Introduction
Hepatitis B Virus (HBV) is currently a leading healthcare problem affecting more than 240 million people worldwide, despite the availability of a highly effective vaccine [1]. Drug therapies are available on the market for the treatment of HBV, but these rarely provide a cure. The development of novel compounds is hampered by the lack of easily accessible animal models and sophisticated in vitro tools that recapitulate the full viral life cycle [3]. Patient isolates of HBV are infrequently used in vitro and HBV cultures, but could prove useful for identifying novel therapies for a cure of HBV.

Here we aimed to identify HBV positive patient isolates capable of infecting primary human hepatocytes in a 3D microfluidic device, and compared this with an infection launched from HepG2.2.15 derived virus. The same cell lots were infected with infectious inoculum and cultured for 14 days.

Methods
Cryopreserved primary human hepatocytes (PHH) were obtained from Life Technologies (USA) or GPS Hepatic Biosciences (USA). 0.6 x 10⁸ hepatocytes were seeded into each LiverChip® well in Williams’ E medium containing supplements. Infection was launched using commercially available HBV positive serum (SeraLab and Quest Biomedical) or HBV genotype D derived from China). For HBV DNA, total DNA was extracted from cell culture medium using QIAamp MinElute Virus Spin Kit along with a reagents and specific primers for pgRNA and total HBV RNA.

Assays
HBV surface antigen (HBsAg) was measured using a CLIA according to the manufacturer’s instructions (AutoBio Diagnostics, China). For HBV DNA, total DNA was extracted from cell culture medium using QIAamp MinElute Virus Spin Kit along with a standard curve generated with Accurex HBV panel. qPCR was run with TaqMan reagent and specific primers and probes. Total RNA was extracted using the RNeasy mini kit (Qiagen). RNA was converted to cDNA using the high capacity cDNA synthesis kit (Life Technologies). Gene expression profiles were quantified in equivalent cDNA samples using either TaqMan specific assays or SYBR reagents and specific probes and primers for total HBV RNA and pre-genomic (pg) RNA on an ABI QuantStudio 6 real time PCR system. Cells were stained with 50 µg/ml Phalloidin-TRITC, counterstained with Hoechst and imaged on an Upright Zeiss LSM 510 confocal microscope.

Results 1 – Hepatocyte Microtissues in LiverChip®
PHH cultured in 3D (in the LiverChip® platform) for 14 days form microtissues, demonstrating polarity of cells and the presence of bile canaliculi (Fig. 2). This has been identified as essential for effective HBV infection and particularly the presence of the hepatic basolateral sodium-dependent-bile transporter, sodium taurocholate co-transporting polypeptide (NTCP) [4]. PHH cultured in LiverChip® have been shown to express higher levels of NTCP than those cultured in 2D (data previously presented).

Results 2 – HBV Infection in LiverChip®
HBV DNA levels were assessed from 10 human HBV positive serum and plasma samples identifying varying levels of HBV DNA (Fig. 3A). Using these 10 patient samples, infections were launched with an addition of 10 % serum or plasma resulting in a range of HBsAg levels at Day 8 or 9 in culture (Fig. 3B). Data from further experiments with Serum 5, demonstrates greater cumulative HBsAg over 7 days than that measured in the inoculum indicating the presence of a productive infection (Fig. 3C). Interestingly, a positive infection with patient inoculum was not correlated with HBV DNA levels in patient serum. Both Serum 4 and Plasma 1 had higher HBV DNA levels than Serum 5, but did not yield a positive infection.

Results 3 – Human Serum
PHH were infected with HBV positive patient serum (Fig. 4, Serum 5) at MOI 1.3 and 3.6 in LiverChip®. HBV DNA and HBsAg in the cell culture supernatant were measured over a 14 day period post infection (Fig. 4). Despite the presence of HBV DNA, replication intermediates (Fig. 5) and HBsAg, no HBeAg was measureable. No HBsAg mutations were found to be present, to account for the lack of antigen production.

Results 4 – HepG2.2.15 derived virus
PHH were infected with concentrated HepG2.2.15 derived virus at an MOI of 80 in LiverChip®, HBV DNA, HBsAg and HBsAg in the cell culture supernatant was measured over a 14 day period post infection (Fig. 6).

Conclusions
• Phenotypic analysis of PHH cultured in LiverChip® indicate that microtissues should be susceptible to HBV infection.
• One patient inoculum was identified from 10 samples, as capable of producing a positive HBV infection in PHH cultured in LiverChip® platform.
• PHH infected with patient inoculum produced HBV DNA for up to 14 days as well as HBsAg. There was an absence of HBeAg production, despite the lack of relevant genetic mutations.
• HepG2.2.15 derived virus also produced a productive infection in LiverChip®, but at a lower level than demonstrated with patient inoculum. HBV DNA, HBsAg and HBsAg were present.
• Both patient inoculum and HepG2.2.15 derived virus produce positive HBV infection in LiverChip® microtissues, useful for clinical testing of novel treatments for the disease.