HCV BLOT 3.0

WESTERN BLOT ASSAY

Instructions For Use

CE
0123

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MAD0011-ENG-6

Note: Changes Highlighted.

REF (18 tests kit) : 11130-018
(36 tests kit) : 11130-036

NAME AND INTENDED USE

The **MP Diagnostics HCV BLOT 3.0** is a qualitative enzyme immunoassay for the *in vitro* detection of antibodies to hepatitis C virus (HCV) in human serum or plasma. It is intended for use as a more specific supplemental / confirmatory test on specimens found repeatedly reactive using specific procedures such as Enzyme-Linked Immunosorbent Assays (ELISA).

The MP Diagnostics HCV Blot 3.0 is intended to be used by professional to aid in the diagnosis of HCV infection.

The MP Diagnostics HCV Blot 3.0 is intended to be used manually or semi-automated using the validated AutoBlot System.

INTRODUCTION

HCV has been identified as the major cause of parenterally transmitted non-A, non-B (NANB) hepatitis. Hepatitis C virus infection occurs in all WHO regions. The highest burden of disease is in the Eastern Mediterranean Region and European Region, with 12 million people chronically infected in each region. In the South-East Asia Region and the Western Pacific Region, an estimated 10 million people in each region are chronically infected. Nine million people are chronically infected in the African Region and 5 million the Region of the Americas. The hepatitis C virus is a bloodborne virus. It is most commonly transmitted through the reuse or inadequate sterilization of medical equipment, especially syringes and needles in healthcare settings, the transfusion of unscreened blood and blood products, and injecting drug use through the sharing of injection equipment. Most people do not have symptoms in the first weeks after injection. It can take between two weeks and six months to have symptoms. When symptoms do appear, they may include fever, feeling very tired, loss of appetite, nausea and vomiting, abdominal pain, dark urine, pale faeces, joint pain, jaundice (yellowing of the skin or eyes.[8]

Screening tests typically are now available for the diagnosis of Hepatitis C infection. These screening tests typically involve antigens from the structural region (capsid) as well as one or more specific antigens from non-structural regions of the virus (NS3, NS4, NS5). Repeatedly reactive samples from screening tests require additional and more specific tests to confirm HCV seropositivity, since false-positive reactions are possible with currently available HCV ELISA screening tests.

Supplementary confirmatory tests should include individual viral antigens as well as appropriate negative controls. The **MP Diagnostics HCV BLOT 3.0** includes structural and non-structural antigens from HCV and is intended as supplemental confirmatory assay for presence of antibodies to HCV.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on **MP Diagnostics** products and packaging.

Use-by date *Synonym for this :* Expiry Date	IVD In vitro diagnostic medical device
Batch Code *Synonyms for this are:* Lot Number Batch Number	REF Catalogue Number
Temperature Limit	Attention. See Instruction for Use
Manufacturer	EU REP Authorised Representative in the European Community
Contains sufficient for <n> tests	Consult instructions for use
Do not reuse	UDI Unique Device Identifier

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips contain five recombinant HCV proteins, including Core, NS3-1, NS3-2, 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 or 36 strips
CONTROL −	**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 +	**REACTIVE CONTROL** Inactivated human serum with high titer antibodies to HCV. Non-reactive for anti-HIV-1/2, anti-HTLV-I/II and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)
BUF STOCK 10x	**STOCK BUFFER CONCENTRATE (10x)** Tris buffer with heat inactivated normal goat serum. Contains thimerosal as preservative.	1 bottle (20 ml)
BUF WASH 20x T	**WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20. Contains thimerosal as preservative.	1 bottle (70 ml)
CONJUGATE	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase. Contains sodium azide preservative.	1 vial (120 µl)
SUBS BCIP / NBT	**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)
	Instructions For Use	1 copy
	Forceps	1 piece
	Incubation Tray *	

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

* Incubation trays provided but packed separately from the kit.

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, 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 ***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.

Pursuant to EC regulation 1272/2008 (CLP), hazardous components are classified and labelled as follows:

Component:	**Nitrocellulose strips**
Signal Word:	**Danger**
Pictogram:	
Hazard Statements:	H228 Flammable solid
Precautionary Statements:	P210 Keep away from heat/ sparks/open flames/hot surfaces. – No smoking. P280 Wear protective gloves/protective clothing/ eye protection/face protection.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	100% Nitrocellulose

Component:	**STOCK BUFFER CONCENTRATE (10x) WASH BUFFER CONCENTRATE (20x)**
Signal Word:	Warning
Pictogram:	
Hazard Statements:	H373 May cause damage to organs through prolonged or repeated exposure

Precautionary Statements:	P260 Do not breathe dust/ fume/gas/mist/vapours/ spray. P501 Dispose of contents/ container in accordance with local/regional/national/ international regulations.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	0.1% Thimerosal

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. Please DO NOT reuse incubation trays.

ANALYTICAL PRECAUTIONS

1. Serum, EDTA plasma or citrate plasma samples can be used. Heparin plasma samples must not be used as they may cause a non-specific reactions to the HCV core band.
2. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instruction Manual. 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 patients' samples 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 MP Diagnostics 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.
3. The opened components with proper handling and storage are stable until the expiry date given on the kit.

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 must 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 are recommended to 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 for not more than 60 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
- AutoBlot System 36 (Product code: EMC019) / AutoBlot System 48 (Product code: EMC020) and the accessories of the systems (please approach local representative or manufacturer for the details)(Optional)

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.

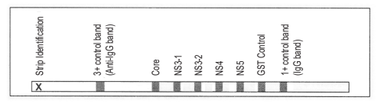
2. **NON-REACTIVE CONTROL**
   Only the IgG control band and the anti-IgG control band should be reactive on the Non-Reactive strip. (Fig. 1b)

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 1a).

Specifications assigned to MP Diagnostics HCV Blot 3.0 Test Controls are traceable via internal production/QC procedures. The preparation of MP Diagnostics HCV Blot 3.0 Test Controls is described by detailed internal production/QC procedures.

INTERPRETATION OF RESULTS

The following is a diagram of the antigens and controls coated on MP Diagnostics HCV BLOT 3.0



Locate and identify the intensity of the control bands. The 3+ intensity is the anti-IgG band and the 1+ intensity band is the IgG control band. These should be visible on all strips. The intensity of any reactive band is compared to these two bands for reference. Comparison with these bands is performed in order to assign a reactivity rating to each antigen on the strip.

PATTERN	INTERPRETATION
1) No reactivity	-
2) Reactivity < 1+ control	±
3) Reactivity = 1+ control	1+
4) Reactivity > 1+ and <3+ control	2+
5) Reactivity = 3+ control	3+
6) Reactivity > 3+ control	4+

BLOT PROFILE	INTERPRETATION
No bands of 1+ or greater reactivity	**NEGATIVE**
1+ or greater reactivity to 2 or more HCV antigens OR POSITIVE 2+ or greater reactivity to Core band only	**POSITIVE**
Any single HCV band of 1+ or greater reactivity but does not meet criteria for POSITIVE	**INDETERMINATE**

Reactivity to GST control band alone is considered **NEGATIVE.**

GST control band reactivity of 1+ or more and 1+ or greater reactivity on one or more HCV antigens is considered **INDETERMINATE.**

A sample which has been found non-reactive on another manufacturer's HCV screening assay or confirmatory assay may be found reactive on MP Diagnostics HCV BLOT 3.0 due to the presence of unique epitopes in this confirmatory test.

SPECIFIC PERFORMANCE CHARACTERISTICS

Overall Diagnostic Performance
A total of 550 HCV positive specimens and 583 HCV negative specimens were tested with MP Diagnostics HCV Blot 3.0; the overall diagnostic sensitivity is 99.8% and the overall diagnostic specificity is ≥99.9%.

Performance	MP Diagnostics HCV Blot 3.0	
	Performance	95% CI
Diagnostic Sensitivity*	99.82% (549/550)	98.99% to 100.00%
Diagnostic Specificity**	100% (583/583)	99.37% to 100%
Positive Predictive Value (PPV)	100%	99.33% to 100%
Negative Predictive Value (NPV)	99.83%	98.80% to 99.98%

*HCV Positive specimens including various HCV genotype-1 to 6.
**HCV Negative specimens including Normal Blood Donor, Cross-Reactive specimen/Interfering specimen/Pregnancy specimen

Specimens for Diagnostic Specificity

Category	Specificity sample size
Normal Blood Donors	215 #7
Hospitalized Specimen	213 #13
Cross-reactive (HTLV-I/II, HIV-1, HIV-2, HAV, HBsAg, Pregnancy specimens)	138
Interference	
Triglyceride	1
Hemoglobin	1
Total Protein	1
RF	2
IgG	2
Bilirubin	2
Lipemic	2
E.coli	6
Total	583

Note: Indeterminate results included in the calculation. # denotes no. of indeterminate result.

Six (6) different exogenous interfering substances (Acetaminophen, Caffeine, Ibuprofen, Tetracycline Hydrochloride, Ethanol, Rifampicin) were spiked into HCV Neg- and Pos+ samples and the results showed that no interfering effect was noticed from these substances.

Sero-conversion panels/Low titer panel
A total of fourteen (14) sero-conversion panels were tested with MP Diagnostics HCV Blot 3.0. The reactivity and average days detection from 1st bleed of MP Diagnostics HCV Blot 3.0 were found to be better or comparable with the commercially available supplemental HCV test device.

Another fifteen (15) seroconversion panels were tested with MP Diagnostics HCV Blot 3.0. 14 seroconversion panels were tested with HCV positive; 1 seroconversion panel was tested with HCV indeterminate.

A total of four (4) HCV Low Titer panels were evaluated on MP Diagnostics HCV Blot 3.0 and other commercially available supplemental HCV test devices. The performance of MP Diagnostics HCV Blot 3.0 were found to be better or comparable with the commercially available supplemental HCV test device.

Analytical sensitivity
With the titration of three (3) HCV-positive specimens, the reactivity endpoint for MP Diagnostics HCV Blot 3.0 was found to be at 1:800 and 1:1280 dilution, respectively.

Precision
The inter-assay (between-run) and intra-assay (within-run, within-day, and day-to-day) reproducibility of MP Diagnostics HCV Blot 3.0 has been evaluated using a set of control panel members. All results obtained consistently fall within the acceptable criteria, indicating the MP Diagnostics HCV Blot 3.0 is robust, reproducible, and consistent in three (3) lots study.

LIMITATIONS OF THE METHOD

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

A **NEGATIVE** result does not exclude the possibility of exposure to or infection with HCV, negative result could probably due to early infection before sero-conversion when the antibody level is too low to be detected.

An **INDETERMINATE** result should not be used as a basis for diagnosis of HCV infection. Reactivity of ≥1+ on one HCV antigen only may be non-specific reactivity, an indication of past resolved infection, or an indication of early seroconversion. We recommend retesting two to six months later using a fresh specimen. **INDETERMINATE** sera can be tested by PCR to further determine whether a person has been exposed to or infected with HCV.

A positive result does not distinguish past and current infection, nucleic acid test (NAT) for HCV ribonucleic acid (RNA) is needed to confirm active infection and the need for treatment. [7]

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no 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 purpose. Natural or legal persons may claim compensation for damage caused by a defective device in accordance with applicable Union and national law. Authorised representative shall be legally liable for defective devices on the same basis as, and jointly and severally with, the manufacturer if manufacturer has not complied its obligations listed in Regulation (EU) 2017/746.

TECHNICAL PROBLEMS / COMPLAINTS / SERIOUS INCIDENTS

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.

Should there be any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Summary of Safety and Performance (Reference no.: SSP-MP Diagnostics HCV Blot 3.0) is available on EUDAMED or can be obtained from MP Biomedicals.

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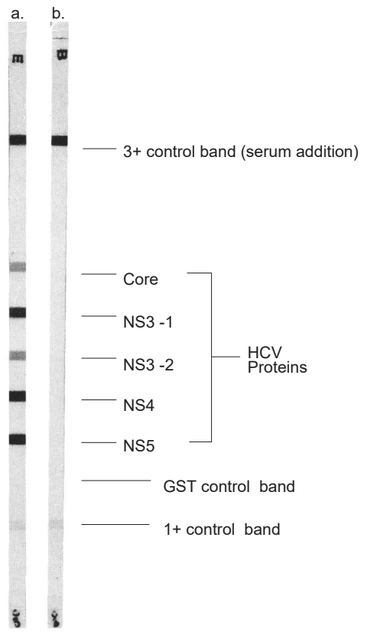
FIGURE 1



Viral specific bands as visualized with:

a. Reactive Control
b. 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 appearrance of white spots in reactive zones big enough to prevent 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.