For the detection of antibodies to HIV-2 in serum or plasma samples.

**NAME AND INTENDED USE**

The **MP DIAGNOSTICS (MPD) HIV-2 WESTERN BLOT (VERSION 1.2)** is a qualitative enzyme immunoassay for the *in-vitro* detection of specific antibodies to HIV-2 in human serum or plasma. The MPD HIV-2 Western Blot is intended for use as a more specific test on human serum or plasma specimens found to be repeatedly reactive using screening procedures like the Enzyme-Linked Immunosorbertent Assay (ELISA).

**INTRODUCTION**

Human Immunodeficiency Virus Type 2 (HIV-2) infection was first described in 1985 in asymptomatic prostitutes from Senegal. The virus was subsequently isolated in 1986 from patients with AIDS-like symptoms in Guinea Bissau and Cape Verde. HIV-2 is related to, but distinct from HIV-1, the prototype AIDS virus. As such HIV-2 has many molecular, biological and serological similarities with HIV-1.

Reports have shown that the infection is not limited to Africa and that HIV-2 seropositive individuals have been identified in Europe and the United States. Several screening tests for simultaneous detection of HIV-1 and HIV-2 antibodies are now widely available. More specific test, like the MP Diagnostics Western Blot procedure utilizing native HIV-2 viral proteins is thus necessary to ascertain HIV-2 positivity for specimens found repeatedly reactive from screening assays.

**CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE**

The nitrocellulose strips are incorporated with separated, bound antigenic proteins from partially purified inactivated HIV-2 using electrophoretic blotting. Individual nitrocellulose strips are incubated with diluted serum or plasma specimens and
controls. Specific antibodies to HIV-2, if present in the specimens, will bind to the HIV-2 proteins on the strips.

The strips are washed to remove unbound materials. Antibodies that bind specifically to HIV-2 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 is sensitive enough to detect marginal amounts of HIV-2 specific antibodies in serum or plasma.

**KIT COMPONENTS**

1. **NITROCELLULOSE STRIPS**
   - Incorporate with HIV-2 viral lysate.
   - Available in 18 & 36 strips
   - Keep dry and away from light.

2. **NON-REACTIVE CONTROL**
   - Inactivated normal human serum. Non-reactive for Hepatitis B surface antigen (HBsAg) and antibodies to HIV-1, HIV-2, and HCV.
   - Contains sodium azide and thimerosal as preservatives.

3. **STRONG REACTIVE CONTROL**
   - Inactivated human serum with high titered antibodies to HIV-2. Non-reactive for HBsAg & anti-HCV.
   - Contains sodium azide and thimerosal as preservatives.

4. **STOCK BUFFER CONCENTRATE (10X)**
   - Tris Buffer with heat inactivated normal goat serum. Contains thimerosal as preservative.

5. **WASH BUFFER CONCENTRATE (20X)**
   - Tris with Tween-20. Contains thimerosal as preservative.

6. **CONJUGATE**
   - Goat anti-human IgG conjugated with alkaline phosphatase. Contains sodium azide as preservative.

7. **SUBSTRATE**
   - Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).

8. **BLOTTING POWDER**
   - Non-fat dry milk

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**Non-Reactive Control**

Inactivated normal human serum. Non-reactive (80 ul) for Hepatitis B surface antigen (HBsAg) and antibodies to HIV-1, HIV-2, and HCV. Contains sodium azide and thimerosal as preservatives.

**Strong Reactive Control**

Inactivated human serum with high titered antibodies to HIV-2. Non-reactive for HBsAg & anti-HCV. Contains sodium azide and thimerosal as preservatives.

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**Conjugate**

Goat anti-human IgG conjugated with alkaline phosphatase. Contains sodium azide as preservative.

**Substrate**

Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).
Volume of reagents provided are sufficient for 4 runs.

**WARNINGS AND PRECAUTIONS**

1. For *in vitro* diagnostic use only.
2. For Professional use only
3. Please refer to the product labelling for information on potentially hazardous components

**HEALTH AND SAFETY INFORMATION**

⚠️ **CAUTION:** This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection. **HANDLE ASSAY SPECIMENS, STRONG REACTIVE AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS.** It is recommended that the components and test specimens be handled using good laboratory working practices. They should be disposed of in accordance with established safety procedures.

The **Strong Reactive Control** and **Non-Reactive Control** contain Thimerosal and Sodium azide while Stock Buffer Concentrate and Wash Buffer Concentrate contain Thimerosal and Conjugate contains Sodium azide. Sodium azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water to prevent metal azide buildup in plumbing system. The following are the appropriate Risk (R) phrases.

R22 Harmful if swallowed.

The **Substrate** contains 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium which is classified per applicable European Economic Community (EEC) Directives as harmful (Xn). The following are the appropriate Risk (R) phrases.

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.

1. Avoid Microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.

2. Do not pipette by mouth.

3. Handle test specimens, nitrocellulose strips, Reactive and Non-Reactive Controls as potentially infectious agents.
4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in bio-hazard waste-bags. Wash hands thoroughly afterwards.

5. It is highly recommended that this assay be performed in a biohazard cabinet.

6. Keep materials away from food and drink.

7. In case of accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.

8. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.

9. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with 1% sodium hypochlorite solution before work is resumed. Sodium hypochlorite should not be used on acid containing spills unless the area is wiped dry with absorbent paper first. Material used (including disposable gloves) should be disposed off as potentially biohazardous material. Do not autoclave material containing sodium hypochlorite.

10. Autoclave all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.

11. Decontaminate all used chemicals and reagents by adding sufficient volume of sodium hypochlorite to make a final concentration of at least 1%. Leave for 30 minutes to ensure effective decontamination.

12. We do not recommend re-use of incubation trays.

ANALYTICAL PRECAUTIONS

1. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instruction Manual. Deviations from the procedure may lead to aberrant results.

2. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and Western Blot strips are matched for optimal performance. Use only the reagents supplied with the kit.

3. Do not use kit components beyond the expiry date printed on the kit box.

4. Avoid microbial contamination of the reagents, when opening and removing aliquots from the original vials or bottles, as this will prematurely reduce the shelf life of the kits and give erroneous results. Use aseptic techniques including pipettes or disposable pipette tips when drawing aliquots from vials.

5. The kit controls should be assayed concurrently with patients’ samples for each test run.
6. Use a new pipette tip for each specimen aliquot to prevent cross contamination.

7. For best results dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.

8. It is recommended that glassware to be used with the reagents should be washed with 2M hydrochloric acid and rinsed thoroughly with distilled or deionised water prior to use.

9. Use only reagent grade quality, deionised or distilled water to dilute reagents.

10. All reagents must be mixed well before use.

11. Working Conjugate solution, Diluted Wash Buffer and Blotting Buffer should be **prepared fresh prior to use**.

12. The Working Conjugate solution should be prepared using a polypropylene container or beaker.

13. Do not expose reagents or perform test in an area containing a high level of chemical disinfectant fumes (e.g. hypochlorite fumes) during storage or during incubation steps. Contact inhibits colour reaction. Also do not expose reagents to strong light.

14. The assay should preferably be performed at room temperature (25°C ± 3°C).

15. Make sure that the test strips are laid with the numbers on the strips facing upwards.

16. For Western Blot Assay, it is important to use a rocking platform shaker and not a rotary shaker. Otherwise, performance of the kit will be compromised. The recommended speed and tilt angle of the shaker are 12 to 16 cycles per minute, and 5 to 10 degrees, respectively.

17. Ensure that automated equipment if used is validated before use.

18. Ensure that the specimens are added away from the strip. Tray can be tilted and specimen added where the buffer is collected at lower end. This prevents dark spot formation due to specimen addition on the strip.

19. Avoid the use of self-defrosting freezers for the storage of samples.

20. We do not recommend the use of diluted or lyophilized samples, as they may give false results. If they form part of or a whole QC panel, they should be validated.
STORAGE INSTRUCTIONS

1. Store MPD HIV-2 Western Blot kit and its components at 2-8°C when not in use.

2. All test reagents and strips when stored at 2°C to 8°C, are stable until the expiry date given on the kit. Do not freeze reagents.

A. Antigen strips
   • Avoid unnecessary exposure of antigen strips to light.

B. Reagents
   • Store reagents in their original vials or bottles, and they should be capped for storage.
   • Dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
   • Precipitates may form when the Substrate is stored at 2°C to 8°C. This will not affect the performance of the kit.

Caution: Avoid unnecessary exposure of substrate to light.

MATERIALS REQUIRED BUT NOT PROVIDED

• Deionized or distilled water
• Disposable gloves
• Rocking platform (designed with a rocking speed range of 12 to 16 cycles per minute, and which moves through a 5° to 10° tilt to wash strips evenly)
• Pipettors and tips of appropriate volume
• Aspirator with sodium hypochlorite trap
• 56°C water bath (optional)
• Sodium hypochlorite for decontamination

SPECIMEN HANDLING AND STORAGE (OPTIONAL)

Serum or plasma samples should be stored at 2°C to 8°C if the test is to be run within 7 days of collection or frozen at -20°C or colder if the test is to be delayed for more than 7 days. Clear, non-hemolyzed samples are preferred. Lipemic, icteric or contaminated (particulate) samples should be filtered (0.45µm) or centrifuged before testing.

Patients’ specimens can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follows:
1. Loosen caps of specimen containers.
2. Heat specimen to 56°C for 30 minutes in a water bath.
3. Allow specimen to cool before retightening caps.
4. Specimen can be stored frozen until analysis.

We recommend that the patients’ specimen should not undergo repeated freeze-thaw cycles prior to testing.

### PREPARATION OF REAGENTS

1. **DILUTED WASH BUFFER**
   (a) DILUTED WASH BUFFER should be prepared fresh prior to use.
   (b) Dilute 1 volume of WASH BUFFER CONCENTRATE (20X) with 19 volumes of reagent grade water. Mix well.

2. **BLOTTING BUFFER**
   (a) BLOTTING BUFFER should be prepared fresh prior to use.
   (b) Dilute 1 volume of STOCK BUFFER CONCENTRATE (10X) with 9 volumes of reagent grade water. Mix well.
   (c) Add 1 g of BLOTTING POWDER to every 20 ml of the diluted STOCK BUFFER prepared in step 2(b) above. Stir to ensure powder dissolves completely.
   (d) Stir again before dispensing.

3. **WORKING CONJUGATE SOLUTION**
   Note: Prepare solution in polypropylene container / beaker.
   (a) WORKING CONJUGATE SOLUTION should be prepared fresh prior to use.
   (b) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 into BLOTTING BUFFER, for example, 5 µl CONJUGATE to 5ml BLOTTING BUFFER.

4. **SUBSTRATE SOLUTION (ready to use)**
   (a) Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

### ASSAY PROCEDURE - RAPID ASSAY

**Note:**

a) Users can use either the rapid or overnight assay to run the tests. HIV bands are more developed and more bands may appear with the overnight assay, but the overall performance of the two assays is the same.

b) Aspirate all used chemicals and reagents into a trap containing Sodium hypochlorite.

c) All incubations are to be carried out on a rocking platform.
Caution:

Some samples cause dark patches on the spot of the strip where they are added. To avoid this problem, one should ensure the following:

i. Sample should be added only after BLOTTING BUFFER is added.

ii. Tilt the tray slightly by elevating either the top or bottom end of the tray. The Blotting Buffer will flow to the lower end of the tray. Add the sample where the Blotting Buffer is collected. When all the samples are added, return the tray back to its original flat position. Always ensure that the strips are kept wet during the process.

iii. Alternatively, if tilting the tray is not desired, the samples may be added to the top or bottom end of the well. This way if dark patches showed, the reading of the strip results will not be affected.

Procedure:

1. Add 2 ml of DILUTED WASH BUFFER to each well.  
2. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Strong Reactive and Non-Reactive controls.  
3. Incubate the strips for 1 to 2 minutes at room temperature (25 ± 3°C) on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration. (Note: Do not allow the strips to dry. Failure may result in watery marks on developed strips for some specimens.)  
4. Add 2 ml of BLOTTING BUFFER to each well.  
5. Add 20 µl each of patients’ sera or controls to appropriate wells. Care should be taken to ensure specimens are not added directly onto the strips.  
6. Cover the tray with the cover provided and incubate for 1 hour at room temperature (25 ± 3°C) on the rocking platform.  
7. Carefully uncover the tray to avoid splashing or mixing of samples. Tilt the tray to aspirate the mixture from the wells. Change aspirator tips between samples to avoid cross-contamination.  
8. Wash each strip 3 times with 2 ml of DILUTED WASH BUFFER allowing 5 minutes soak on the rocking platform between each wash.  
9. Add 2 ml of WORKING CONJUGATE SOLUTION to each
well.

10. Cover tray and incubate for 1 hour at room temperature (25 ± 3 °C) on the rocking platform.  
   60 minutes

11. Aspirate CONJUGATE from the wells. Wash as in step 8.  
   3 x 2 ml

12. Add 2 ml of SUBSTRATE SOLUTION to each well.  
   2 ml

13. Cover tray and incubate for 15 minutes on the rocking platform.  
   (Note: The reaction can be stopped before 15 minutes if all the bands are visible.)  
   15 minutes

14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step).  
   3 x 2 ml

15. Using forceps, gently remove strips onto paper towels. Cover with paper towels and dry. Alternatively, allow strips to dry in the wells of the tray.

16. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive tape over the developed bands. Observe the bands (See Interpretation of Results) and grade the results. For storage, keep the strips in the dark.

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**ALTERNATIVE PROCEDURE - OVERNIGHT ASSAY**

**Procedure:**

1. Add 2 ml of DILUTED WASH BUFFER to each well.  
   2 ml

2. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Strong Reactive and Non-Reactive controls.

3. Incubate the strips for 1 to 2 minutes at room temperature (25 ± 3 °C) on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration.  
   (Note: Do not allow the strips to dry. Failure may result in watery marks on developed strips for some specimens.)  
   2 minutes

4. Add 2 ml of BLOTTING BUFFER to each well.  
   2 ml

5. Add 20 µl each of patients’ sera or controls to appropriate wells.  
   20 µl
6. Cover the tray with the cover provided and incubate overnight (16 - 20 hours) at room temperature (25 ± 3°C) on the rocking platform.

7. Carefully uncover the tray to avoid splashing or mixing of samples. Tilt the tray to aspirate the mixture from the wells. Change aspirator tips between samples to avoid cross-contamination.

8. Wash each strip 3 times with 2ml of DILUTED WASH BUFFER allowing 5 minutes soak on the rocking platform between each wash. 3 x 2 ml

9. Add 2 ml of WORKING CONJUGATE SOLUTION to each well.

10. Cover tray and incubate for 30 minutes at room temperature (25 ± 3°C) on the rocking platform. 30 minutes

11. Aspirate CONJUGATE from the wells. Wash as in step 8. 3 x 2 ml

12. Add 2 ml of SUBSTRATE SOLUTION to each well. 2 ml

13. Cover tray and incubate for 15 minutes on the rocking platform. (Note: The reaction can be stopped before 15 minutes if all the bands are visible.) 15 minutes

14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step). 3 x 2 ml

15. Using forceps, gently remove strips onto paper towels. Cover with paper towels and dry. Alternatively, allow strips to dry in the wells of the tray.

16. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive tape over the developed bands. Observe the bands (See Interpretation of Results) and grade the results. For storage, keep the strips in the dark.
SUMMARY OF ASSAY PROTOCOLS

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Qty</th>
<th>Room Temp Rapid Assay</th>
<th>Room Temp Overnight Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrocellulose strip</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>2 ml</td>
<td>1 - 2 mins</td>
<td>1 - 2 mins</td>
</tr>
<tr>
<td>Blotting Buffer</td>
<td>2 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Specimen</td>
<td>20 µl</td>
<td>60 mins</td>
<td>Overnight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16 - 20 hours)</td>
<td></td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>3 x 2 ml</td>
<td>3 x 5 mins</td>
<td>3 x 5 mins</td>
</tr>
<tr>
<td>Conjugate</td>
<td>2 ml</td>
<td>60 mins</td>
<td>30 mins</td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>3 x 2 ml</td>
<td>3 x 5 mins</td>
<td>3 x 5 mins</td>
</tr>
<tr>
<td>Substrate (Ready to use)</td>
<td>2 ml</td>
<td>15 mins (or less)</td>
<td>15 mins (or less)</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>3 x 2 ml</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS

<table>
<thead>
<tr>
<th>Reagents</th>
<th>NUMBER OF STRIPS TO BE USED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>1X Wash Buffer (ml)</td>
<td>60</td>
</tr>
<tr>
<td>1X Blotting Buffer (ml)</td>
<td>20</td>
</tr>
<tr>
<td>Conjugate (ul)</td>
<td>11</td>
</tr>
<tr>
<td>Substrate (ml)</td>
<td>11</td>
</tr>
<tr>
<td>Blotting Powder (g)</td>
<td>1</td>
</tr>
</tbody>
</table>

QUALITY CONTROL

We recommend that the Non-Reactive & Strong Reactive controls be run with every batch of assay. In order for the results obtained from any assay to be considered valid, the following conditions must be met:

1. **NON-REACTIVE CONTROL**
   No viral specific bands should be observed on the Non-Reactive control strip.

2. **STRONG REACTIVE CONTROL**
   All relevant molecular weight bands must be evident. Figure 1 provides a guide to the relative positioning of bands visualized with the MPD HIV-2 Western Blot (Version 1.2) kit and permits identification of bands observed for the STRONG REACTIVE CONTROL. The bands are p26, gp36, p53, p56, p68, gp80 and gp125.
**INTERPRETATION OF RESULTS**

The major HIV-2 gene products that have been identified are:

<table>
<thead>
<tr>
<th>GAG</th>
<th>POL</th>
<th>ENV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORE ANTIGENS</td>
<td>ENDONUCLEASE POLYMERASE ANTIGENS</td>
<td>ENVELOPE ANTIGENS</td>
</tr>
<tr>
<td>p16, p26, p56</td>
<td>p34, p53, p68</td>
<td>gp36, gp80, gp125</td>
</tr>
</tbody>
</table>

The criteria for interpretation of a positive HIV-2 specimen may differ according to various national regulatory bodies. It is recommended that national policies be followed in the interpretation of results. However, the recently proposed WHO criteria for interpretation has been widely used. The WHO criteria can be used in the interpretation of results of test specimens performed with the MPD HIV-2 Western Blot (Version 1.2).

Each strip is compared to the strips used with the NON-REACTIVE AND STRONG REACTIVE CONTROLS for that run.

The result of blotting is then interpreted as NEGATIVE, INDETERMINATE or POSITIVE based on the pattern which is present:

<table>
<thead>
<tr>
<th>PATTERN</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) No viral specific bands present</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>2) 2 of the 3 envelope bands (gp36, gp80, gp125)</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>3) Any bands present but pattern does not meet criteria for POSITIVE</td>
<td>INDETERMINATE</td>
</tr>
</tbody>
</table>

Individuals seropositive for either HIV-1 or HIV-2 may display cross-reactivity with either viral proteins. The GAG and POL genes of HIV-1 and HIV-2 are highly conserved, resulting in a high degree of serological cross-reactivity with the GAG and POL encoded proteins. There is a greater divergence for the amino acids of the envelope glycoproteins and cross-reactivity against those glycoproteins are considered to occur less often.

However reports (4, 5) have shown that cross-reactivity against the envelope glycoproteins occur more often than previously believed. Thus sera showing extensive cross-reactivity indicating dual infection should be interpreted carefully. Further confirmation by characterization of gene sequences via polymerase chain reactions...
(PCR) procedure is recommended in such highly cross-reactive specimens to indicate infection with either HIV-1, HIV-2 or dually infected.

**LIMITATIONS OF THE PROCEDURE**

Optimal assay performance requires the strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.

Individuals with POSITIVE blots for antibodies to HIV-2 should be referred for medical evaluation. A NEGATIVE blot does not exclude the possibility of infection with HIV-2. INDETERMINATE blots should not be used as the basis for diagnosis of HIV-1 infection. It is recommended that REPEAT TESTING using the original specimen or FOLLOW-UP TESTING be performed for such sera.

**LIMITED EXPRESSED WARRANTY DISCLAIMER**

The manufacturer makes no express warranty other than that the test kit will function as an *in-vitro* diagnostic assay within the specifications and limitations described in the product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any other purposes. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss howsoever caused by the product in the use or in the application thereof.

**TECHNICAL PROBLEMS / COMPLAINTS**

Should there be a technical problem / complaint, please do the following:

1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.

**REFERENCES**


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FIGURE 1

HIV-2 REACTIVE PROFILE
TROUBLE SHOOTING CHART

**Dark spots develop on strips**

- White patches develop on strips
- Expected bands do not develop or are of weak intensity
- Non-specific bands develop and not HIV-2 indicative
- Strong Background develops on strip in the absence or presence of positive bands
- Bands develop on negative control
- Strips are defective
- Strong Background develops on strip in the absence or presence of positive bands

- Bands develop on negative control
- Strips are defective
- Strips left to dry after pre-soaking step prior to adding Blotting Buffer

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

- Positive control weak
- Positive control OK

- The problem is probably caused by the reagents.
  1. Reagents not properly prepared.
  2. Wrong conjugate dilution.
  3. Unstable reagents due to improper temperature exposure.
  4. Conjugate contaminated with human IgG.
  5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.
  6. Trays, reagent(s) or water having high phosphate concentration.
  7. Rotary platform used instead of Rocking platform

- The problem is probably caused by test sample.
  1. Wrong test sample dilution.
  2. Test sample contaminated with conjugates.
  3. Tests samples severely immune-complexed.
  4. Test sample deteriorated or denatured due to repeated freeze-thaw or improper storage.
  5. Rotary platform used instead of Rocking platform
  6. Test sample may be an ELISA “false” positive

**Non-specific bands develop and not HIV-2 indicative**

- Sample is too strong and reacts with trace amounts of intermediates.
- Sample cross reacts with H-9 proteins present in viral preparation (e.g., HLA-ABC, HLA-DR)

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

- Overdeveloped strips (stop reaction sooner).
- Incomplete washing.

**Bands develop on negative control**

- Tray wells or Control may have been crossed contaminated.

**Strips are defective**

- They are cracked.
- They contain air bubbles which cause the appearance of white spots in reactive zones big enough to prevent any detection.
- They show dark spots due to fungal growth upon initial opening of the strip tubes. However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user’s site

**Strips are defective**

- They are cracked.
- They contain air bubbles which cause the appearance of white spots in reactive zones big enough to prevent any detection.
- They show dark spots due to fungal growth upon initial opening of the strip tubes. However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user’s site

**Watery marks on developed strips**

- Overdeveloped strips (stop reaction sooner).
- Incomplete washing.

**Positive control weak**

- The problem is probably caused by the reagents.
  1. Reagents not properly prepared.
  2. Wrong conjugate dilution.
  3. Unstable reagents due to improper temperature exposure.
  4. Conjugate contaminated with human IgG.
  5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.
  6. Trays, reagent(s) or water having high phosphate concentration.
  7. Rotary platform used instead of Rocking platform

**Positive control OK**

- The problem is probably caused by test sample.
  1. Wrong test sample dilution.
  2. Test sample contaminated with conjugates.
  3. Tests samples severely immune-complexed.
  4. Test sample deteriorated or denatured due to repeated freeze-thaw or improper storage.
  5. Rotary platform used instead of Rocking platform
  6. Test sample may be an ELISA “false” positive

**Non-specific bands and/or dark background develop on strips**

- They are cracked.
- They contain air bubbles which cause the appearance of white spots in reactive zones big enough to prevent any detection.
- They show dark spots due to fungal growth upon initial opening of the strip tubes. However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user’s site

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

- Positive control weak
- Positive control OK

- The problem is probably caused by the reagents.
  1. Reagents not properly prepared.
  2. Wrong conjugate dilution.
  3. Unstable reagents due to improper temperature exposure.
  4. Conjugate contaminated with human IgG.
  5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.
  6. Trays, reagent(s) or water having high phosphate concentration.
  7. Rotary platform used instead of Rocking platform

- The problem is probably caused by test sample.
  1. Wrong test sample dilution.
  2. Test sample contaminated with conjugates.
  3. Tests samples severely immune-complexed.
  4. Test sample deteriorated or denatured due to repeated freeze-thaw or improper storage.
  5. Rotary platform used instead of Rocking platform
  6. Test sample may be an ELISA “false” positive

**Watery marks on developed strips**

- Overdeveloped strips (stop reaction sooner).
- Incomplete washing.

**Non-specific bands develop on strips**

- Sample is too strong and reacts with trace amounts of intermediates.
- Sample cross reacts with H-9 proteins present in viral preparation (e.g., HLA-ABC, HLA-DR)

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