WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: MP Diagnostics HIV Blot 2.2
Number: PQDx 0198-071-00

Abstract

**MP Diagnostics HIV Blot 2.2** with product codes 11030-018 and 11030-036, manufactured by MP Biomedicals Asia Pacific Pte. Ltd, **CE marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 4 April 2016.

MP Diagnostics HIV Blot 2.2 kit is a qualitative enzyme immunoassay for the in vitro detection of antibodies to HIV-1 and HIV-2 in human serum or plasma. It is intended for use as a supplemental assay on human serum or plasma specimens found repeatedly reactive using EIA. The separated specific HIV-1 viral antigens incorporate onto the strips via electrophoretic and electrotransblot procedures combined with a specific HIV-2 synthetic peptide on the same strip allow for further delineation of the antibody responses to specific viral proteins. Each strip also includes an internal specimen addition control to minimize the risk of false negatives due to operations errors and to ensure the addition of specimens.

The nitrocellulose strips are incorporated with separated bound antigenic proteins from partially unpurified inactivated HIV-1 using electrophoretic blotting, plus a specific HIV-2 synthetic peptide on the same strips. Individual nitrocellulose strips are incubated with diluted specimens and controls. Specific antibodies to HIV-1 and HIV-2 if present in the specimens will bind to the HIV-1 proteins and HIV-2 peptide on the strips. The strips are washed to remove unbound materials. Antibodies that bind specifically to HIV proteins can be visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate BCIP/NBT. This method has the sensitivity to detect marginal amounts of HIV specific antibodies in serum or plasma.

The test kit contains:

<table>
<thead>
<tr>
<th>Component</th>
<th>18 tests (product code 11030-018)</th>
<th>36 tests (product code 11030-036)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nitrocellulose Strips:</strong></td>
<td>18 strips</td>
<td>36 strips</td>
</tr>
<tr>
<td>Incorporated with HIV-1 viral lysate, a specific HIV-2 envelope peptide and specimen addition control band.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-reactive control:</strong></td>
<td>1 vial (80μl)</td>
<td>1 vial (80μl)</td>
</tr>
</tbody>
</table>
Inactivated normal human serum non-reactive for hepatitis B surface antigen (HBsAg), antibodies to HIV-1/2, and anti-HCV. Contains sodium azide and thimerosal as preservatives.

**Strong reactive control:**
Inactivated normal human serum with high titered antibodies to HIV-1 and HIV-2 and non-reactive for HBsAg, and anti-HCV. Contains sodium azide and thimerosal as preservatives.

- 1 vial (80μl)

**Weak reactive control:**
Inactivated normal human serum with high titered antibodies to HIV-1 ONLY and non-reactive for HBsAg, anti-HIV-2 and anti-HCV. Contains sodium azide and thimerosal as preservatives.

- 1 vial (80μl)

**Stock Buffer concentrate (10x):**
Tris buffer with heat inactivated normal goat serum. Contains thimerosal as preservative.

- 1 bottle (20ml)

**Wash buffer concentrate (20x):**
Tris buffer with Tween-20. Contains thimerosal as preservative.

- 1 bottle (70ml)

**Conjugate:**
Goat anti-human IgG conjugated with alkaline phosphate. Contains thimerosal as preservative.

- 1 vial (160μl)

**Substrate:**
Solution of 5-bromo-4-chloro-3-indoyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).

- 1 bottle (100ml)

**Blotting powder:**
Non-fat dry milk

- 10 packets (1g each)

**Incubation tray:**
9 wells each

- 2 trays

**Instruction for use:**

- 1 copy

**Forceps:**

- 1 pair

**Storage:**
The test kit should be stored at 2-8 °C.

**Shelf-life:**
24 months.
WHO special warning:
WHO reviewed the instructions for use that were current at the time of WHO prequalification, and a number of changes were suggested. Most but not all changes were made by the manufacturer (outstanding comments relate to general layout and nomenclature).

Summary of prequalification status for MP Diagnostics HIV Blot 2.2

<table>
<thead>
<tr>
<th>Status on PQ list</th>
<th>Initial acceptance</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>4 April 2016</td>
<td>listed</td>
<td></td>
</tr>
<tr>
<td>10 October 2014</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>05 June 2015</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>MR</td>
<td></td>
</tr>
</tbody>
</table>

MR: Meets Requirements
NA: Not Applicable

MP Diagnostics HIV Blot 2.2 was accepted for the WHO list of in vitro prequalified diagnostics on the basis of data submitted and publicly available information.

Background information

MP Biomedicals Asia Pacific Pte. Ltd, submitted an application for prequalification of MP Diagnostics HIV Blot 2.2. Based on the established prioritization criteria, MP Diagnostics HIV Blot 2.2 was given priority for prequalification.

Product dossier assessment

MP Biomedicals Asia Pacific Pte. Ltd, submitted a product dossier for MP Diagnostics HIV Blot 2.2 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 MP Diagnostics HIV Blot 2.2 for prequalification.

The manufacturer committed to amend and submit additional documentation on the following issues:
1. The Manufacturer will investigate the effect of transport conditions on HIV Blot 2.2 shelf life including a higher temperature range (expected completion date Q4 2017).

2. The manufacturer will commence testing all material used in the production of positive and negative test kit controls using state-of-the-art methods (i.e. nucleic acid detection).

**Manufacturing site inspection**

A comprehensive inspection was performed at the site of manufacture (2 Pioneer Place, Singapore) of MP Diagnostics HIV Blot 2.2 in November 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 14 July 2015.

The manufacturer committed conduct the following studies which will be reviewed at the next re-inspection: drop and shock testing for HIV Blot 2.2.

**Laboratory evaluation**

The objective of the performance laboratory evaluation is to assess the performance and operational characteristics of commercially available in-vitro diagnostics for the purpose of advising the governments of WHO Member States on these issues. In particular, suitability for use in resource-limited settings will be assessed.

Based on the risk level associated with the use of the supplemental assays, the known general performance of supplemental assays and role of the supplemental assays in patient care in resource-limited settings, it was decided that WHO will not conduct performance evaluations of these assays as part of the prequalification assessment process.

Consequently, laboratory evaluation of MP Diagnostics HIV Blot 2.2 was not conducted.
Labelling

1. Labels
2. Instructions for use
MP Diagnostics
HIV BLOT 2.2
WESTERN BLOT ASSAY

For detection and identification of IgG antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2).

For den Nachweis und die Identifizierung von IgG-Antikörpern gegen das humane Immunodefizienz-Virus Typ 1 (HIV-1) und Typ 2 (HIV-2).

Pour la détection et l’identification des anticorps IgG du virus d’immunodéficience humaine de type 1 (VH1-1) et de type 2 (VH2-2).

Per la determinazione e l’identificazione degli anticorpi IgG del virus dell’immunodeficienza umana di tipo 1 (VH1-1) e di tipo 2 (VH2-2).

Para la detección e identificación de anticuerpos IgG contra el virus de inmunodeficiencia humana tipo 1 (VH1-1) e tipo 2 (VH2-2).

For the detection and identification of IgG antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2).

Antigen Strips

<table>
<thead>
<tr>
<th>Ref</th>
<th>Lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 strips</td>
<td>36 strips</td>
</tr>
</tbody>
</table>

Control -

| 80μl x 1 | 80μl x 1 |

Control +

| 80μl x 1 | 80μl x 1 |

Control Weak

| 80μl x 1 | 80μl x 1 |

Buf Stock 10x

| 20ml x 1 | 20ml x 1 |

Buf Wash 20x

| 70ml x 1 | 70ml x 1 |

Conjugate

| 160μl x 1 | 160μl x 1 |

Subs SCIP / NBT

| 100ml x 1 | 100ml x 1 |

Powder Blotting

| 1g x 10 | 1g x 10 |

Colour Code:
- Yellow 012C
- P485
- P021
- Black

Size: 280mm(W) x 83mm(H) 2015-07-22
1. INTRODUCTION

Screening tests are widely available for detecting antibodies to both HIV-1 and HIV-2 viruses. Usually, they are based on enzyme immunoassay (EIA) technology, using specific antigens from HIV proteins and monoclonal antibodies directed against these antigens. Many different antigens are used for specific reagents, which can provide for better sensitivity, specificity, and results. The assay is a qualitative test designed to detect specific antibodies to both HIV-1 and HIV-2 in serum, plasma, or other body fluids without regard to the patient’s clinical history or the type of exposure.

The assay is used in diagnostic laboratories to identify patients with HIV infection and to follow the course of the disease. It is also used in research to study the immune response to HIV infection and to evaluate the effectiveness of new treatments and vaccines.

2. MATERIALS AND METHODS

2.1. Reagents

2.1.1. Irradiated human plasma.

2.1.2. Washing buffer: 1x saline solution.

2.1.3. Blocking buffer: 1x Tris-buffered saline solution.

2.1.4. Diluted conjugate:

2.1.5. Substrate solution:

2.1.6. Counterstain solution:

2.1.7. Others:

2.2. Equipment

2.2.1. Microtiter plate reader.

2.2.2. Incubator.

2.2.3. Centrifuge.

2.2.4. Pipettes.

2.2.5. Gloves.

2.2.6. Plastic boxes.

2.2.7. Other.

3. PROCEDURE

3.1. Preparation of reagents

3.1.1. Diluted conjugate:

3.1.2. Substrate solution:

3.1.3. Counterstain solution:

3.2. Preparation of samples

3.2.1. Irradiated human plasma:

3.2.2. Washing buffer:

3.2.3. Blocking buffer:

3.2.4. Others:

3.3. Assay procedure

3.3.1. Microtiter plate reader:

3.3.2. Incubator:

3.3.3. Centrifuge:

3.3.4. Pipettes:

3.3.5. Gloves:

3.3.6. Plastic boxes:

3.3.7. Other:

4. INTERPRETATION OF RESULTS

4.1._positive_result:

4.2._negative_result:

5. LIMITATIONS

5.1. Limitations of the assay:

5.2. Limitations of the test:

6. QUALITY CONTROL

6.1. Positive control:

6.2. Negative control:

6.3. Internal control:

6.4. Standardization:

7. STORAGE AND HANDLING

7.1. Storage:

7.2. Handling:

8. SAFETY AND DISPOSAL

8.1. Safety precautions:

8.2. Disposal:

9. REFERENCES

9.1. Literature:

9.2. Other:

10. APPENDIX

10.1. Table of reagents:

10.2. Table of equipment:

10.3. Table of procedures:

11. NOMENCLATURE

11.1. Abbreviations:

11.2. Symbols:

12. ACKNOWLEDGEMENTS

12.1. Acknowledgments:

13. CONFLICT OF INTEREST

13.1. Financial relationships:

13.2. Non-financial relationships:

14. APPENDIX A: ADDITIONAL INFORMATION

14.1. Additional information:

15. APPENDIX B: ADDITIONAL FIGURES

15.1. Figure A:

15.2. Figure B:

15.3. Figure C:

16. APPENDIX C: ADDITIONAL TABLES

16.1. Table A:

16.2. Table B:

17. APPENDIX D: ADDITIONAL REFERENCES

17.1. References:

18. APPENDIX E: ADDITIONAL APPENDICES

18.1. Appendix E:

19. APPENDIX F: ADDITIONAL APPENDICES

19.1. Appendix F:

20. APPENDIX G: ADDITIONAL APPENDICES

20.1. Appendix G:

21. APPENDIX H: ADDITIONAL APPENDICES

21.1. Appendix H:

22. APPENDIX I: ADDITIONAL APPENDICES

22.1. Appendix I:

23. APPENDIX J: ADDITIONAL APPENDICES

23.1. Appendix J:

24. APPENDIX K: ADDITIONAL APPENDICES

24.1. Appendix K:

25. APPENDIX L: ADDITIONAL APPENDICES

25.1. Appendix L:

26. APPENDIX M: ADDITIONAL APPENDICES

26.1. Appendix M:

27. APPENDIX N: ADDITIONAL APPENDICES

27.1. Appendix N:

28. APPENDIX O: ADDITIONAL APPENDICES

28.1. Appendix O:

29. APPENDIX P: ADDITIONAL APPENDICES

29.1. Appendix P:

30. APPENDIX Q: ADDITIONAL APPENDICES

30.1. Appendix Q:

31. APPENDIX R: ADDITIONAL APPENDICES

31.1. Appendix R:

32. APPENDIX S: ADDITIONAL APPENDICES

32.1. Appendix S:

33. APPENDIX T: ADDITIONAL APPENDICES

33.1. Appendix T:

34. APPENDIX U: ADDITIONAL APPENDICES

34.1. Appendix U:

35. APPENDIX V: ADDITIONAL APPENDICES

35.1. Appendix V:

36. APPENDIX W: ADDITIONAL APPENDICES

36.1. Appendix W:

37. APPENDIX X: ADDITIONAL APPENDICES

37.1. Appendix X:

38. APPENDIX Y: ADDITIONAL APPENDICES

38.1. Appendix Y:

39. APPENDIX Z: ADDITIONAL APPENDICES

39.1. Appendix Z:
It is unlikely to detect gp41 in the absence of gp160 because the gp41 appears as a diffuse band. Any sharp and discreet band present (n = 74) is the polymeric form of gp41 and the concentration of gp160 is higher than gp41 on the MP Diagnostics HIV BLOT 2.2. gp41 is stained in a HIV-2 strip. For representing the gp41, samples with gp160 and gp41, gp160 and gp41, gp41 only, gp160 only and gp41 or no antigen are stained to compare its reactivity with the molecular weight of the gp41 band in a single case, but not with gp160. The gp41 band is visible in both gp160 and gp41. 2. The gp41 band is visible as a diffuse band. Any sharp and discreet band present (n = 74)

Table 1: HIV-1 positive samples (209 samples)

<table>
<thead>
<tr>
<th>Source</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>208</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 indicative samples</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: HIV-1 positive samples (209 samples)

<table>
<thead>
<tr>
<th>Source</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>208</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 indicative samples</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3: HIV-1 positive samples (209 samples)

<table>
<thead>
<tr>
<th>Source</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>208</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 indicative samples</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4: HIV-1 positive samples (209 samples)

<table>
<thead>
<tr>
<th>Source</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>208</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 indicative samples</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5: HIV-1 positive samples (209 samples)

<table>
<thead>
<tr>
<th>Source</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>208</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 indicative samples</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6: Potential interfering and pregnant women samples (81 samples)

<table>
<thead>
<tr>
<th>Source</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>208</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 indicative samples</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 positive samples</td>
<td>202</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 indicative samples</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV-2 negative samples</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
**Troubleshooting Chart**

<table>
<thead>
<tr>
<th>White positive develop on strips</th>
<th>Non-specific bands develop and not HIV-9 indicative</th>
<th>Strong Background develop on strip in the absence or presence of positive bands</th>
<th>Bands other than the Serum Control band develops on negative control</th>
<th>Sharp, discrete band at gp120 region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expected bands do not develop or are of weak intensity:</strong></td>
<td><strong>Check positive-control</strong></td>
<td><strong>Overloading of negative sample or erroneous higher conjugate concentration or erroneous blocking buffer or over incubation:</strong></td>
<td><strong>Overdeveloped strips (stop reaction sooner):</strong></td>
<td><strong>Try wash or Control may have been caused contaminated:</strong></td>
</tr>
<tr>
<td>1. Strips were flipped over during assay.</td>
<td>2. Strips not properly washed before wash step.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Strips not properly washed after Band/Protein.</td>
<td>4. Strips not properly washed before washing.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Positive control weak:</strong></th>
<th><strong>Positive control OK:</strong></th>
<th><strong>Unidentified bands:</strong></th>
<th><strong>Non-specific bands and/or dark background develop on strips:</strong></th>
<th><strong>Strips are defective:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>The problem is probably caused by the reagents.</td>
<td>The problem is probably caused by test sample.</td>
<td>1. Bands such as Blotting Buffer and blotting conjugate Solution are not properly prepared.</td>
<td>1. They are cracked.</td>
<td></td>
</tr>
<tr>
<td>1. Reagents such as Blotting Buffer and Blotting conjugate Solution are not properly prepared.</td>
<td>2. Wrong conjugate dilution.</td>
<td>2. Bands not added.</td>
<td>2. They contain air bubbles which cause the appearance of white spots in reactive zones big enough to prevent any detection.</td>
<td></td>
</tr>
<tr>
<td>3. Undesirable reagents are present.</td>
<td>3. Incorrect conjugate dilution.</td>
<td>3. Serum not added.</td>
<td>3. They show dark spots due to fungal growth upon initial opening of the strip tubes.</td>
<td></td>
</tr>
<tr>
<td>4. Conjugate contaminated with human IgG.</td>
<td>4. Incubation time too long due to repeated freezing.</td>
<td>4. Strips not added.</td>
<td>However, if dark spots develop sometime later after the initial opening of the tube then the problem is due to improper strip storage conditions at the user’s site.</td>
<td></td>
</tr>
<tr>
<td>5. Incubation temperature greater than 30°C.</td>
<td>5. Rotary platform used instead of Rocking platform.</td>
<td>5. Substrate not added.</td>
<td>6. Rotary platform used instead of Rocking platform.</td>
<td></td>
</tr>
<tr>
<td>6. Rotary platform used instead of Rocking platform.</td>
<td>7. Watery marks on developed strips.</td>
<td>7. Overdeveloped strips.</td>
<td>7. If sample is HIV seronegative by Western Blot, the screening assays which gave HIV seropositive results are actually false positive.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bands other than the Serum Control band develops on negative control</th>
<th><strong>Strong Background develop on strip in the absence or presence of positive bands:</strong></th>
<th><strong>Non-specific bands develop and not HIV-9 indicative:</strong></th>
<th><strong>Expected bands do not develop or are of weak intensity:</strong></th>
<th><strong>Check positive-control:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Overdeveloped strips (stop reaction sooner).</td>
<td>2. Incomplete washing.</td>
<td>1. Overloading of negative sample or erroneous higher conjugate concentration or erroneous blocking buffer or over incubation.</td>
<td>2. Strips not properly washed before wash step.</td>
<td>3. Strips not properly washed after Band/Protein.</td>
</tr>
<tr>
<td>3. Serum not added.</td>
<td>4. Substrate not added.</td>
<td>3. Strips not properly washed after Band/Protein.</td>
<td>4. Strips not properly washed after Band/Protein.</td>
<td>4. Strips not properly washed after Band/Protein.</td>
</tr>
</tbody>
</table>

**Note:** This is not caused by hook effect because hook effect will result in false negative result. **Watery marks on developed strips**

- Strips left to dry after pre-soaking step prior to adding Blotting Buffer.

**Expected bands do not develop or are of weak intensity:**

- Check positive-control.

**Strong Background develop on strip in the absence or presence of positive bands:**

- Overloading of negative sample or erroneous higher conjugate concentration or erroneous blocking buffer or over incubation.

**Non-specific bands develop and not HIV-9 indicative:**

- Overloading of negative sample or erroneous higher conjugate concentration or erroneous blocking buffer or over incubation.

**Absence of Serum Control Band:**

- Strips defective.

**Bands other than the Serum Control band develops on negative control:**

- Overdeveloped strips (stop reaction sooner).

**Expected bands do not develop or are of weak intensity:**

- Check positive-control.

**Strong Background develop on strip in the absence or presence of positive bands:**

- Overdeveloped strips (stop reaction sooner).

**Non-specific bands develop and not HIV-9 indicative:**

- Overloading of negative sample or erroneous higher conjugate concentration or erroneous blocking buffer or over incubation.

**Absence of Serum Control Band:**

- Try wash or Control may have been caused contaminated.

**Bands other than the Serum Control band develops on negative control:**

- Overdeveloped strips (stop reaction sooner).

**Expected bands do not develop or are of weak intensity:**

- Check positive-control.

**Strong Background develop on strip in the absence or presence of positive bands:**

- Overdeveloped strips (stop reaction sooner).

**Non-specific bands develop and not HIV-9 indicative:**

- Overloading of negative sample or erroneous higher conjugate concentration or erroneous blocking buffer or over incubation.

**Absence of Serum Control Band:**

- Try wash or Control may have been caused contaminated.

**Bands other than the Serum Control band develops on negative control:**

- Overdeveloped strips (stop reaction sooner).