FAQ

INNO-LiPA HBV

- WHICH MATERIALS DO I NEED WHICH ARE NOT PROVIDED?

  In the package insert, you can find an overview of required materials which are not provided.

  In summary, to perform the PCR, you need a DNA thermal cycler, vortex, DNA polymerase.

  To perform the LiPA hybridization test you need a warm water bath with shaking platform and orbital, reciprocal or rocking platform shaker. You can also choose to automate the hybridization/liquid dispensing, by use of an automate distributed by Fujirebio Europe N.V. More details can be found in the package insert.

- WHICH EXTRACTION METHOD IS ADVISED TO USE?

  For the DNA extraction, prior to use of the INNO-LiPA™ HBV Multi-DR kit, Qiagen QIAamp® DNA Blood Mini Kit (Cat. no: 51104) is recommended. See manufacturers protocol and please follow instructions for use of the extraction method.

  The DNA extraction used to optimize the INNO-LiPA™ HBV GT and INNO-LiPA™ HBV Precore test is the High Pure PCR Template Preparation Kit (Roche Diagnostics; Catalog no.: 1796 828).

  For all tests, other DNA extraction procedures can be used. When using other commercially available extraction methods, the method should be validated in your laboratory.

- WHAT KIND OF TAQ POLYMERASE IS ADVISED TO USE?

  For the amplification of the INNO-LiPA™ HBV Multi-DR kit, the use of Qiagen HotStarTaq® DNA polymerase (Cat. no:203203), 10x PCR buffer (Cat. no: 203203) is recommended.

  For the amplification of the INNO-LiPA™ HBV GT kit and the INNO-LiPA™ HBV Precore, Taq DNA polymerase and Taq amplification buffer from by Stratagene (Catalog n.: 600 132) are recommended for use.

  When using other commercially available DNA polymerases, additional validation in your laboratory is required.

- ARE THERE ANY CONTROLS INCLUDED IN THE KIT?

  Every strip contains a conjugate control line, to control the correct performance of the test.

  The strip also contains a line for amplification control, to control the presence of amplified PCR product. There are no separate positive or negative controls included in the kit.

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- CAN I AUTOMATE THE ASSAY?

  Yes, next to running the assay manually, the hybridization and dispensing of reagents can be automated. Fujirebio Europe N.V. has 2 automates available for performing the test. The *Auto*-LiPA^{TM}48 allows you to test up to 48 samples, the AutoBlot3000H can run up to 20 tests. These instruments automate the hybridization and liquid handling parts of the assay. For more information about the automates, please see section automation.

- CAN I USE ONE SINGLE AMPLIFICATION FOR ALL 3 HBV KITS?

  The three kits were initially designed with different amplification protocols and cannot be performed in the same amplification run.

  However, upon request of our customers, we optimized a protocol for INNO-LiPA^{TM} HBV Genotyping. Since the HBV target region for drug resistance encompasses the region for detecting HBV genotypes, the amplicon generated with INNO-LiPA^{TM} HBV Multi-DR can also be used on the INNO-LiPA^{TM} HBV Genotyping strip. For more information, please contact your local distributor or customer support.

  Poster: “A sensitive single-round PCR for use with both the INNO-LIPA HBV DRv2 and INNO-LiPA HBV genotyping assay 2006.”

- CAN I RUN VERSANT HCV GENOTYPING 2.0 TOGETHER WITH INNO-LIPA^{TM} HBV?

  To test the INNO-LiPA^{TM} HBV tests, a hybridization temperature of 49°C is recommended whereas the hybridization temperature for Versant HCV Genotyping 2.0 is 50°C. Therefore it is not recommended to perform both tests in the same run.

- WHAT IS THE LOWER LIMIT OF DETECTION?

  Based on in-house data:

  - INNO-LiPA^{TM} HBV Genotyping and INNO-LiPA^{TM} HBV PreCore: 100 IU/ml (500 copies/ml)
  - INNO-LiPA^{TM} HBV DR v2: 200 IU/ml (990 copies/ml). Though that is an average across all probes.

- WHY IS IT IMPORTANT TO DETECT DRUG RESISTANCE MUTATIONS FOR HEPATITIS B?

  Patients treated with nucleos(t)ide analogues can develop resistant mutations, resulting in a partial or non-response to the drugs. At different time points of the treatment, it is advisable to detect (the emergence of) possible mutations in order to provide the patient with the best available treatment.

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When to test?

- Before antiviral therapy in treatment-naive patients or in non-naive patients
- During antiviral therapy to predict viral breakthrough or to change drugs / add drugs after viral breakthrough
- After liver transplantation to investigate the cause of post-transplant viral breakthrough.

- WHICH MUTATIONS ARE DETECTED BY OUR STRIPS IN THE INNO-LIPA™ HBV MULTI-DR KIT?

The INNO-LiPA™ Multi-DR kit contains two different strips (called HBV DR v2 and HBV DR v3), which can be tested together.

The INNO-LiPA™ HBV DR v2 strip is a very specific and sensitive tool for simultaneous detection of hepatitis B virus wild-type, mutations, or polymorphisms at codons 80, 173, 180, 181, 204 and 236.

The INNO-LiPA™ HBV DR v3 strip has been developed for the simultaneous detection of wild type, mutations, or polymorphisms at codons 184, 202 and 250 as well as wild type and mutant motifs for the polymorphism A194T.

- HOW CAN DETECTION OF HBV GENOTYPING HELP TO GUIDE THE TREATMENT?

HBV genotypes A and B are associated with higher rates of anti-HBe seroconversion and HBsAg loss compared to genotypes D and C, respectively, after treatment with PEG-IFN (EASL guidelines 2012).

So the choice to treat with interferon should be driven by the HBV genotype.

- WHAT IS A HBV PRECORE MUTATION? WHY IS IT USEFUL?

Some background: The C gene of the HBV genome has a precore and a core region. If translation is initiated at the precore region, the protein product is HBeAg. If translation begins with the core region, HBcAg is the protein product .

There are two types of mutations: basal core promoter mutations (mutation A1762T and G1764A/T) and precore stop codon mutation (PC codon 28). The double mutation at positions 1762 and 1764 results in decreased transcription of mRNA, which in turn leads to diminished formation of HBeAg. This decrease in HBeAg production can be up to 70%.

A change from G-->A at position 1896 (= Trp) results in a stop at codon 28 of the precore region. In this case, no HBeAg protein is produced.

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Detecting these mutations can confirm if a patient with no or low levels of HBeAg has either HBeAg negative disease due to mutations or is responding to the treatment (=no mutations). Furthermore these mutations are an indication for the risk of hepatocellular carcinoma (HCC) which is 3.79-fold higher in patients with BCP mutations compared to patients without mutations. Patients without PC/BCP mutations are also good candidates for interferon treatment.*

* Ozasa et al. Hepatology 2006;44(2):326-34 - Malik et al. PLoS One 2012;7(6):e39028 - Hayashi et al. Intervirology 2009;52(1):22-8 - Xiao et al. J Med Virol 2011;83(9):1544-50 - Ren et al. J Viral Hepat 2010;17(12):887-95

- WHAT IS THE DIFFERENCE OF INNO-LIPA™ HBV COMPARED WITH SEQUENCING?

  INNO-LiPA™ can detect 10% minor variants (Pas et al. J Clin Virol 2002;25:63-71). Sequencing can only detect 20% minor variants. This means that INNO-LiPA™ is a very sensitive method allowing to detect upcoming mutations in a very early stage and to easily detect samples with mixed genotypes.

- DO YOU HAVE PICTURES OF SOME RESULTS?

  INNO-LiPA HBV Genotyping

  INNO-LiPA HBV Multi-DR

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INNO-LiPA HBV PreCore