FaStep® HEV-P23 **Hepatitis E Rapid Test (Device,Serum/Plasma)**

For professional in vitro diagnostic use only.

INTENDED USE

The HEV IgM Rapid Test Device (Serum/Plasma) is an *in vitro* diagnostic rapid immunochormatographic assay for the qualitative detection of specific anti-HEV IgM in human serum and plasma specimens. The product may be used as an aid in the diagnosis of hepatitis E. A reactive result should be confirmed by other supplemental assay(s).

INTRODUCTION

Hepatitis E is inflammation of the liver caused by infection with the hepatitis E virus (HEV).[1] It is spread by fecally contaminated water within endemic areas or through the consumption of uncooked or undercooked meat.[2,3,4] The course of infection has two phases, the prodromal phase and the icteric phase. The infection is self-limited. Whether protective immunoglobulins develop against future re-infection remains unknown. The overall case fatality rate is 4%, although pregnant women and liver transplant recipients may be at substantially higher risk.

HEV infection is usually diagnosed by detecting specific anti-HEV antibodies (IgM and IgG). Analysis of HEV RNA in biologic specimens such as stools, serum, and liver biopsy using NATs is also used for diagnosis.

Anti-HEV IgM appears during acute stage of the infection and is detectable four days after the onset of jaundice and persists for up to five months.[5] However, strongly positive reactions are rare after three months.[6] The phenomenon of long-lasting and protective antibodies to HEV was observed which greatly enhance the understanding to the diagnosis, epidemiology, zoonosis-related studies and vaccine development. The anti-HEV IgM assays could be used as first line tools for the routine diagnosis of acute HEV infection, even in immunocompromised patients.

The HEV IgM Rapid Test is a lateral flow immunoassay for the direct detection of anti-HEV IgM. It will provide a presumptive diagnosis of acute infection of hepatitis E.

PRINCIPLE

The HEV IgM Rapid Test Device detects specific anti-HEV IgM through visual interpretation of color development on the internal strip. HEV antigens are immobilized on the test region of the membrane.

When specimen and then buffer is added to the sample well on the test panel, anti-HEV IgM, if present, will bind to the anti-human IgM antibodies conjugated to colored particles on the sample pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by HEV antigen immobilized at the detection zone. A colored bind will form at the test region. The presence of this colored band indicates a positive result, while its absence indicates a negative result.

As liquid continues to migrate down the test strip, the control line appears. The appearance of this colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS

Materials Provided

- Test devices
- Droppers
- Buffer
- Package insert

Materials Required But Not Provided

- Specimen collection equipment and container
- Timer
- Centrifuge

WARNINGS AND PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Read these instructions carefully before performing the test.
- Do not use the test beyond the expiration date.
- Do not use the test if the packaging is damaged.
- Do not reuse tests.
- Apply standard biosafety precautions when handling and disposing of potentially infectious materials.
- Handle all specimens as potentially infectious.
- Wear protective clothing such as gloves, laboratory coats, and eye protection when specimens are being assayed.
- The test device and accessory should be disposed in a proper biohazard waste container after testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled.
- Avoid splashes and clean up spills immediately with appropriate disinfectant.
- The buffer contains 0.02% sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Humidity and temperature can adversely affect results.
- Do not use any other specimen than those specified. For plasma, EDTA, sodium citrate, sodium heparin or potassium oxalate can be used as anticoagulant.
- Used testing materials should be discarded in accordance with local regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- Do not freeze the kit.
- Protect the kit from humidity.
- Care should be taken to protect the components of the kit from contamination. Do not use the test if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND PREPARATION

- The HEV IgM Rapid Test Device (Serum/Plasma) is intended for use with human serum or plasma specimens only.
- Collect specimens according to safe phlebotomy procedure.
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to one week. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Multiple freeze/ thaw cycles should be avoided. freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated sera may cause erroneous results.

TEST PROCEDURE

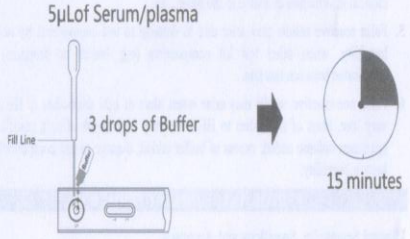
Bring the test device, buffer and specimen to room temperature (15-30°C) prior to testing.

1. Remove the test device from its sealed pouch and use it as soon as possible. For best results, the assay should be performed within one hour.
2. Place the test device on a clean, level surface. Label with specimen ID.

   Using the provided disposable dropper, draw the specimen up to the Fill Line, and transfer serum/plasma (approximately 5 uL), to the sample well (S), then add 3 drops of buffer and start the timer.

Avoid trapping air bubbles in the sample well (S), and do not add any solution to the result area.

3. Wait for the colored band(s) to appear. **Read results at 15 minutes.**



INTERPRETATION OF RESULTS

POSITIVE: Two colored lines appear. One line should always appear in the control region (C), and another line appears in the test region (T).

NEGATIVE: Only one colored line appears in the control region (C). No apparent colored line appears in the test region (T).

INVALID: No line appears in the control region (C). Results from any test which has not produced a control line at the specified reading time must be discarded. Please review the test procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. However, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and the concentration of analytes in the specimen cannot be determined.

2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control line failure.

QUALITY CONTROL

- An internal procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique. The control line does not control for the addition of adequate volume of specimen.
- External quality controls are not supplied with this kit. It is recommended that quality controls be tested as a good laboratory practice.

LIMITATIONS

1. The HEV IgM Rapid Test Device (Serum/Plasma) is for professional *in vitro* diagnostic use only, and should be used for the qualitative detection of IgM antibodies to HEV in human serum and plasma.
2. The HEV IgM Rapid Test Device (Serum/Plasma) will only indicate the presence of IgM antibodies to HEV in the specimen and should not be used as the sole criteria for the diagnosis of HEV infection.
3. For confirmation of test results, specimens should undergo further testing using different assays, such as EIA or molecular assays.
4. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5. False reactive results may arise due to damage to test components by heat or humidity, when other test kit components (e.g. buffer or droppers) are substituted between test kits.
6. False non-reactive results may arise when titers of IgM antibodies to HEV are very low, titers of antibodies to HEV are very high (hook effect), insufficient specimen volume added, excess of buffer added, damage to test components by heat or humidity.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

The HEV IgM Rapid Test Device (Serum/Plasma) was evaluated with a total of 359 specimens. The testing results were summarized as below:

HEV IgM Rapid Test vs. EIA

		EIA		Total
		Positive	Negative	
HEV IgM Rapid Test	Positive	210	1	211
	Negative	3	145	148
Total		213	146	359

Relative Sensitivity: 98.6% (95.9%-99.5%)*

Relative Specificity: 99.3% (96.2%-98.9%)*

Overall Agreement: 98.9% (97.2%-99.6%)*

*95% Confidence Interval

Interfering Substances

The HEV IgM Rapid Test Device (Serum/ Plasma) is not affected by all the analytes listed below.

Analyate	Concentration	Analyate	Concentration
Albumin	60 g/L	EDTA	3.4 umol/L
Bilirubin	0.236 g/L	Heparin	3000 U/L
Hemoglobin	2 g/L	Sodium citrate	5 mg/mL
Triglycerides	33.2 g/L	Potassium oxalate	2 mg/mL
Acetylsalicylic acid	3.62 mmol/L	Rifampicin	78.1 umol/L
Ascorbic acid	342 umol/L	Isoniazid	292 umol/L
Amoxicillin	206 umol/L	Ethambutol	58.7 umol/L
Aspirin	4.34 mmol/L	Quinine	148 umol/L
Fluconazole	245 umol/L	Coffee (caffeine)	308 umol/L
Ibuprofen	2425 umol/L	Alcohol (ethanol)	86.8 mmol/L
Loratadine	0.78 umol/L	Visual hemolysis	NA
Nadolol	3.88 umol/L	Icteric	NA
Naproxen	2170 umol/L	Lipemic	NA
Paroxetine	3.04 umol/L		

Cross-reactivity

The HEV IgM Rapid Test Device (Serum/ Plasma) have no cross-reactivity with unrelated medical infections listed below.

anti-HAV IgM +	Yellow fever +	Chagas +
HBsAg +	anti-Zika virus +	Toxoplasmosis +
anti-HCV +	Chikungunya +	HAMA +
anti-Rubella IgM +	Syphilis +	RF +
anti-CMV IgM +	anti-Dengue virus +	ANA+
anti-HSV-I IgM +	Tuberculosis +	EBV IgG +
anti-HSV-II IgM +	*P. vivax* +	*P. falciparum* +

Precision

Intra-Assay (one kit lot)

Within-run precision has been determined by using 10 replicates of 3 specimens: a negative, a low IgM positive and a high IgM positive. One kit lot of the HEV IgM Rapid Test Device (Serum/Plasma) has been tested using above specimens. The specimens were correctly identified >99% of the time.

Inter-Assay (three different kit lots)

Between-run precision has been determined by 10 replicates of 3 specimens: a negative, a low IgM positive and a high IgM positive. Three different kit lots of the HEV IgM Rapid Test Device (Serum/Plasma) have been tested using above specimens. The specimens were correctly identified >99% of the time.

LITERATURE REFERENCES

1. "Hepatitis E". www.niaid.nih.gov. Retrieved 2016-02-23.
2. Harrison TJ. Hepatitis E virus -- an update. *Liver*. 1999 Jun. 19(3):171-6
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4. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E.*Lancet*. 2012 Jun 30. 379(9835):2477-88.
5. Favorov MO, Fields HA, Purdy MA, et al. Serologic identification of hepatitis E virus infections in epidemic and endemic settings. J Med Virol. 1992;36(4):246–250.
6. Dalton HR, Stableforth W, Thurairajah P, et al. Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. Eur J Gastroenterol Hepatol. 2008;20(8):784–790.

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests

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