies to HIV-1. Test line '2' is pre-coated with HIV-2 recombinant antigen (gp36) for the detection of antibodies to HIV-2. The Control line is pre-coated with a control line protein.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the strip. HIV-1 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV-1 conjugates. The immune-complex is then captured on the membrane by the pre-coated HIV-1 antigen forming a purple colored line at test line "1", indicating a HIV-1 antibody positive or reactive test result. Lack of color development on test line "1" suggests an HIV-1 antibody negative or non-reactive result. HIV-2 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV-2 conjugates. The immune-complex is then captured on the membrane by the pre-coated HIV-2 antigen forming a purple colored line at test line "2", indicating a HIV-2 antibody positive or reactive test result. Lack of color development on test line "2" suggests a HIV-2 antibody negative or non-reactive result.

The test contains an internal control line ("C"), which should exhibit a purple colored line of the immune-complex of the control proteins regardless of color development on the test lines. If the "C" line does not develop, the test result is invalid and the specimen must be retested with another device. The control line test in device is not a specimen addition control. Since only one control line is present, the result is not invalidated by the absence of one color band. Therefore, interpretation of the control line test result is not required.

Storage and Stability:
The First Response® HIV 1-2.O Card Test Rapid Test is limited to the qualitative detection of HIV-1 or HIV-2 antibodies in human serum, plasma or whole blood. The test kit is not automated and does not require any laboratory equipment. The test kit is not intended for use with human tissues, cells, or cell culture supernatant.

Materials Provided:
- 30 cassette packages (foil pouches), each containing: 1 device
- 1 desiccant
- 1 specimen transfer device (sample pipette)
- 1 assay buffer bottle - 2.5 ml
- 30 sterile single-use lancets (optional)
- 30 alcohol swabs (optional)
- 1 instructions for use

Materials Required but Not Provided:
- New pair of disposable gloves
- Pen
- Timer
- Extra lancets and alcohol swabs, if needed
- Sharps disposal box
- Venipuncture blood collection materials and precision pipette plus tips (if whole blood is collected by venipuncture)
- Biohazardous waste container

Test Procedure:
1) Bring the First Response® HIV 1-2.O Card Test kit components to room temperature prior to testing.
2) Remove the test device and the sample pipette from the foil pouch and place them on a flat, clean and dry surface.
3) Slowly add 20 μl (two drops) of whole blood or 10μl (one drop) of serum or plasma to the sample well (S) using the sample pipette. Dispose of the used sample pipette as biohazardous waste.
4) Add 35 μl (one drop) of the assay buffer to the sample well (S). Dispose of the used assay buffer as biohazardous waste.
5) Observe for development of colored bands in the results window.
6) Interpret the test results at 15 minutes. After recording the results, dispose of the test device as biohazardous waste.
7) Do not interpret after 15 minutes.

Interpretation of the Test:
Non-reactive Result
If one or more color line appears at control line "C" as in the figure, the specimen is non-reactive.

Reactive Results
If two color lines appear, one at control line "C" and the other at test line HIV-1 "1" as in the figure, the specimen is reactive for antibodies to HIV-1. Interpret a faint line as a reactive line.

If two color lines appear, one at control line "C" and the other at test line HIV-2 "2" as in the figure, the specimen is reactive for antibodies to HIV-2. Interpret a faint line as a reactive line.

Invalid Results:
If no color line appears at the control line "C" within the stipulated time then the result is invalid. The result is also invalid if a color band appears only at test line "1" and/or "2". If there is high background coloring and incomplete migration along the test strip then the result is invalid.

Limitations:
1) The “Test Procedure” and “Interpretation of the Test” sections must be followed closely. Failure to follow the procedure may lead to inaccurate test results.
2) First Response® HIV 1-2.O Card Test is designed to detect antibodies to HIV-1 and HIV-2 in human serum, plasma and whole blood. Other body fluids or pooled specimens may not give accurate results.
3) The First Response® HIV 1-2.O Card Test Rapid Test is limited to the qualitative detection of HIV-1 or HIV-2 antibodies in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer of the specimen.
4) There is potential for interference when testing is performed with hemolytic specimens. Hemolytic specimens may produce a reddish background even after the reading time.
5) Highly lipemic samples or turbid samples shall be centrifuged and the resultant supernatant shall be used for testing.

Specimen Collection and Storage:
1) Capillary blood collection: Clean the area to be lanced with an alcohol swab and allow it to air dry. Pierce the skin with a sterile lancet provided. Wipe away first drop of blood and use second drop for collection. Take a 20 μl (two drops) sample pipette provided and squeeze the large bulb. Immerse the open end in the blood drop and then release the pressure to draw blood into the sample pipette.
2) Venous Blood collection: Collect the whole blood by venipuncture in to collection tube containing anticoagulants (EDTA, Heparin, ACD and Sodium Citrate anticoagulants have been validated for use with this test).
3) Plasma collection: Collect the whole blood in collection tubes containing anticoagulants (such as EDTA, Heparin, ACD or Sodium Citrate) by venipuncture. Centrifuge the tube at 3000 RPM for 10-15 min to obtain plasma (supernatant).
4) Serum: Collect whole blood in collection tubes without having any anticoagulants by venipuncture. Keep the tube in an upright position for 30 minutes and then centrifuge it at 3000 rpm for 10-15 minutes to obtain serum (supernatant).
5) Whole blood specimens collected in appropriate anticoagulant may be used for testing immediately or may be stored at 2-8 °C for up to 3 days. Do not freeze whole blood specimen.
6) If serum or plasma specimens are not immediately tested, they should be refrigerated at 2-8 °C. For storage periods greater than three days, freezing at -20 °C is recommended. They should be brought to room temperature prior to use.
7) Serum or plasma specimens containing precipitate or high lipemia may yield inconsistent test results. Such specimens must ALWAYS be centrifuged prior to assaying.

Sample Pipette
[Diagram of sample pipette]

First Response® HIV 1-2.O CARD TEST
I05FRC30
(A.1) In-house Evaluation: First Response® HIV-1,2.O Card Test has been tested using an in-house panel of Positive and Negative clinical samples confirmed by a leading commercial anti-HIV-1 and -2 ELISA kit. The result shows that First Response® HIV-1,2.O Card Test is very accurate compared to other commercial ELISA kit. In a comparison of the First Response® HIV-1,2.O Card Test versus a leading commercial anti-HIV-1 and -2 ELISA and rapid test, results gave a sensitivity of 100%, a specificity of 100% and total agreement of 100%.

(A.2) External Evaluation: The Performance of First Response®HIV-1,2.O Card Test was evaluated at various external laboratories and institutes. The summary of reports is as follows:

(B.1) Analytical Specificity: Sensitivity:

(3) A non-reactive result for an individual subject indicates an absence of detectable HIV-1 or HIV-2 antibodies. However, a non-reactive result can occur if the quantity of the HIV-1 or HIV-2 antibodies present in the sample is below the detection limits of the assay or the antibodies that are detected are not present during the stage of disease in which a sample is collected.

6) Immunochromatographic testing alone cannot be used to diagnose AIDS even if the antibodies against HIV-1 and/or HIV-2 are present in a patient's sample. A non-reactive result at any time does not preclude the possibility of HIV-1 or HIV-2 infection.

7) All three test lines (1, 2 and C) may develop when tested with samples containing high titers of HIV-1 antibodies. However, reactive test results, especially for both HIV-1 and HIV-2 may not indicate mixed infection. False positives are possible but may result from the cross-reactivity of HIV-1 and HIV-2 because of the similarity of their genomic structure. To differentiate virus type or co-infection accurately, one must perform a confirmatory test such as Western blot or PCR.

8) EDTA, Heparin, ACD or Sodium Citrate anticoagulants have been validated for use with this test.

9) False negative results may arise because of the hook effect due to very high titer of antibodies in the sample.

10) Although a reactive result may indicate infection with HIV-1 or HIV-2 virus, a diagnosis of AIDS can only be made by clinical grounds. A reactive result for an individual subject indicates an absence of detectable HIV-1 or HIV-2 antibodies. However, a non-reactive result can occur if the quantity of the HIV-1 or HIV-2 antibodies present in the sample is below the detection limits of the assay or the antibodies that are detected are not present during the stage of disease in which a sample is collected.

Performance Characteristics:

(A) Clinical Specificity and Sensitivity

(B) Analytical Specificity and Sensitivity:

(B.1) Analytical Specificity: Sensitivity:

Precise:

References:


