HCV BLOT 3.0
Instructions For Use

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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MAD 0012-ENG-2

Note: Changes Highlighted

REF (18 tests kit) : 11132-018
(36 tests kit) : 11132-036

NAME AND INTENDED USE

The **MP Diagnostics (MPD) HCV BLOT 3.0** is a qualitative enzyme immunoassay for the *in vitro* detection of antibodies to HCV in human serum or plasma.

This kit is supplied for research use only. It is not intended for use as in the diagnosis or prognosis of disease. In particular, the test cannot be used to evaluate blood specimens for the purpose of donor screening, or as a confirmatory diagnostic.

INTRODUCTION

HCV has been identified as the major cause of parental transmitted non-A, non-B (NANB) hepatitis. Screening tests typically are now available for the diagnosis of Hepatitis C infection.

The **MP Diagnostics HCV BLOT 3.0** includes structural and non-structural antigens from HCV and is to be used as an informational research test on serum or plasma specimens. Each strip also includes an internal sample addition control and an reagent addition control, to minimize the risk of false negatives due to operational errors and to ensure sample addition.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on **MP Diagnostics** products and packaging. These symbols are the most common ones appearing on medical devices and their packaging. They are explained in more detail in the British and European Standard BS EN 980: 2008 and International Standard ISO15223:2007.

Use by
Synonym for this:
Expiry Date

LOT
Batch Code
Synonyms for this are:
Lot Number
Batch Number

Temperature Limitation

Manufacturer

Contains sufficient for <n> tests

REF Catalogue Number

Caution

Consult Instructions for Use

Do not reuse

Harmful (Xn) / Irritant (Xi)

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips contain four recombinant HCV proteins form the Capsid, NS3, NS4, and NS5 regions of the HCV genome. The HCV proteins are expressed as GST fusion proteins, so a GST control band is included to indicate reactivity to native GST. The blots also contain an IgG control band and an anti-IgG band. Individual nitrocellulose strips are incubated with diluted serum or plasma specimens and controls. Specific antibodies to HCV, if present in the specimen, will bind to the HCV proteins on the strips. The strips are washed to remove unbound materials and then incubated with affinity purified anti-human IgG conjugated with alkaline phosphatase. The conjugate antibody will bind to any antigen-antibody complexes formed on the blots. Unbound conjugate is removed by washing. A BCIP/NBT substrate is added to visualise reactive protein bands on the blots.

KIT COMPONENTS

	Component Description	Quantity Provided
ANTIGEN STRIPS	**NITROCELLULOSE STRIPS** Incorporated with HCV recombinant structural and non structural antigens. Two serum addition control (anti-human IgG and human IgG) bands. Keep dry and away from light.	Available in 18 & 36 strips
CONTROL − Xn	**NON-REACTIVE CONTROL** Inactivated normal human serum non-reactive for Hepatitis B surface antigen (HBsAg), antibodies to HIV-1, HIV-2 & HCV. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)
CONTROL + Xn	**REACTIVE CONTROL** Inactivated human serum with high titered antibodies to HCV. Non-reactive for anti-HIV-1/2, and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)
BUF STOCK 10x Xn	**STOCK BUFFER CONCENTRATE (10x)** Tris buffer with heat inactivated normal goat serum. Contains thimerosal as preservative.	1 bottle (20 ml)
BUF WASH 20x Xn	**WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20. Contains thimerosal as preservative.	1 bottle (70 ml)
CONJUGATE Xn	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase.	1 vial (120 µl)
SUBS BCIP / NBT Xn	**SUBSTRATE** Solution of 5-bromo-4-chloro-3-indolyl- phosphate (BCIP) and nitroblue tetrazolium (NBT).	1 bottle (100 ml)
POWDER BLOTTING	**BLOTTING POWDER** Non-fat dry milk	10 packets (1g each)
	Incubation Tray, 9 wells each	2 or 4 trays
	Instructions for Use	1 copy
	Forceps	1 pair

Note : Volume of reagents provided are sufficient for 4 runs.

WARNINGS AND PRECAUTIONS

1. For *Research Use* only. It is not intended for use in the diagnosis or prognosis of disease.
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, REACTIVE, AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS.** It is recommended that the components and test specimens be handled using universal precautions. They should be disposed in accordance with established safety procedures.

The ***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.

The ***Substrate*** contains 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. These preparations are not hazardous and does not contain hazardous ingredients at concentration used according to European Economic Community (EEC) Directive.

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. Serum, EDTA plasma or citrate plasma samples can be used. Heparin plasma samples preferably should not be used as they may cause a non-specific reaction to the HCV core band.
2. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instruction For Use. Deviations from the procedure may lead to aberrant results.
3. **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.
4. Do not use kit components beyond the expiry date printed on the kit box.
5. 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.
6. The kit controls should be assayed concurrently with test specimens for each test run.

7. Use a new pipette tip for each specimen aliquot to prevent cross contamination.
8. For best results dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
9. 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.
10. Use only reagent grade quality, deionised or distilled water to dilute reagents.
11. All reagents must be mixed well before use.
12. Working Conjugate solution, Diluted Wash Buffer and Blotting Buffer should be **prepared fresh prior to use**.
13. The Working Conjugate solution should be prepared using a polypropylene container or beaker.
14. 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.
15. The assay should preferably be performed at room temperature (25°C ± 3°C).
16. Make sure that the test strips are laid with the numbers on the strips facing upwards.
17. 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.
18. Ensure that automated equipment if used is validated before use.
19. 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.
20. Avoid the use of self-defrosting freezers for the storage of reagents and samples.

STORAGE

1. Store **MPD HCV BLOT 3.0** kit and its components at 2°C to 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.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Serum, EDTA plasma or citrate plasma samples can be used. Heparin plasma samples preferably should not be used as they may cause a non-specific reaction to the HCV Core band.

Before storage, ensure that blood clot or blood cells have been separated by centrifugation.

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 or bacterial) samples should be filtered (0.45µm) or centrifuged before testing.

Samples can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follows:
1. Loosen cap of sample container.
2. Heat-inactivate sample at 56°C for 30 minutes in a water bath.
3. Allow sample to cool down before retightening caps.
4. Sample can be stored frozen until analysis.

Repeated freeze-thawing of sample is not recommended.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

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

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, 5µl 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

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

b) 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. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Reactive, and Non-Reactive controls.	
2. Add 2 ml of DILUTED WASH BUFFER to each well.	**2 ml**
3. Incubate the strips for at least 1-2 minutes at room temperature (25 ± 3°C) on a rocking platform (speed of 12 to 16 oscillations per minute). Remove buffer by aspiration.	**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. Care should be taken to ensure specimens are not added directly on the strips.	**20 µl**
6. Cover the tray with the cover provided and incubate for 1 hour at room temperature (25 ± 3°C) on the rocking platform.	**60 minutes**
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.	**3 x 2 ml**
9. Add 2 ml of WORKING CONJUGATE SOLUTION to each well.	**2 ml**
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.	**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

Reagents	Qty	Duration
Nitrocellulose strip	1	-
Wash Buffer	2 ml	2 mins
Blotting Buffer	2 ml	-
Specimen	20 µl	60 mins
Wash Buffer	3 x 2 ml	3 x 5 mins
Conjugate	2 ml	60 mins
Wash Buffer	3 x 2 ml	3 x 5 mins
Substrate (Ready to use)	2 ml	15 mins
Distilled Water	3 x 2 ml	-

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS

Reagents	NUMBER OF STRIPS TO BE USED						
	3	6	9	15	20	27	36
1X Wash Buffer (ml)	60	100	140	240	300	400	520
1X Blotting Buffer (ml)	20	40	60	80	100	120	160
Conjugate (µl)	11	17	23	35	45	59	77
Substrate (ml)	11	17	23	35	45	59	77
Blotting Powder (g)	1	2	3	4	5	6	8

QUALITY CONTROL

The Non-Reactive & Reactive Controls should be run with every assay. In order for the results obtained from any assay to be considered valid, the following conditions must be met:

1. **CONTROL BANDS (Anti-IgG and IgG CONTROL)**
   The two control bands (anti-IgG and IgG, refer to diagram below) should be differentially reactive on all blots. The presence of the anti-IgG band indicates that serum was added in the initial incubation step. The absence of the anti-IgG control band and the presence of the IgG on a blot would indicate the failure to add the patient serum. If there are no bands on any of the blots in an assay, including the control blots, then this could indicate failure in technique or in one of the kit reagents.

2. **NON-REACTIVE CONTROL**
   Only the IgG control band and the anti-IgG control band should be reactive on the Non-Reactive strip. (Fig. 1c)

3. **REACTIVE CONTROL**
   All of the recombinant HCV protein bands should be reactive with the Reactive Control serum. In addition, the anti-IgG and IgG control bands should be reactive. The GST control band should be non-reactive. (Fig 1b).

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.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no express warranty other than that the test kit will function as a Research Use Only assay within the specifications and limitations described in the product Instructions For Use when used in accordance with the instruction contained therein. The manufacturer disclaims any warranty expressed or implied, including such express or implied warranty with respect to merchantability, fitness for use or implied utility for any other purpose. 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. The manufacturer makes no representation expressed or implied, that this product will not infringe the intellectual property rights of the third parties.

TECHNICAL PROBLEMS / COMPLAINTS

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

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

BIBLIOGRAPHY

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4. Van der Poel C. L. Reesink H., W., Schaasberg W. et al. 1990. Infectivity of blood seropositives for hepatitis C virus antibodies. Lancet; 335:558-560.
5. Colombo M., Kuo G., Choo Q-L., et al. 1989. Prevalence of antibodies to hepatitis C virus in Italian patients with heptocellular carcinoma, Lancet; 2: 1006-8.
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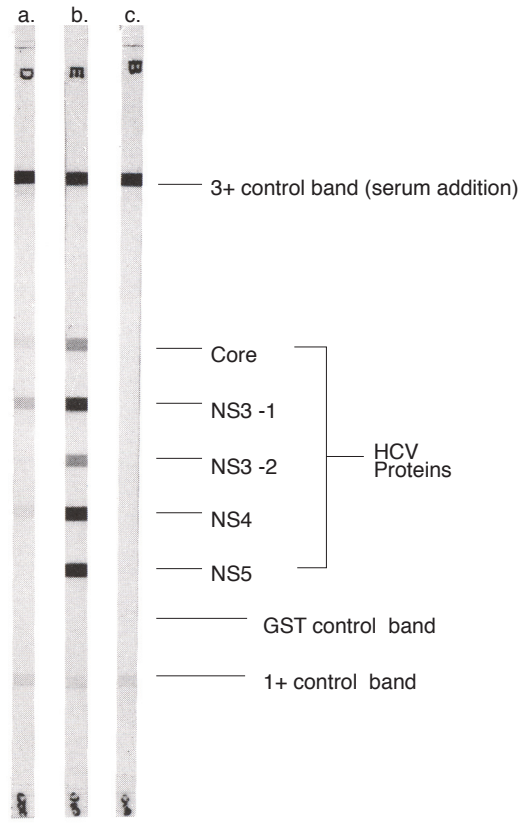
FIGURE 1



Viral specific bands as visualized with:
a. Weak Reactive Serum.
b. Strong Reactive Control.
c. Non-Reactive Control.
(Note: This position of GST band is indicated, but the band itself is not visible since these sera are non-reactive with GST).

TROUBLE SHOOTING CHART

Dark spots develop on strips

1. Bacterial or fungal contamination of test sample.
2. Precipitation of immune complexes in aged test sample.
3. Bacterial or fungal contamination on strip due to improper storage.
4. Strips physically damaged, cracked or scratched.
5. Strips not properly washed between assay steps.

White patches develop on strips

1. Strips was flipped over during assay.
2. Trays not properly washed before use.
3. Poor dissolution of Blotting Powder.

Expected bands do not develop or are of weak intensity.

Check positive control

- 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 conjugate.
  3. Test samples severely immune-complexed.
  4. Test sample IgG 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.

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

1. overdeveloped strips (stop reaction sooner).
2. Incomplete washing.

Absence of Serum Control Band

1. Serum not added.
2. Strips flipped over during assay.
3. Conjugate not added.
4. Substrate not added.

Bands other than the Serum Control band develops on negative control

Tray wells or Control may have been crossed contaminated.

Strips are defective

1. They are cracked.
2. They contain air bubbles which cause the appearance of white spots in reactive zones big enough to prevent any detection.
3. 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.