Using these conditions our assay is able to detect concentrations of ESAT-6 from 0.1 to 1 µg/mL.

Conclusion: Optimal conditions of the assay are being improved to increase the sensitivity to detect antigenic fractions that may be associated to other serological markers for global evaluation of patients looking for tuberculosis laboratory evidences. The sensitivity level achieved for ESAT-6 is being evaluated in patients with tuberculosis to determine its clinical applicability for TB diagnosis.

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75.039
Variation in liver histopathology in chronic HBV-infected individuals with normal liver function tests correlates with HBV replication
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Background: Clinical and laboratory parameters may not reflect disease activity and imaging studies are insensitive indicators of fibrosis in the precirrhotic liver. The biopsy enables the pathologist and physician to define the extent of disease by grading the necroinflammatory activity and staging the fibrosis and thus determine the progression of liver disease. The aim of this study is to characterize the liver histopathological profiles and their correlation with hepatitis B virus (HBV) replication in chronic HBV-infected individuals with normal liver function tests (LFT).

Methods: We performed a percutaneous liver biopsy in 60 chronic HBV-infected individuals with normal LFT. The biopsied tissue was processed for histological examination including grade of disease activity and stage of fibrosis. HBV markers were detected with ELISA. Serum HBV DNA load was assessed with quantitative real-time polymerase chain reaction.

Results: The histological findings from mild to moderate grade of severity and stage of progression were the most common histological findings. Twenty five cases were with grade 1 stage 0 (G1S0), 25 cases with G1S1, 8 cases with G2S1 and 2 with G2S2. The patients with serum HBV DNA positive had significant severity in the grade of disease activity and stage of fibrosis in liver tissue than those in the patients with HBV DNA negative (P<0.05). In the same way, there was an significant difference between the patients with HBeAg-positive and —negative groups (P<0.05). The patients with the highest serum HBV viral load had the most severe necroinflammatory activity and fibrosis than those in the patients with lower viral load (P<0.05). No significant differences were observed between sex groups and age groups (P>0.05, respectively).

Conclusion: The histological abnormal findings such as hepatic inflammation and fibrosis were common in chronic HBV-infected individuals with normal LFT. The severity of necroinflammatory activity and fibrosis correlates with HBV replication and viral load and HBeAg expression status.

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75.040
Short primers for amplification of diverse virus strains
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Background: Due to the high range of viral diversity no universal, highly conserved primer set exists that is able to amplify all sequenced viral targets. We have performed a computational study that predicted that approximately 3,700 18-mers would be necessary to produce amplicons across the sequenced viral database. However, by decreasing the length of the primer from the traditional 18-mer to a 10-mer the number of primers significantly decreases to approximately 1,000. Shortmers show promise for acting as universal primers that can discriminate both DNA and RNA viruses at the serotype level. As a demonstration of the specificity of shortmers for viral identification we designed 10 and 11- mers that were capable of amplifying three serotypes of Blue Tongue Virus in traditional Reverse Transcription PCR (RT-PCR).

Methods: An in-house program, the Multiplex Primer Prediction (MPP) algorithm, was used to identify a primer set capable of amplifying various serotypes of the Blue Tongue Virus (BTV). The RNA of BTV strains 2, 13 and 17 was extracted in-house and selected as the template for Reverse Transcription PCR (RT-PCR) reactions. The primer set was predicted to amplify a 231 bp product from BTV 2, 13 and 17.

Results: Using RT-PCR and gel electrophoresis we visually verified the presence of ~231 bp products from the singleplex reactions containing the shortmer primer set and individual templates BTV 2, 13 and 17. The target amplicons were then gel extracted and analyzed with using Sanger sequencing, using the forward 11-mer primer. Results indicated that the BTV2 amplicon was 95% homologous to its predicted amplicon, the BTV13 amplicon was 97% homologous for its predicted amplicon sequence and the BTV 17 amplicon was 97% homologous for its predicted amplicon. The percent homology of amplicon to predicted sequence was less than 100% due to gaps in the sequence reads.

Conclusion: We have succesfully predicted short primers that are capable of serotype-level viral detection. As a streamlined version of this analysis, we are currently adapting this assay to run on Luminex platform.

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