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**Dates:** Received: 04 January, 2017; Accepted: 08 February, 2017; Published: 09 February, 2017

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## Short Communication

# Attention to the problem of the inaccurate testing caused by virus mutation strains in clinical laboratory settings

## Summary

A few virus strain mutations for many viruses can cause the problem of the inaccurate testing in clinical laboratory settings. Therefore, we put forward the following strategies and recommendations including re-examination using different methods or a comparative study or a parallel control study by different kits, and performing virus nucleic acid sequencing analysis if necessary.

In recent years, in vitro diagnostic test for the virus has made significant progress [1]. From a methodological point of view, these diagnostic tools were more and more accurate and reliable, but there are still some problems that should be paid attention to, especially for the problem of the inaccurate testing caused by virus strain mutation in clinical laboratory settings.

At present, the most common methods of virus testing in clinical laboratory settings include as following: serological diagnosis based on antigen or antibody such as ELISA [2-4], and nucleic acid test methods including PCR [5], RT-PCR [6, 7], real-time PCR [8-13], and molecular hybridization [14-16], etc. However, there may be different test results when different kits or test methods were used.

In theory, many viruses such as human papillomavirus (HPV) [15], hepatitis B virus (HBV) [17], hepatitis C virus HCV [18] and herpes simplex virus (HSV) [19] have many genotypes and nucleic acid polymorphisms, resulting in immunological diversity of immune antigen or antibody, and a few mutated proteins cannot be combined with antigen or antibody of diagnostic reagents and lead to a failure in testing. As has been recently reported that: there occurred inconsistent detection results of HBV for 30 blood donors among two ELISA reagents and HBV quantitative real-time PCR detection kit [19]. The

further research showed that the mutations of S gene in HBV resulted in inaccurate results [20].

However, virus nucleic acid polymorphisms can cause a difficulty in design of PCR primers and probes. Imperfect primer design would decrease amplification efficiency. In fact, for many viruses, due to nucleic acid variability, the matched primers covering all viruses cannot be achieved. For example, the reported primers and probe designed with S gene target gene of HBV sequence in a HBV fluorescent quantitative PCR kit widely used in clinical settings of China are as following:

P1: 5 '-ATCCTGCTGCTATGCCTCATCTT- 3';

P2: 5 '-ACAGTGGGGAAAGCCCTACGAA -3';

Probe: 5 '-TGGCTAGTTTACTAGTGCCATTTG- 3'.

But according to the reported primers and probe, we conducted the similarity alignment of DNA sequence in Genebank and found that their bases cannot strictly match with DNA sequence in few HBV strains. Similarly, HPV has too great nucleic acid variability to design perfect primers and probe in theory, suggesting that vitro diagnostic kits had some defects due to the low efficiency amplification even false negative results caused by the problem of primers. Similarly, mutations in viral nucleic acids can lead to peptide mutations in the antigen or antibody, resulting in incorrect diagnostic results, which affects the accuracy of immune diagnostic kits in vitro. Thus, the following strategies and recommendations are putted forward: i) when being clinical diagnosed as viral infection and highly suspected test results, re-examination should be performed in other ways or diagnostic tools; ii) for some extremely important diagnostic experiments in vitro, there must rely on different methods to improve the sensitivity and accuracy of diagnosis. For example, HBV diagnostic assay for blood donor is vital to blood safety, and thereby the tests should use two different reagents. HBV ELISA and nucleic acid testing are recommended as reliable tools, which can

significantly reduce the spread of occult HBV infection and improve blood transfusion security; iii) pays more attention to the diagnostic performance analysis for clinically diagnostic reagents and conducts a comparative study or a parallel control study using different kits; and iv) investigates the reasons for incorrect experimental results, and conducts virus nucleic acid sequencing analysis if necessary.

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