Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 11, pp.22303-22305, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IDENTIFICATION OF PLASMA MATERIAL FOR DEVELOPMENT OF INDIGENOUS HBV STANDARD FOR NUCLEIC ACID TECHNIQUES

***Manoj Kumar Rajput, Praveen Gautam, Anurag Singh, Mohit Sharma, Akanksha, Reba Chhabra and Surinder Singh**

National Institute of Biologicals, Noida, India

ARTICLE INFO

Article History:
Received 26th August, 2015
Received in revised form
05th September, 2015
Accepted 26th October, 2015
Published online 30th November, 2015

Key words:
HBV,
Reference standard,
NAT.

ABSTRACT

This is the first report on identification of plasma material for development of indigenous HBV standard for nucleic acid techniques. The HBsAg positive plasma bag was collected from National Capital Region. The hepatitis B virus in the plasma was quantitated by using four different lots of two different assays. The mean viral load determined was 5.59 log10 IU/ml. The plasma material was found to have genotype-D of the HBV. The nucleotide sequence was submitted under accession no. LK995378.

***Citation*:** Manoj Kumar Rajput, Praveen Gautam, Anurag Singh, Mohit Sharma, Akanksha, Reba Chhabra, Surinder Singh, 2015. "Identification of plasma material for development of indigenous hbv standard for nucleic acid techniques", *International Journal of Current Research*, 7, (11), 22303-22305

INTRODUCTION

The molecular diagnostics have played crucial role in diagnosis of HBV infection and patient management for around two decades. Many laboratories around the world are using in-house developed molecular assays to detect and quantitate HBV in patient samples. The commercial molecular assays constitute only few percent part of diagnostics as a whole and this little part is also dominated by non-transfusion transmitted infections. In commercial as well as in in-house developed molecular assays the basic components remains the same and in almost all cases, the components belong to different producers or manufacturers. Most of the in-house assays, lacked adequate reproducibility and comparability (Best *et al*., 2000; Schiram *et al*., 2002; Zaaijer *et al*., 1993). Therefore, reference materials are required to calibrate the concentration of components as per their activity in the reaction. The development of reference materials have lagged. The international standards available from World Health Organization (WHO) are few. These standards have played an important role in standardization and evaluation of the sensitivity of molecular assays. The WHO international standards are developed according to.

****Corresponding author: Manoj Kumar Rajput,***
National Institute of Biologicals, Noida, India.

"The WHO guidelines for the preparation and establishment of reference standards for biological substances" (WHO Technical Report Series, no.932, 2006, P 80). This document came into existence in 1978. Till date it has been revised three times, first in 1986, second in 1990 and third in 2004. During the process of revision various meetings of experts were conducted and the suggestion made by them are incorporated. The suggestions were made by experts based on their experience and findings on the subject. Their laboratory data plays an important role in the revision. The expert committee consists of representatives from various countries, health departments, manufacturers, standards organizations. The document describes the common principles for the development and establishment of all WHO biological reference materials. The description about quantification of transfusion transmitted viruses by molecular assays was incorporated during 2004 revision.

MATERIALS AND METHODS

Sample collection and viral load determination

Plasma from the blood bags found positive for HBsAg, during the screening at blood bank located in NCR was selected. The viral load of plasma was determined using cobas TaqMan HBV test for use with high pure system and cobas Ampliprep/cobas TaqMan HBV test.

Sequencing

HBV DNA was extracted using QIAmpMinElute virus spin kit (QIAGEN, Germany). The genomic region encoding for *precore/core* and partial *pol* genes was amplified using *Taq* PCR kit (New England Biolabs) with 20 pmoles of each forward primer (5'-GAGGAGTTGGGGGAGGAGATTA-3'; ntd. 1734-1755) and reverse primer (5'-AGGCGCTACGTG TTGTTTCTC-3'; ntd. 2785-2805). The thermal cycling was performed in GeneAmp PCR system 9700 (Applied Biosystems) with: denaturation at 94 ºC for 5 min followed by 40 cycles consisting of 94 ºC for 1 min, 58 ºC for 1 min, 72 ºC for 1.5 min and final extension at 72 ºC for 7 min. The HBV DNA amplicons were purified using QIA quick PCR purification kit (QIAGEN, Germany). The nucleotide sequence of amplified product was determined in both directions by using BigDye Terminator v3.1 Cycle Sequencing kit and Genetic Analyzer 3130xl (Applied Biosystems). The nucleotide sequence was analysed using BLAST. The sequence was submitted to EMBL/GenBank under accession number LK995378.

RESULTS

This is the first report on identification of material for development of HBV standard for nucleic acid techniques. The viral load of HBsAg positive plasma was determined using four qualitative assays (Two different lots of Cobas TaqMan HBV test and and two different lots of Cobas Ampliprep/ Cobas Taqman HBV test; Table 1). Lot A of Cobas TaqMan HBV test showed viral load of 5.55 log10 IU/ml, lot B of Cobas TaqMan HBV test showed viral load of 5.64 log10 IU/ml, Lot X of Cobas Ampliprep/Cobas TaqMan HBV Test showed viral load of 5.49 log 10 IU/ml and lot Y of Cobas Ampliprep/Cobas TaqMan HBV Test showed viral load of 5.67 log10 IU/ml.

The alignment revealed that the sequence belong to conserved core protein region of HBV genotype-D. The accession number assigned by GenBnak to our sequence is LK995378. Further the sequence is classified to belong to sub-genotype-D3 of genotype-D based on its clustering with sub-genotype D3 sequences.

DISCUSSION

Since the establishment of first WHO HBV international standard in 1999, even after 16 years there is no report on development of national or indigenous HBV reference standard for nucleic acid techniques. There is need for development of local HBV reference standard because (1) to decrease the cost of getting reference material (2) genotype specificity or local origin nature. The indigenous reference material developed by our laboratory would be ten times cheaper than the WHO HBV standard. The WHO international standard for HBV was established in 1999. It was an HBV genotype-A single donor high tittered plasma diluted into cryosupernatant and lyophilized.

The development study was planned by National Institute for Biological Standards & Controls (NIBSC), UK, a WHO collaborating centre. The data of a variety of commercial and laboratory developed molecular assays using single heminested or nested primers were analysed. The data of the most of the assays was qualitative. The first WHO HBV DNA international standard (NIBSC code 97/746) was assigned the value of 6 log10 IU/ml after reconstitution. The mean assayed value was 6.42 log10 IU/ml (Saldana *et al*., 2001). The first HBV DNA standard was replaced by 2nd HBV DNA standard (97/750) in 2006. It was a second lyophilization preparation of original material. The second HBV DNA standard was also assigned the value of 6 log10 IU/ml but the mean assayed value was slightly reduced to 6.30 log10 equivalents per ml.

Table 1. Viral determination by different tests

S.No	Name of Test Kit	Lot/ Batch	Viral Load	Average	Mean
1.	Cobas TaqMan HBV Test	A	5.55 log10 IU/ml		
2.	Cobas TaqMan HBV Test	B	5.64 log10 IU/ml	5.60 log10 IU/ml	
3.	Cobas Ampliprep/Cobas TaqMan HBV Test	X	5.49 log10 IU/ml		
4.	Cobas Ampliprep /Cobas TaqMan HBV Test	Y	5.67 log10 IU/ml	5.58 log10 IU/ml	5.59 IU/ml

The average viral load of two lots of Cobas TaqMan HBV test was 5.6 log10 IU/ml and the average viral load of Cobas Ampliprep/Cobas TaqMan HBV test was 5.58 log10 IU/ml. The overall average viral load detected was 5.59 log10 IU/ml. The nucleotide sequencing of HBV present in the material was done to reveal its genotype. The 735-bp nucleotide sequence was determined by Sanger's dideoxy method. This nucleotide sequence was aligned with the HBV nucleotide sequences present in GenBank using Basic Local Alignment Search Tool (BLAST). The nucleotide sequence was found to have more than 98% homology to nucleotide sequences of HBV genotype D in the GenBank. Since the sample was collected from national capital region, it was expected to contain HBV genotype D because, HBV genotype D is dominant in northern India (Chattopadhyay *et al*., 2006). The homology to HBV genotype D was confirmed at both nucleotide as well as amino acid level.

The 3rd WHO HBV standard (10/264) was introduced in 2014. This material has been assigned a unitage of 5.93 log10 IU/ml when reconstituted (Heermann *et al*., 1999; Fryer *et al*., 2011). Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. WHO do not assign an expiry date to its international reference materials as per its policy. The origin of biological material used for preparation of all versions of WHO HBV standard is United Kingdom. All WHO HBV standards comprise of dilution of the Eurohep reference R1, which contains genotype A of HBV. The HBV genotype A is dominant in UK therefor as per their needs it is more suitable but in larger part of India HBV genotype D is dominant. Therefore, the reference material containing genotype D would be more suitable as per local needs. In this study we propose plasma material which can be considered to establish first indigenous national HBV reference material for nucleic acid techniques.

The plasma has been collected from National Capital Region. The material has been characterized for viral load and genotype. Four different lots of two types of assay were used to quantify the infectious agent. The similar procedure is followed by WHO to determine the viral load of WHO HBV reference standard (Chudy *et al*., 2009).

The recent WHO HBV standard was assigned value of 5.93 log10 IU/ml. The calculate value of our material is 5.59 log10 IU/ml. The material identified by us is similar to WHO HBV standard in terms of viral load and it consist of HBV genotype D. Therefore, the material possesses all properties to be considered as reference material and most importantly the origin of the identified material is local. Thus it fulfil all the requirements expected from a reference standard. It also fulfils the general requirements of ISO guide 34 (ISO Guide 34: 2009, General requirement for the competence of reference material producers).

REFERENCES

Best, S.J., Gust, A.P., Johnson, E.I.M., McGavin, C.H. and Dax, E. 2000. Quality of Human immunodeficiency virus viral load testing in Australia. *J. Clin. Microbiol*., 38: 4015-4020.

Chattopadhyay, S., Das, B.C. and Kar, P. 2006. Hepatitis B virus genotypes in chronic liver disease patients from New Delhi, India. *World J. of Gastroenterol*., 12: 6702-6706.

Chudy, M., Hanschmann, K.M., Kress, J., Gerlich, W. and Nubling, C.M. 2009. Collaborative study to establish a World Health Organization International Genotype Panel for hepatitis B virus nucleic acid amplification technique (NAT)-based assays. WHO ECBS Report 2009;WHO/BS/09.2121.

Fryer, J.F., Heath, A.B., Wilkinson, D.E., Minor, P.D. and the collaborative study group. 2011. Collaborative study to evaluate the proposed 3rd WHO International Standard for hepatitis B virus (HBV) for nucleic acid amplification technology (NAT)-based assays. WHO ECBS Report 2011; WHO/BS/2011.2170.

Heermann, K.H., Gerlich, W.H., Chudy, M., Schaefer, S. and Thomssen, R. 1999. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. *J. Clin. Microbiol*., 37: 68-73.

ISO Guide 34: 2009. General requirement for the competence of reference material producers.

Saldana, J., Gerlach, W., Lelie, N., Dawson, P., Heerman, K. and Health, A. 2001. WHO collaborative study group: An international collaborative study to establish a world health organization international standard for hepatitis B virus DNA nucleic acid amplification techniques. Vox Sang., 92: 141-150.

Schiram, J., van Loon, A.M., Valetnine-Thon. E., Klapper, P., Reid, J. and Cleator, G. 2002. External quality assessment program for qualitative and quantitative detection of hepatitis C virus RNA in diagnostic virology. *J. Clin. Microbiol*., 40: 2973-2980.

World Health Organization: Annex II Recommendations for the preparation, Characterization and Establishment of International and other Biological Reference Standards (revised 2004). WHO Technical Report Series, no.932. Geneva, WHO, 2006, P 80.

Zaaijer, H.L., Cuypers, H.T., Reesink, H.W., Winkel, I.N., Gerken, G. and Lelie, P.N. 1993. Relaibility of polymerase chain reaction for detection of hepatitis C virus. Lancet., 341: 722-724.

****