

ANSWERING REVIEWERS

July 4, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 1221188-review.doc).

Title: S-phase arrest may response for promotion HBV replication after vincristine treatment

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11188

The manuscript has been improved according to the suggestions of reviewers:

1 Revision has been made according to the suggestions of the reviewer

(1) The primers and conditions of PCR and RT PCR are unclear.

According to the reviewers' requirements, I have added the detail information about the PCR and RT PCR in the articles as follows: The primers were designed specifically to the conserved region of HBV gene: forward primer(F2150):5'-CCTAGTAGTCAGTTATGTCAAC-3';

reverse primer(R2300):5'-TCTATAAGCTGGAGGAGTGCGA-3'.

The plasmid pneo-CH9 / HBV1.1 in different concentration (5×10², 5×10³, 5×10⁴, 5×10⁵, 5×10⁶, 5×10⁷ copies/μl) was taken as template to make the standard curve. Cycling parameters : initial denaturation 95 °C, 3 min; subsequent steps 94 °C, 15 s; annealing at 65 °C, 30 s; extension at 72 °C, 20 s; 10 cycles; then degeneration of 94 °C, 15 s; annealing from 65 °C to 55 °C (start, from 65 °C, 1 °C lower after each cycle) for 30 s; extension at 72 °C, 20 s; 30 cycles; at the same time, reading the fluorescence.

(2) How were Dane particles distinguished from nucleocapsid?

The outer layer of Dane particle is HBs-Ag, while the outer layer of HBV nucleocapsid is HBc-Ag. There are two kinds of ELISA kits to detect these two particles respectively. One is coated with HBsAb, so it can only capture the Dane particle and free HBs-Ag. Then the captured particles would be lysed and the HBc-Ag exposed and can be detected by HBc-Ab. So the level of HBc-Ab here represented the level of Dane particle.

Another kit is coated with HBc-Ab. When the samples was added into the kit and the cell membrane lysis buffer was added into each well immediately too. So the Dane particle changed into nucleocapsid and the HBc-Ag exposed. Therefore, this kit can detected the total HBcAg from the Dane particle and the HBV nucleocapsid.

(3) How did the authors calculate the relative HBV antigen levels?

The levels of HBV HBsAg and HBe-Ag in culture medium were assessed by ELISA. Following the manufacturer's protocol (Kehua biotec Inc, Shanghai, China), read the OD value of each well in the microplate reader at 450 nm. Take the absorbance of the OD value for the vertical (Y), HBs-Ag or HBe-Ag standard concentration corresponding to the abscissa (X), do the corresponding curve. Then get the relative HBV antigen levels according to the standard curve.

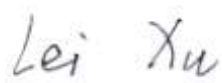
(4) What is rc, ds, and ss in Fig 2. What does the red color, dash area, and lines mean?

HBV DNA in cells contains three different forms including relaxed circular (rc DNA), double-stranded (ds DNA) and single-stranded (ss DNA). I have added this explanation in the figure

legends. The dash area, and lines represent the contents of rc, ds, or ss DNA respectively. And there wasn't red color in the fig.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

A handwritten signature in black ink that reads "Lei Xu". The letters are cursive and slightly slanted to the right.

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