HEV IgM ELISA

Note Changes Highlighted

NAME AND INTENDED USE

The MP Diagnostics (MPD) HEV IgM ELISA is an enzyme-linked immunosorbent assay intended for the detection of IgM antibodies to Hepatitis E Virus (HEV) in human serum or plasma.

INTRODUCTION

Major epidemics of enterically transmitted non-A, non-B hepatitis (ET-NANBH) have been found to occur in developing regions such as Asia, the former USSR, Central America and Africa (1,2). Sporadic cases have also been reported in developed nations, including Australia, the United Kingdom and the United States (3,4,5). Cases in developed nations have generally been associated with travel to endemic regions.

The course of the infection is generally acute and self-limiting without chronic sequelae. There is, however, a high incidence of mortality in pregnant women in the third trimester, about 10-20% (1) and a mortality rate of 1-2% in the general population, which is 10 times that of Hepatitis A (HAV). With the cloning of the etiological agent of ET-NANBH and the identification of type common viral epitopes (6,7), specific diagnostic tools have been developed to detect antibodies to hepatitis E virus (HEV).

Studies with Egyptian children from Benha in 1986 revealed that previous exposure to HEV will elicit an IgG response (8) which may be transient and disappear in 6 months but can sometimes last up to 8 years or more as seen in a recent study in Taiwan (9). The IgM response has been shown to be limited to the acute phase of HEV infection. Previously, detection of the acute response in HEV infection has been through observation of viral particles in the stool of infected individuals using IEM or by PCR (10,11). This method requires expensive equipment and technique-dependent expertise. Furthermore, the shedding of the viral particles is usually in small quantities and may not be of sufficient titer to be detected. The MP Diagnostics HEV IgM ELISA utilizes recombinant HEV antigens from the structural region of the viral genome to detect the presence of IgM antibodies associated with acute infection.

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The wells of the polystyrene microplate strips are coated with three recombinant HEV antigens which correspond to the structural regions of the Hepatitis E Virus. Human serum or plasma, diluted in diluent buffer, are incubated in these coated wells. HEV specific antibodies, if present, will bind to the solid phase HEV antigens. The wells are thoroughly washed to remove unbound materials and a mouse monoclonal anti-human IgM labeled with horseradish peroxidase is added to the wells. This labeled antibody will bind to any antigen-antibody complexes previously formed and excess unbound labeled antibodies are removed by washing. A substrate solution containing 3, 3’, 5, 5’-tetramethylbenzidine (TMB) is then added to each well. The presence of specific antibodies is indicated by the presence of a blue colour after substrate addition. Reaction is terminated by addition of hydrochloric acid. The intensity of the colour is measured spectrophotometrically at 450nm and is proportional to the amount of antibodies present in the specimen.
**KIT COMPONENTS**

<table>
<thead>
<tr>
<th>Component Description</th>
<th>Quantity Provided</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MICROPLATE</strong></td>
<td></td>
</tr>
<tr>
<td>HEV MICROPLATE</td>
<td>1 plate (96 wells)</td>
</tr>
<tr>
<td></td>
<td>Twelve 8-well strips per plate, sealed in an aluminum pouch with desiccant. Each microplate well contains adsorbed recombinant HEV proteins. Store at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>CONTROLs</strong></td>
<td></td>
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<tr>
<td>NON-REACTIVE CONTROL</td>
<td>1 vial (160 µl)</td>
</tr>
<tr>
<td></td>
<td>Inactivated normal human serum, non-reactive for anti-HCV, anti-HEV, HBsAg and anti-HIV 1. Contains thimerosal and sodium azide as preservatives. Store at 2°C to 8°C.</td>
</tr>
<tr>
<td>REACTIVE CONTROL</td>
<td>1 vial (80 µl)</td>
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<tr>
<td></td>
<td>Inactivated human serum containing a high titer of IgM antibodies specific for HEV. Contains thimerosal and sodium azide as preservatives. Store at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>DILUENT</strong></td>
<td>1 bottle (100 ml)</td>
</tr>
<tr>
<td></td>
<td>Tris based saline solution containing heat treated normal goat serum, bovine serum albumin and stabilizers. Contains Bronidox™ as preservative. Store at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>PLATE WASH CONCENTRATE (20X)</strong></td>
<td>1 bottle (120 ml)</td>
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<tr>
<td></td>
<td>Phosphate buffered saline with Tween-20. Contains chloroacetamide as preservative. Store at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>CONJUGATE IgM</strong></td>
<td>1 vial (70 µl)</td>
</tr>
<tr>
<td></td>
<td>Mouse monoclonal anti-human IgM labeled with horseradish peroxidase. Contains 0.02% thimerosal as preservative. Store at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>SUBSTRATE</strong></td>
<td>1 bottle (12.5 ml)</td>
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<tr>
<td></td>
<td>Buffer containing 3, 3', 5, 5'-tetramethylbenzidine. Store in the dark at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>SOLN STOP HCN</strong></td>
<td>1 bottle (30 ml)</td>
</tr>
<tr>
<td></td>
<td>1N Hydrochloric acid solution. Store in the dark at 2°C to 8°C.</td>
</tr>
<tr>
<td><strong>PLATE COVERS</strong></td>
<td>4 pieces</td>
</tr>
<tr>
<td></td>
<td>Adhesive covers for microplate during incubation.</td>
</tr>
<tr>
<td><strong>INSTRUCTION MANUAL</strong></td>
<td>1 copy</td>
</tr>
</tbody>
</table>

**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. 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.

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 assay specimens, microplates, Reactive and Non-Reactive Controls as potentially infectious agents.
4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard 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 an 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. Never add water to Stop Solution.
10. 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 contaminating 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.

Bronidox™ is a Trade Mark of Henkel Chemical Co.
11. Autoclave all used and contaminated materials at 121°C, 15 psi for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.

12. 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.

**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 microplates 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. To prevent cross contamination, use a new pipette tip for each specimen aliquoted to, and do not touch the top or the bottom of the strips, the edge of the wells or the liquid in the wells with fingers or pipette tips.

6. 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.

7. For best results allow all reagents and samples to reach room temperature (25°C ± 3°C) before use. Immediately after use return to 2°C to 8°C storage.

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

9. All reagents must be mixed well before use.

10. Working Conjugate and Diluted Wash Buffer should be **prepared fresh prior to use.**

11. 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.

12. Do not remove microplates from the storage bag until immediately before use. Opened, unused strips should be stored at 2°C to 8°C in its storage bag with the desiccant provided.

13. The kit controls should be assayed concurrently with patients’ samples for each test run.

14. Care should be taken to avoid touching or splashing the rim of the well with conjugate. Do not “blow out” from the micropipette. It is recommended to use reverse pipetting whenever possible.

15. Use of highly haemolyzed samples, incomplete clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.

16. **DO NOT USE A WATER BATH TO INCUBATE PLATES.**

17. CO₂ incubators must not be used.

18. During 37°C incubation, evaporation must be prevented. Cover plates with adhesive covers provided.

19. Avoid repeatedly opening and closing the incubator door during incubation steps.

20. Do not store the stop solution in a shallow dish or return it to a stock bottle after use.

21. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Remove any bubbles in the well, e.g. by gentle tapping.

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

23. Routine maintenance of aspiration/ wash system is strongly recommended to prevent carryover from highly reactive specimens to non-reactive specimens.
STORAGE INSTRUCTIONS

1. Store MPD HEV IgM ELISA kit and its components at 2°C to 8°C when not in use.
2. All test reagents and microplates when stored at 2°C to 8°C are stable until the expiry date given on the kit. Do not freeze the reagents.
3. Crystals may form when Plate Wash Concentrate (20x) is stored at 2°C to 8°C. These must be dissolved by warming at 37°C prior to use.
4. Precipitate may form when the Diluent is stored at 2°C to 8°C. This will not affect the performance of the kit.
5. Opened, unused microplate strips must be stored with the desiccant provided at 2°C to 8°C in a closed pouch.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Samples should be stored 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-haemolysed samples are preferred. Lipemic, icteric or contaminated (particulate) samples should be filtered (0.45µm) or centrifuged before testing.

Patients’ sera can be inactivated but this is not a requirement for optimal test performance.
Inactivate sera as follows:
1. Loosen caps of serum containers.
2. Heat serum to 56°C for 30 minutes in a water bath.
3. Allow serum to cool before retightening caps.
4. Serum can be stored frozen until analysis.

We recommend that the patients’ sera should not undergo repeated freeze-thaw cycles.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable absorbent bench top paper and paper towels.
2. Polypropylene tubes or containers.
3. Graduated pipettes: 5 ml, 10 ml.
4. Multichannel pipettor capable of delivering 50 µl, 100 µl, and 200 µl.
5. Pipettor capable of delivering 1-1000 µl.
6. Disposable pipette tips.
7. Reagent reservoirs (troughs) with a capacity of 25 ml.
8. Deionised or distilled water, reagent grade quality.
9. Flasks: 500 ml, 1 litre.
10. A 37°C incubator.
11. A dual (A450-A620) or single (A450) wavelength microassay plate reader.
12. Sodium hypochlorite (5%) solution or liquid household bleach.

PREPARATION OF REAGENTS

1. WORKING CONJUGATE
   a. WORKING CONJUGATE should be prepared fresh prior to use.
   b. To prepare diluted conjugate, make a 1:200 dilution of conjugate with diluent provided in the kit.
   c. Use only polypropylene containers or tubes.
   d. Refer to chart for working conjugate preparation.

   CONJUGATE PREPARATION CHART

<table>
<thead>
<tr>
<th>Number of tests</th>
<th>Vol. of Conjugate (µl)</th>
<th>Vol. of Diluent (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>15</td>
<td>3.0</td>
</tr>
<tr>
<td>48</td>
<td>30</td>
<td>6.0</td>
</tr>
<tr>
<td>72</td>
<td>45</td>
<td>9.0</td>
</tr>
<tr>
<td>96</td>
<td>60</td>
<td>12.0</td>
</tr>
</tbody>
</table>

2. DILUTED WASH BUFFER
   a. DILUTED WASH BUFFER should be prepared fresh prior to use.
   b. Dilute 1 volume of PLATE WASH CONCENTRATE with 19 volumes of distilled water (reagent grade quality). Mix well. Approximately 400 ml of wash buffer is required to wash 1 plate.
**ASSAY PROCEDURE**

**IMPORTANT:** Immunoassays of this nature are temperature-sensitive and time-dependent. Strict adherence to the assay procedure will ensure optimal assay performance. Deviations from the recommended procedure may lead to aberrant results.

1. Remove microplate from the aluminium bag.

2. Shake specimen and control vials before use.

3. Fill a reagent reservoir with DILUENT. Using a multichannel pipettor, add 200 µl of DILUENT to all wells.

4. Wells A1 and B1 are ‘BLANKS’ DO NOT ADD SPECIMEN TO THESE WELLS. Add an additional 10 µl of diluent to these wells.

5. Add 10 µl of specimen to the assigned well, starting at well H1. This will give a final specimen dilution of 1:21. DO NOT PLACE SPECIMEN INTO AN EMPTY WELL.

6. After the test specimen have been added, add 10 µl of NON-REACTIVE CONTROL per well to wells C1, D1 & E1.

7. Add 10 µl of REACTIVE CONTROL per well to wells F1 and G1. Mix thoroughly by tapping gently on all sides of microplate, taking care to keep the plate flat on the bench-top.

8. Carefully cover the microplate with a plate cover provided to prevent evaporation during incubation.

9. Incubate for 30 minutes at 37°C (Do not use a 37°C water bath for incubation).

10. Prepare WORKING CONJUGATE as described in the PREPARATION OF REAGENTS prior to washing the microplate.

11. Remove and discard the plate cover and wash the microplate with DILUTED WASH BUFFER using one of the two recommended methods.

A. Automated or Semi-automatic Microplate Washer - Aspirate completely the contents of all wells by lowering the aspirator tip gently to the bottom of each well. BE CAREFUL NOT TO SCRATCH THE INSIDE OF THE WELL SURFACE. Fill the entire plate with at least 300 µl/well, then aspirate immediately in the same order. Perform this cycle six (6) times.

12. Blot dry by inverting the microplate and tapping firmly onto absorbent paper. All residual plate wash buffer should be blotted dry. Colour formation can be inhibited during the substrate incubation by residual plate wash buffer.

13. Fill a reagent reservoir with the WORKING CONJUGATE. Using a multichannel pipettor, add 100 µl of WORKING CONJUGATE to each well. Apply another plate cover.

14. Incubate the microplate for 30 minutes at 37°C (Do Not use a 37°C water bath for incubation).

15. Remove and discard the plate cover. Repeat the wash procedure as in Step 11 and Step 12.

16. Fill a reagent reservoir with SUBSTRATE solution. Using a multichannel pipettor, add 100 µl of SUBSTRATE solution to each well. Apply a plate cover.

17. Incubate for 15 minutes in the dark at room temperature (25 ± 3°C).

18. Remove and discard the plate cover.

19. Using a multichannel pipettor, add 100 µl of STOP SOLUTION to each well. Mix gently by tapping the plate.

20. Determine the Absorbance for each well at 450nm. If a dual filter instrument is used, the reference wavelength should be 620 nm.

**NOTE:** Absorbance should be read within 10 minutes upon addition of the STOP SOLUTION.
1. The BLANK and the REACTIVE CONTROL should be assayed in duplicate, whereas the NON REACTIVE CONTROL in triplicate on each plate with each run of specimens.

2. Blank values must have an absorbance of ≤ 0.100.

3. Non-Reactive Control values must have an absorbance ≤ 0.100 after subtracting the Blank.

4. Each of the 2 Reactive Control values must have absorbance ≥ 0.500 after subtracting the Blank.

5. For the assay to be valid, the difference between the mean absorbances of the Reactive Control and the Non-Reactive Control (RCx - NRCx) should be 0.400 or greater. If not, technique may be suspect and the assay must be repeated. If RCx - NRCx is consistently low, deterioration of reagents may be suspected.

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed.

THE MEAN ABSORBANCE VALUES OF THE BLANK MUST BE SUBTRACTED FROM BOTH THE CONTROLS AND THE SPECIMENS ABSORBANCE VALUES BEFORE INTERPRETATION OF RESULTS.

The presence or absence of IgM antibodies specific for HEV is determined by relating the absorbance of the specimens to the CUT-OFF VALUE of the plate.

The CUT-OFF VALUE for MPD HEV IgM ELISA is calculated as 0.400 + the Mean Absorbance of the Non-Reactive Control.

Individual Non-Reactive Control values should be less than or equal to 0.100 unit. If one of the Non-Reactive Control value does not meet the above criteria it must be excluded as aberrant. The Non-Reactive Control Mean (NRCx) should then be recalculated using the remaining individual Non-Reactive Control values. All remaining individual Non-Reactive Control values must meet the criteria or the assay is invalid and must be repeated.

Individual Reactive Control values must be greater than or equal to 0.500 units. If one Reactive Control value does not meet either of the above criteria, the assay is invalid and must be repeated.

For the assay to be valid, the RCx - NRCx value should be 0.400 or greater. If not, improper technique or deterioration of reagents may be suspected and the assay should be repeated.

Finally, reactive specimens which are Non-Reactive on retesting are considered negative by the criteria of the MPD HEV IgM ELISA.

CALCULATION OF RESULTS

1. Calculation of Non-Reactive Control Mean Absorbance (NRCx)

   Example:  
<table>
<thead>
<tr>
<th>Well No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.010</td>
</tr>
<tr>
<td>D1</td>
<td>0.012</td>
</tr>
<tr>
<td>E1</td>
<td>0.008</td>
</tr>
<tr>
<td>Total</td>
<td>0.030</td>
</tr>
<tr>
<td>Mean</td>
<td>0.030/3 = 0.010 (NRCx)</td>
</tr>
</tbody>
</table>

2. Calculation of Reactive Control Mean Absorbance (RCx)

   Example:  
<table>
<thead>
<tr>
<th>Well No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.758</td>
</tr>
<tr>
<td>G1</td>
<td>0.732</td>
</tr>
<tr>
<td>Total</td>
<td>1.490</td>
</tr>
<tr>
<td>Mean</td>
<td>1.490 / 2 = 0.745 ( RCx )</td>
</tr>
</tbody>
</table>

3. Calculation of the difference between RCx and NRCx

   Example:  
   | NRCx     | 0.010 |
   | RCx      | 0.745 |
   | RCx-NRCx | 0.745 - 0.010 |
   |          | 0.735 |

4. Calculation of CUT - OFF value

   CUT- OFF Value = 0.400 + NRCx

   Example :  
   | NRCx | 0.010 |
   | CUT-OFF value | 0.400 + 0.010 |
   |        | 0.410 |

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the CUT - OFF value are considered Non-Reactive by the MPD HEV IgM ELISA.
2. Specimens with absorbance values greater than or equal to the CUT - OFF value are considered initially reactive by the criteria of the MPD HEV IgM ELISA and should be retested in duplicate before interpretation.
3. Specimens found Reactive on retesting are to be interpreted as repeatedly reactive for IgM antibodies to HEV by the criteria of the MPD HEV IgM ELISA.
4. Initially reactive specimens which are Non-Reactive on retesting are considered negative by the criteria of the MPD HEV IgM ELISA.
SPECIFIC PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity
The detection of viremia in blood or fecal specimens during the initial phase of the infection have been used to establish the presence of Hepatitis E. However, the detection of viremia can only be successful if fecal or serum samples are collected early enough, preferably during the first 14 days post-onset of symptoms (12). Based on the detection of viremia, the sensitivity of MPD HEV IgM ELISA is determined based on the ability to detect the number of positives from this well-characterized panel of sera. Out of 152 samples collected within the 14-day window period, 141 samples were reactive by the MPD HEV IgM ELISA. This represents a sensitivity of 93%.

The seroreactivity among healthy individuals in low-risk populations tends to be quite low, approximately ≤ 1%, while the seroreactivity among healthy individuals within endemic areas tend to be somewhat higher. Seroreactivity indicates recent exposure to the Hepatitis E virus.

LIMITATION OF THE METHOD

Repeatedly reactive results from the MPD HEV IgM ELISA is presumptive evidence of IgM antibodies to HEV in the specimen. A NON-REACTIVE result from the MPD HEV IgM ELISA indicates the likely absence of detectable IgM antibodies to HEV in the specimen. A NEGATIVE result does not exclude the possibility of exposure to or infection with HEV.

Falsely reactive results can be suspected with a test kit of this nature. The proportions of false reagents will depend on the sensitivity and the specificity of the test kit. For most diagnostic assays, the higher the prevalence of antibody in a population, the lower the proportion of falsely reactive samples.

Based on internal studies, presence of rheumatoid factor (RF) or high titers of IgG was found not to affect the performance of MPD HEV IgM ELISA. The use of IgG removal methods (e.g. RFRR) which require the sample to be diluted may affect the sensitivity performance of MPD HEV IgM ELISA.

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 express or implied, including such express 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. The manufacturer makes no representation express 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 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.

BIBLIOGRAPHY
