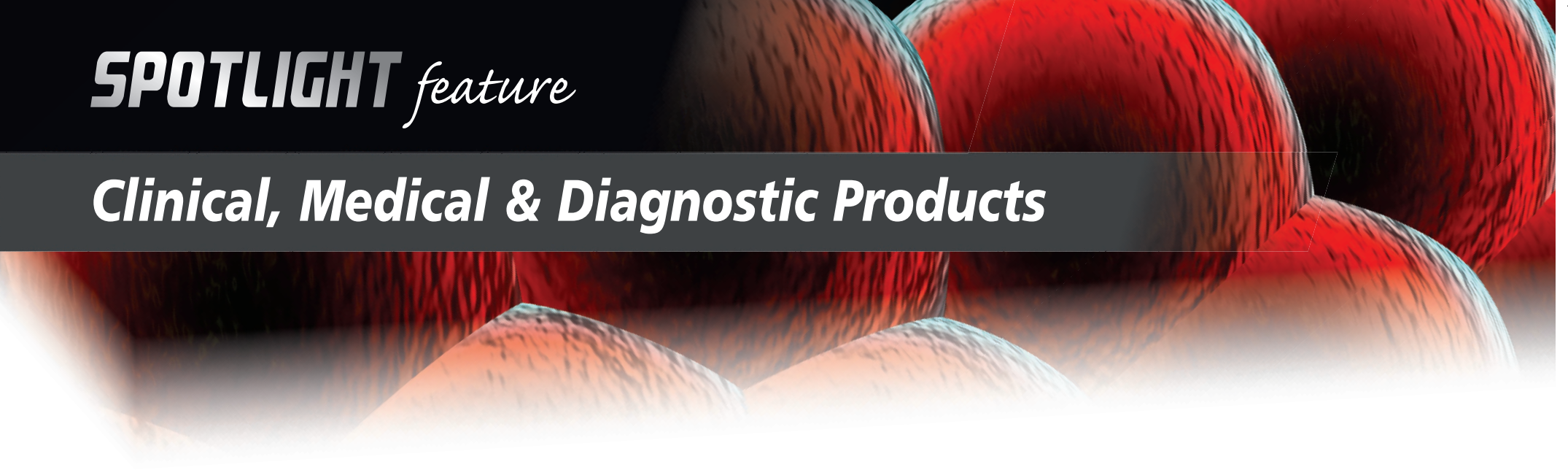
SPOTLIGHT feature

Clinical, Medical & Diagnostic Products



Clinical Evaluation of a Multiplex Real-time PCR Assay for the Detection and Quantification of Hepatitis E Virus

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Every year an estimated 20 million individuals are infected by the hepatitis E virus. Over 3.3 million symptomatic cases of hepatitis E and 56,600 hepatitis E related deaths occur as a result [1]. Hepatitis E is a liver disease caused by the hepatitis E virus: a non-enveloped, positive-sense, single-stranded ribonucleic acid (RNA) virus transmitted mainly through the faecal-oral route, via contaminated water and some foods such as pork.

Following exposure to the hepatitis E virus the incubation period ranges from 3 to 8 weeks, and the virus can cause both acute sporadic and epidemic viral hepatitis. Symptomatic infection is most common in young adults aged 15-40 years, with typical signs and symptoms including jaundice, anorexia, enlarged liver, abdominal pain and tenderness, nausea, vomiting and fever.

Outbreaks and sporadic cases of hepatitis E occur around the world. Disease prevalence is highest in developing countries where accesses to essential water, sanitation, hygiene and health services are limited [1]. In developed countries hepatitis E carries a different threat, as disease transmission can occur through the transfusion of blood products. Hepatitis E is usually self-limiting and is not considered to be fatal. However, it is imperative to avoid transmission and disease progression in immunosuppressed, pregnant and transplant patients, and in cases where HBV or HCV infection is negative but symptoms persist [2]. These groups of patients are more susceptible to acute liver failure which can cause death. Therefore it is essential that diagnostics methods utilised are sensitive and specific, to allow accurate patient diagnosis, and prompt treatment options to be employed.

Diagnosing hepatitis E

The clinical signs, symptoms and laboratory findings for the diagnosis of hepatitis E often overlap with other etiologies, which can make confirming a diagnosis problematic. Diagnostic methods fall into two categories: direct and indirect. Direct methods of detecting the virus include real-time polymerase chain reaction (RT-PCR), whilst indirect methods include detection of the anti-HEV IgM and IgG antibodies. Anti-HEV IgM and IgG are reliable methods of diagnosis in immune-competent hosts, however within immunocompromised hosts false negatives are frequently presented, posing a diagnostic challenge [3].

To diagnose HEV infection efficiently in immunocompromised patients, RT-PCR is becoming the gold standard test due to its accurate, sensitive and fast diagnosis [4]. Choosing the right assay that offers specific and accurate results in a timely fashion is an important factor within RT-PCR, and for precise diagnosis, as any degree of performance variability can ultimately lead to inaccurate diagnoses with disastrous consequences.

A range of assays from various manufacturers are currently on the market to detect the hepatitis E virus, one of which is an assay produced by Fast-track diagnostics. An internal evaluation performed by Fast-track diagnostics showed that 100% of samples were correctly detected by the assay, exhibiting specificity, sensitivity and precision of analysis. Following internal evaluation, the same variables were measured in an external quality control evaluation. The Quality Control for Molecular Diagnostics (QCMD) confirmed the results observed in the internal evaluation, as shown in *Table 1*.

Table 1. Number of samples correctly detected by FTD HEV assay.

Evaluation type	Total samples tested	Correctly confirmed positive or negative	% Correctly detected
External evaluation – Quality Control Molecular Diagnostics (QCMD)	8	8	100
Internal evaluation – specificity, sensitivity and precision analysis	92	92	100

The following comparison study highlights the results of a comparison between the Fast-track diagnostics assay, a competitor assay and in-house assay already used at MHH, to detect hepatitis E virus [5].

A Comparison Study to Clinically Evaluate a Novel Fast-track Diagnostics Multiplex Real-Time PCR Assay

Objective

The MHH, relies on prompt and efficient disease diagnosis, to provide effective patient care. To ensure the most efficient assay was being utilised for accurate patient diagnosis, an external comparison study to evaluate the Fast-track diagnostics Hepatitis E RNA (FTD HEV) assay, against their HEV in-house singleplex assay (MHH) and a competitor assay (detecting HEV and internal control) as reference [3].

Methods

One hundred and four clinical specimens as represented in *Table 2* including EDTA blood, serum, stool, ascites and CPDA were extracted, and were tested with two competitor assays against the in-house assay currently used by MHH.

200µl input volume was extracted and eluted in 75µl and tested on a medium to high-throughput PCR platform. Fast-track diagnostics hepatitis E assay was tested with Fast-track mastermix (Fast-track diagnostics), and the PCR setups were performed in accordance with the instructions of the kit or assay.

Table 2. Overview of the clinical samples tested by FTD HEV assay, MHH in-house assay 1 and competitor kit.

	Clinical Specimens (tested)				
Kit	Serum	EDTA blood	Stool	Ascites	CPDA
FTD HEV assay	16	60	21	2	1
MHH in-house assay	7	59	15	1	0
Competitor kit	10	27	10	1	1

Results

The FTD HEV assay had already displayed positive results in terms of sensitivity, specificity and precision as proven in internal evaluations conducted by Fast-track diagnostics. The external confirmation by QCMD led to the comparison study, comparing the FTD HEV assay with the in-house assay and competitor kit, to determine the most effective kit to utilise. The results are shown in *Table 3a and 3b*. For the quantification of samples with the FTD HEV assay, three quantification standards given in IU/ml were used, as demonstrated in *Figure 1*.

Table 3a. A comparison of positive and negative samples (EDTA, serum, ascites and stool) detected by FTD Hepatitis E RNA versus MHH in-house assay

Sample type	FTD Hepatitis E RNA assay vs. MHH assay	
	Positive	Negative
EDTA	44	11
	- FTD: 2 additional negatives both confirmed as negative by competitor kit - FTD: 1 additional positive tested negative by MHH and competitor kit	
Serum	6	3
Ascites	10	4
Stool	1	0
	- MHH: 2 additional positives confirmed by competitor kit	

Table 3b. A comparison of positive and negative samples (EDTA, serum, ascites and stool) detected by FTD Hepatitis E RNA versus competitor assay

Sample type	FTD Hepatitis E RNA assay	
	Positive	Negative
EDTA	21	3
	- FTD: 2 additional positives confirmed by MHH as positive - FTD: 1 additional positive tested negative by MHH and competitor kit	
Serum	9	0
CPDA	1	0
Stool	6	1
	- Competitor kit: 2 additional positives confirmed by MHH	

In comparison to the MHH and competitor assays, the FTD HEV assay demonstrated comparably good results, in sensitivity and specificity. For EDTA, serum and CPDA the sensitivity was 100%, however a loss of sensitivity and higher discrepancy of quantity unit logs detected was observed with stool samples. Therefore, due to the results the FTD HEV assay is most suited to the use of EDTA blood, serum, CPDA or ascites as the specimen type to collect for the test of hepatitis E virus.

The quantitative results obtained in this study show the satisfactory performance and usability of the FTD HEV assay. This means that the assay can be confidently selected for use in routine diagnostic procedures, to offer patients prompt and accurate treatment options and improved prognosis.

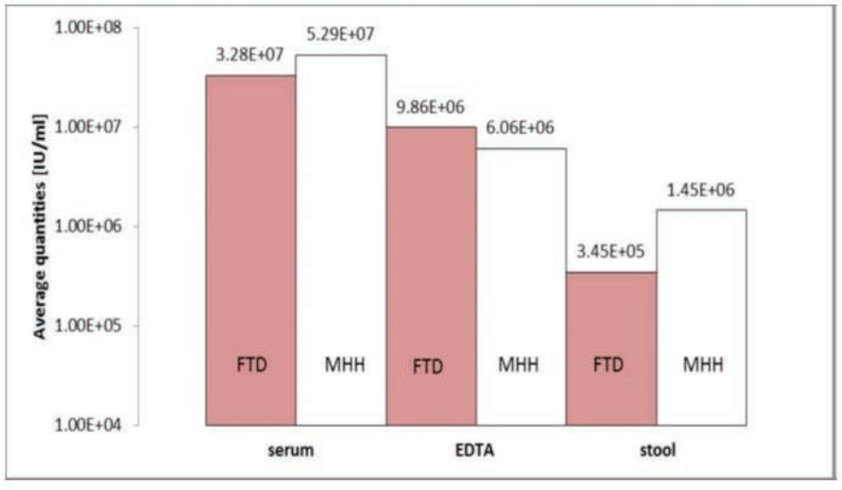

Figure 1. Average quantities (IU/ml) and log10 units from the median difference for the clinical samples tested (serum, EDTA and stool) by FTD Hepatitis E RNA and MHH in-house assay

Conclusion

RT-PCR is an important tool in diagnosing and preventing the spread of infectious diseases, allowing viruses to be identified quicker and more effectively, ensuring precise and effective treatment options can be employed. Choosing an assay that can perform quickly and efficiently is beneficial to the laboratory as it allows test results to be obtained in a timely manner, and guarantees patients receive prompt and accurate treatment, which is important in vulnerable patient groups such as transplant patients in particular.

The results of the recent comparison study, demonstrated that the FTD HEV assay performed with good results compared to the MHH assay and the competitor kit. Since conducting the study, Professor Dr Heiner Wedemeyer has chosen to use the FTD HEV assay for routine diagnostics, allowing MHH to offer efficient diagnosis and suitable treatment options to prevent further disease transmission and ultimately reduce mortality rates.

References

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