Dear Mr Mehta,

Please find enclosed the final report of the WHO evaluation (phase I) of the First Response HIV-1-2-O assay. A summary of the results of the evaluation is given below:

The First Response HIV-1-2-O was evaluated by WHO in the third quarter of 2000. From this evaluation we drew the following conclusions:

The First Response HIV-1-2-O is an immunochromatographic assay for the detection of antibodies to HIV in human serum, plasma or whole blood. A volume of 25μl of serum/plasma or 30μl whole blood is needed to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities. Reading of the results can be done visually.

In this limited evaluation, on a panel of 250 samples we found a sensitivity (95% CI) of 100% (95.5%-100%) and an initial specificity (95% CI) of 98.2% (94.9%-99.6%) compared to the reference assays. The final specificity (95% CI) was 98.8% (95.8%-99.9%) compared to the reference assays. In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.4%.

Thank you very much for submitting the First Response HIV-1-2-O assay for a WHO evaluation. We hope that we may collaborate again in the future.

Yours sincerely

Dr Gaby Vercauteren
Department of Blood Safety and Clinical Technology

ENCLOS: as stated
REPORT OF THE WHO EVALUATION (PHASE I)
OF THE FIRST RESPONSE HIV-1-2-O
(PMC Medical Pty. Ltd)

Date: August 2003

WHO Collaborating Centre for Transfusion Transmitted Infections
Department of Microbiology
Institute of Tropical Medicine
Antwerp
Belgium

Blood Transfusion Safety
Department of Blood Safety and Clinical Technology
World Health Organization
Geneva
Switzerland
1. **Name of the assay**

**First Response HIV-1-2-O**

An *in vitro* qualitative immunochromatographic assay for the detection of antibodies to Human Immunodeficiency Viruses type 1 and 2 in human serum, plasma or whole blood.

At the time of the WHO evaluation, the kit was named BioSign™ HIV-1/HIV-2 WB. Subsequent to the evaluation, WHO was informed by Premier Medical Corporation of the change of kit name to First Response HIV-1-2-O.

2. **Manufacturer**

PMC Medical (India) Pvt. Ltd  
Gala No. 3233, Shree Ganesh Industrial Estate, Kachigam  
Nani-Daman, Daman-396210  
India  
Tel: +91 (02638) 56774  
Fax: +91 (02638) 56775  
E-mail: nnmehata353@aol.com, nnmehata353@hotmail.com

3. **Distributors**

Premier Medical Corporation  
259 Amherst Avenue  
Colonia  
NJ 07067  
USA  
Tel: +1 732 815 0462  
Fax: +1 530 869 7966  
E-mail: nnmehata353@aol.com, nnmehata353@hotmail.com

4. **Price per test and Product Code**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>No. of tests per kit</th>
<th>Price/test Local currency</th>
<th>Price/test US$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD/138</td>
<td>35</td>
<td>Rs 60.00</td>
<td>1.15</td>
</tr>
</tbody>
</table>

5. **General information about the assay**

5.1 **Type of assay**

lateral flow immunochromatographic assay
5.2 Source of antigen
recombinant antigens gp41, gp36 and portions of gp120

5.3 Type of solid phase
immunochromatographic membrane

5.4 Contents
The assay is available in kits of 35 tests.
Each test kit contains 35 test devices, developer solution and directions for use.

5.5 Controls
Control samples:
- Positive and negative controls are not supplied in the kit.
- Each device contains a control line which is an indicator of sample addition and correct performance of the test. This line must be visible for a valid test.

5.6 Conjugate and conjugate diluent
The composition of the conjugate (developer reagent) was not defined
Conjugate diluent: not applicable

5.7 Substrate and substrate diluent
Not applicable

5.8 Dimensions (cm) of kit (w-l-h)
21 / 12.3 / 7

5.9 Labels
No lot number nor expiry date on the outer packaging; no test name on the devices; no test name nor expiry date on the developer reagent vials.

5.10 Quantity of reagents
All reagents are supplied in sufficient quantity.

5.11 Manual of instructions for use of the assay kit
Generally the instructions are clear.

5.12 Storage conditions
The kit may be stored at 4°C - 30°C according to the outer packaging and at 2°C - 30°C according to the package insert.
5.13 Shelf life
The shelf life of the kit following manufacture is 18 months.

6. Operational aspects

6.1 Preparatory work

6.1.1 Reagents
No reagents require preparation.

6.1.2 Stability after opening (°C)
- The device should remain in its original sealed pouch until ready for use.
- Reagents, devices and samples should be at room temperature before use.

6.1.3 Samples

6.1.3.1 Sample type
Whole blood samples anti-coagulated with EDTA were used for this evaluation. The sample types which can be used also include serum, and plasma anticoagulated with EDTA or heparin.

6.1.3.2 Sample size
30 µl of whole blood or 25 µl serum or plasma is required to perform the test.

6.1.4 Number of samples per test run
Minimum number per run : 1 sample
Maximum number per run : 5 samples

6.2 Incubation temperature
Room temperature

6.3 Washing procedure
None

6.4 Readings
The results are read visually.
6.5 Time required to perform the assay (h:min)
Time to test 1 sample: 0:01

6.6 Equipment needed that is not supplied in the kit
micropipette

7. Materials and Methods

7.1 Samples

7.1.1 WHO HIV Whole Blood Test Kit Evaluation Panel (Phase I)

<table>
<thead>
<tr>
<th>HIV-1 Positive</th>
<th>HIV Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>170</td>
<td>250</td>
</tr>
<tr>
<td>32%</td>
<td>68%</td>
<td>100%</td>
</tr>
</tbody>
</table>

7.1.2 Seroconversion panels

Additionally eight anti-HIV-1 seroconversion panels: PRB910, PRB912, PRB914, PRB917, PRB927, PRB928, PRB930 and PRB944 from Boston Biomedica (BBI) were tested.

7.2 Reference assays

Each matched serum specimen included in the evaluation panel was screened with two ELISAs; Vironostika HIV Uniform II plus O (Organon Teknika) and Enzygnost Anti-HIV 1/2 Plus (Dade Behring). Specimens found negative with both ELISAs were considered to be anti-HIV negative. All dually reactive and discordant ELISA results were further characterised by INNO-LIA HIV Confirmation (Innogenetics). When results of the ELISAs and the INNO-LIA HIV Confirmation were all positive the specimen was considered anti-HIV positive.

When the initial ELISA results were discordant and the INNO-LIA HIV Confirmation result and INNOTEST HIV Antigen mAb were negative, the specimen was considered anti-HIV negative. Similarly, when the initial ELISA results were discordant and the INNO-LIA HIV Confirmation result was positive, the specimen was considered anti-HIV positive.

When the INNO-LIA HIV Confirmation result was indeterminate and the INNOTEST HIV Antigen mAb was negative, the specimen was excluded from the panel.

All reference assays were interpreted according to the instructions given by the manufacturer.

The results of the reference assays are shown together with the assay under evaluation results in annexes 1 and 2.
7.3 Lot numbers and expiry date of the evaluated assay kits
Lot number: 300D12 Expriy: 8/2001
(lot number and expiry date of the devices)

7.4 Equipment used during the evaluation
Not applicable.

7.5 Interpretation of the First Response HIV-1-2-O results
The control line on the device must appear pink-purple for a valid test.

A sample is considered anti-HIV positive when two pink-purple lines appear, one in the test window and one in the control window (scores 2, 3 and 4 in annexes 1 - 4).

A valid anti-HIV negative result will show only the line in the control window (score 0 in annexes 1 - 4).

When the reactivity is uncertain the result is anti-HIV indeterminate (score 1 in annexes 1-4)

The assays were performed by one person, the results were read independently by three technicians. When the three technicians interpreted the results differently from each other, the consensus was recorded as that interpretation which occurred 2 out of 3 times. In cases where all three interpretations were different, the result was recorded as indeterminate.

7.5.1 Endpoint stability
The reading endpoint is stable for 10 min.

7.6 Sensitivity-specificity
Sensitivity: The percentage of sera that contain antibody to HIV (reference assays positive) which are positive in this test

Specificity: The percentage of sera that do not contain antibody to HIV (reference assays negative) which are negative in this test

Confidence limits (CL):
95% CLs of the calculated sensitivity and specificity are given in parentheses. Exact 95% confidence limits for Binomial proportions were calculated from the F-distribution (Armitage P. and Berry G. Statistical Methods in Medical Research, 2nd Edition. Blackwell Scientific Publications, Oxford, 1987, page 119).
7.7  Positive and negative predictive values

Positive predictive value (PPV): The probability that when the test is positive, the specimen does contain antibody to HIV. PPVs were calculated using the formula:

\[
PPV = \frac{(\text{prevalence})(\text{sensitivity})}{(\text{prevalence})(\text{sensitivity}) + (1 - \text{prevalence})(1 - \text{specificity})}
\]

Negative predictive value (NPV): The probability that when the test is negative, a specimen does not have antibody to HIV. NPVs were calculated using the formula:

\[
NPV = \frac{(1 - \text{prevalence})(\text{specificity})}{(1 - \text{prevalence})(\text{specificity}) + (\text{prevalence})(1 - \text{sensitivity})}
\]

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected (i.e., the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of serum samples testing false-positive decreases; conversely, the likelihood that a person whose test result is negative is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative.

7.8  Inter-reader variability

The inter-reader variability is calculated when readings are performed without any objective reading instruments. Three persons independently interpret each test result. The inter-reader variability is expressed as the percentage of sera for which initial test results are differently interpreted (i.e., positive, negative or indeterminate) by the independent readers.

7.9  Sensitivity in seroconversion panels

The results obtained with early seroconversion panels using the assays under evaluation were compared with those obtained using Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG), the assay arbitrarily designated the reference for determination of relative sensitivity in these panels. For each seroconversion series (panel) the first specimen in the sample sequence to become reactive with Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG) was assigned the value “0”. Results from the assays under evaluation were compared with Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG) by determining the difference between the specimen assigned value “0” and the relative position in the sample sequence of the first specimen which showed a reactive result with each of the assays under evaluation. For example, if an assay became reactive two specimens earlier in a series than Enzygnost
Anti-HIV 1/2 Plus (Behringwerke AG), the value assigned for that series in that assay was -2. Similarly, if an assay became reactive one specimen later than Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG), the value assigned was +1. The assigned values over the 8 seroconversion series were averaged to determine a mean relative seroconversion sensitivity index for each assay and the 95% confidence limits were determined. The results for the seroconversion panels are presented in Annex 5. Figure 1 shows the results diagrammatically along with the results of 11 other simple/rapid HIV assays recently evaluated by WHO.

8. Results

8.1 Validation of the First Response HIV-1-2-O results.
All tests were valid.

8.2 Comparison of the results with those of the combined reference assays.

8.2.1 Comparison of the First Response HIV-1-2-O initial results with those of the combined reference assays.

<table>
<thead>
<tr>
<th>Reference Assays**</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Response HIV-1-2-O initial results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>167</td>
</tr>
<tr>
<td>?</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

80 170 250

(For a complete listing of all test and reference results see annexes 1, 2 and 4 where specimens producing results discordant from the reference results are highlighted by shading).

Sensitivity (95% CL) \(80/80 \times 100 = 100.0\%\) (95.5% - 100.0%)
Specificity (95% CL) \(167/170 \times 100 = 98.2\%\) (94.9% - 99.6%)
Indeterminate \(1/250 \times 100 = 0.4\%\)

** + : sera positive for anti-HIV
- : sera negative for anti-HIV
? : sera with an indeterminate result
8.2.2 Comparison of the First Response HIV-1-2-O final results with those of the combined reference assays.

<table>
<thead>
<tr>
<th></th>
<th>Reference results**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>80</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>?</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

Sensitivity (95% CL): $\frac{80}{80} \times 100 = 100\%$ (95.5% - 100.0%)
Specificity (95% CL): $\frac{168}{170} \times 100 = 98.8\%$ (95.8% - 99.9%)
Indeterminate (%): $\frac{0}{250} \times 100 = 0\%$

**
+ : sera positive for anti-HIV
- : sera negative for anti-HIV
? : sera with an indeterminate result

Of the two initial false positive results, one became negative and the other remained positive on repeat testing. The second false positive result recorded in the final results was the sample which was indeterminate initially and which gave a clear positive result on repeat testing.

8.3 Positive and negative predictive values

**PPV (0.01% prevalence)**: 0.83
**PPV (6.0% prevalence)**: 84.18

**NPV (0.01% prevalence)**: 100.0
**NPV (6.0% prevalence)**: 100.0

8.4 Inter-reader variability

Initial inter-reader variability: $\frac{1}{250} = 0.4\%$ (see annex 4)
(The specimen showing inter-reader variability is highlighted in **bold italics** in annex 4).
9. **Kit Appraisal by the Technician**

<table>
<thead>
<tr>
<th></th>
<th>Rating*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kit Instructions:</strong></td>
<td>Clarity</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Kit and reagent packaging and labelling:</strong></td>
<td>Clear identif.</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Safety</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>Sample dispensing and volume:</strong></td>
<td>sample type</td>
<td>Anti-coagulated EDTA samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sample volume</td>
<td>30 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample addition control</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Equipment required</strong></td>
<td>Equipment required</td>
<td>micropipette</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Details of equipment required</td>
<td>A micropipette for adding 30µl whole blood or 25µl serum/plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of steps to completion of test:</strong></td>
<td>Number</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>How long is the endpoint stable for?:</strong></td>
<td>Time</td>
<td>10 min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time from start to completion:</strong></td>
<td>Time</td>
<td>1 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recommended maximum tests per run:</strong></td>
<td>Test max.</td>
<td>Not stated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Actual number of tests possible per run:</strong></td>
<td>Test number</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other Comments:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rating key: 1 = poor; 2 = needs improvement; 3 = satisfactory; 4 = good; 5 = excellent*

10. **Additional Comments**

- At WHO we agree with the recommendation that positive and negative control samples should be tested at regular intervals and at certain specific times eg. When commencing testing with a previously unused kit. However, we believe that the control samples should be made available, or a validated source recommended, by Premier Medical Corporation for separate purchase.

- The outer packaging and all perishable kit components should be labelled with the kit name and the expiry date.

- Despite the request that two lot numbers be made available for evaluation, only one was provided.
Summary

The First Response HIV-1-2-O (PMC Medical, India) was evaluated by WHO in the second quarter of 2000. From this preliminary evaluation the following observations can be made:

The First Response HIV-1-2-O is a rapid immunochromatographic assay for the detection of antibodies to HIV in whole blood. A volume of 30 μl of whole blood is needed to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities. Reading of the results can be performed visually.

In this limited Phase I evaluation on a panel of 250 samples we found a sensitivity (95% CL) of 100.0% (95.5% - 100.0%) and an initial specificity (95% CL) of 98.2% (94.9% - 99.6%) compared to the reference assays. The final specificity (95% CL) was 98.8% (95.8% - 99.9%) compared to the reference assays. In this study, 0% of the results were recorded as indeterminate.

One anti-HIV negative sample (WHO990470) was interpreted as positive by three readers when first tested. Two subsequent tests on this sample were interpreted as negative. Laboratory error can not be ruled out as the cause of the initial false positive result.

Results were interpreted independently by three technicians; the inter-reader variability was 0.4%.

The findings of the Phase I evaluation of First Response HIV-1-2-O meet the criteria to proceed to Phase II. The data from the Phase I and II evaluations will be published in an official WHO report of the operational characteristics of commercially available assays to detect antibodies to HIV in whole blood.