別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-10

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 2 月 8 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	An international collaborative study to establish the 2nd World Health Organization International Standard for hepatitis B virus DNA nucleic acid amplification technology-based assays. Baylis, S. A. et al, Vox Sanguinis (2008) [Epub ahead of print]	公表国 英国	
販売名（企業名）						
研究報告の概要	1999 年，WHO は核酸増幅検査 (NAT) による B 型肝炎ウイルス (HBV) DNA 検出のための最初の国際標準用品（サンプル 1）を樹立し，10E6 IU/mL の力価を適用した。その当時，将来的に代替標準品となる可能性があるという発想から，同じ血漿から同じ凍結乾燥条件下で調製した別の DNA 検体（サンプル 2）が保存された。本試験の目的は，長期間保存したこれらサンプルの力価及び安定性を再評価することであった。サンプル 1 及び 2 は，6 ヵ所の分析機関に送付され，4 回に分けて質と量の両面から分析した。全ての分析機関で得られた結果から，これら検体の力価に有意な差は認められなかった。また，安定性試験では両検体ともに非常に安定しており，4℃又は 20℃で 51 ヵ月以上保存した後でも有意な変性は認められなかった。この結果を受けて，WHO は 2006 年 10 月，サンプル 2 を NAT 法による HBV DNA 検出のための新たな国際標準品として樹立した。					使用上の注意記載状況・その他参考事項等 BYL-2008-0308
報告企業の意見			今後の対応			
この試験は新たな国際標準品の樹立を報告するとともに，凍結乾燥により保存された HBV DNA が極めて安定であることが報告されている。			現時点で新たな安全対策上の措置を講じる必要はないと考える。			

Vox Sanguinis (2008)

REPORT

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An international collaborative study to establish the 2nd World Health Organization International Standard for hepatitis B virus DNA nucleic acid amplification technology-based assays

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Vox Sanguinis

Background and Objectives The aim of this study was to replace the 1st World Health Organization International Standard for hepatitis B virus DNA for nucleic acid amplification technique (NAT)-based assays (code 97/746) with a new International Standard. Two lyophilized preparations freeze dried from the same bulk were evaluated in the original collaborative study (coded 97/746 and 97/750, and termed AA and BB, respectively, in the original study). This present study re-evaluates these two preparations in terms of potency and real-time stability.

Materials and Methods The 1st International Standard (97/746) and the second lyophilized preparation (97/750) were coded Samples 1 and 2, respectively, in the present study. The samples were distributed to six laboratories and assayed on four separate occasions. Accelerated thermal degradation samples of the two preparations were examined after long-term storage at 4 °C and 20 °C for more than 51 months.

Results Data were returned from a total of nine different NAT-based assays, five in qualitative format and four in quantitative format. The results of this study confirm the results of the original collaborative study, with no significant differences being found in estimated international units (IU)/ml or polymerase chain reaction-detectable units/ml for the 1st International Standard (Sample 1 in this study) and the proposed replacement preparation, Sample 2 (97/750). Real-time and accelerated degradation studies indicate that both samples are very stable. Storage of both preparations at 20 °C for more than 51 months resulted in no detectable degradation.

Conclusions On the basis of the data presented in this collaborative study, Sample 2 (code 97/750) was established as the 2nd International Standard for hepatitis B virus DNA for NAT-based assays with a potency of 10^6 IU/ml (500 000 IU/vial).

Key words: hepatitis B virus, International Standard, NAT.

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Introduction

The 1st International Standard (IS) for hepatitis B virus (HBV) DNA for nucleic acid amplification technique (NAT)-based assays (code 97/746) was established in 1999 by the

World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) [1]. This standard has been used in the calibration of secondary standards and working reagents, and has been used in the validation of assays for both the qualitative and quantitative detection of HBV DNA in serum and plasma. The standard has been used in the field of blood and blood product safety, as well as in the clinical investigation of HBV infection, both for diagnosis and for monitoring HBV loads in response to antiviral therapy.

In the original collaborative study, three preparations were evaluated. Two of the materials were lyophilized preparations formulated by dilution of R1, the Eurohep reference material [2] in pooled human plasma. This plasma bulk containing HBV was stored at −70 °C until processing. A single bulk material was lyophilized on two separate occasions, 2 weeks apart, using the same processing parameters [1]. These lyophilized preparations, coded 97/746 and 97/750, were termed AA and BB, respectively, in the original collaborative study. A third preparation, termed CC, was a liquid/frozen HBV plasma sample. No significant difference in potency was observed between AA and BB, which had been prepared from the same bulk material, but had been lyophilized on separate occasions. The 1st IS for HBV DNA for NAT-based assays was assigned a potency of 10^6 international units per ml (10^6 IU/ml). In the 50th report of the WHO ECBS [3], it was noted that 97/750 would be reserved as a potential replacement standard in the future. As 97/750 had been fully characterized in the original collaborative study, the WHO ECBS proposed examination of real-time stability data of the 1st IS and the candidate replacement standard 97/750.

In the present collaborative study, the potency and stability of the candidate replacement standard 97/750 is compared to the 1st IS for HBV DNA. The approach for the re-evaluation of 97/750 was agreed on at the 16th meeting of the International Scientific Working Group on the Standardization of Genome Amplification Techniques (SoGAT) in May 2005 [4].

Materials and methods

The 1st IS for HBV DNA for NAT-based assays (97/746) and the proposed replacement (97/750) were lyophilized from the same bulk starting material derived from a high-titre HBV genotype A2 (HBV surface antigen subtype *adw2*) sample (Eurohep R1), diluted in human plasma [1]. This HBV strain has a sequence characteristic of those circulating in central Europe [2].

Collaborative study

Six laboratories participated in the collaborative study and each was requested to assay the 1st IS for HBV DNA (97/746) concurrently with the candidate replacement standard (97/750). The participating laboratories were from five different countries and represented quality control laboratories, a manufacturer of plasma derivatives and an academic institution (a national reference laboratory for hepatitis B and D). Participants were sent four vials of the 1st IS (97/746) and four vials of the candidate replacement standard (97/750), these were coded Samples 1 and 2. The normal temperature for the long-term storage of 97/746 and 97/750 is −20 °C and participants were requested to store the samples under these conditions until analysis. The aim of the study was to determine whether there was any evidence of loss of potency of the two lyophilized preparations during normal storage conditions, since the time they were freeze dried. Participants were requested to test the samples on four separate occasions. The lyophilized samples were reconstituted with 0·5 ml of nuclease-free deionized water and the contents gently agitated for 20 min before analysis. In the case of qualitative assays, participants were requested to perform serial dilutions of the samples in four independent assay runs. In the first qualitative assay run, 10-fold dilutions were performed to determine the end-point for the detection of HBV DNA. In each of the subsequent three assay runs, a minimum of two half-$\log_{10}$ dilutions either side of the predetermined end-point were tested, and results reported as positive or negative. In the case of quantitative assays for HBV DNA, participants were requested to report results in IU/ml and to test the samples without dilution, or prepare dilutions of the samples as necessary, depending on the linear range of assays used. In addition, one laboratory analysed the Eurohep R1 reference in parallel, following continuous storage at −80 °C.

Stability studies

For accelerated thermal degradation studies, vials of 97/746 and 97/750 were incubated at 4 °C and 20 °C, for between 51 months and 56 months. The degradation samples were extracted as previously described [5] and analysed in parallel with samples of the two preparations that had been stored at −20 °C, to provide a baseline for analysis. One set of assay runs was performed using the Artus HBV LC PCR Kit (Qiagen GmbH, Hilden, Germany) and used in accordance with the manufacturer's instructions. A second set of assay runs was performed using previously published primers and probe sequences [5] and amplification reactions were performed using the LightCycler FastStart DNA Master Hybprobe kit (Roche Applied Science, Mannheim, Germany). Standard curves were prepared using serial 10-fold dilutions of the 1st IS for HBV DNA (97/746). The stability studies were performed by two different operators at the National Institute for Biological Standards and Control (NIBSC), UK.

Table 1 Laboratory codes and assay protocols used by participants

Laboratory code	Method	Type
1	Roche COBAS TaqMan HBV test with use of HPS viral nucleic acid kit	Quantitative
2A	Artus HBV LC PCR kit	Quantitative
2B	In-house real-time PCR	Quantitative
3A	Roche COBAS AmpliScreen HBV test	Qualitative
3B	In-house real-time PCR	Qualitative
3C	In-house real-time PCR	Qualitative
4	In-house real-time PCR	Quantitative
5	In-house PCR	Qualitative
6	Roche COBAS AmpliScreen HBV test	Qualitative

PCR, polymerase chain reaction; HBV, hepatitis B virus.
In-house assay details for the following laboratories; 2B, the assay was based on a previously published amplification method [6] targeting the HBs gene with detection using the Roche LightCycler; 3B, qualitative real-time PCR assay amplifying the core region of the HBV genome with detection using the Roche LightCycler; 3C as for 3B, with an initial ultracentrifugation step prior to extraction; 4 real-time PCR amplifying the X region of the HBV genome [7] and detection using the Roche LightCycler; 5, qualitative PCR assay amplifying the HBV core region and detection using capillary electrophoresis.

Table 2 Estimated IU/ml (log_{10}) from quantitative assays

Laboratory number	Sample	
	Sample 1	Sample 2
1	5·99	5·97
2A	6·08	5·99
2B	6·06	5·92
4	5·94	5·86
Mean[a]	6·00	5·93

[a]Results combined for laboratory 2 to give a single mean prior to calculating overall mean of laboratories.

Table 3 Estimated polymerase chain reaction (PCR)-detectable units/ml (log_{10}) for qualitative assays

Laboratory number	Sample	
	Sample 1	Sample 2
3A	6·48	6·58
3B	6·90	6·68
3C	6·56	6·35
5	6·49	6·25
6	6·51	6·59

Results

For the analysis of the results, a code number was allocated at random for each laboratory (Table 1), and does not reflect the numbers assigned to laboratories that participated in the original collaborative study to establish the 1st IS (97/746). Where individual laboratories returned data from more than one assay method, or repeat assays by different operators, the results were analysed separately, and referred to as, for example, laboratories 3A and 3B. Each participating laboratory performed four separate assay runs on the two preparations as requested in the study protocol. The types of assays used by participants are recorded in Table 1; these cover a range of in-house (n = 5) and commercially available tests (n = 4). Where they have been disclosed, details of the assay and region of the HBV genome amplified are indicated (Table 1). Three laboratories (1, 2A, 2B, and 4) returned data from quantitative assays, with results expressed in IU/ml. All calculations were based on the estimates of log_{10} IU/ml, to give overall mean figures for each laboratory. Three laboratories (3A, 3B, 5 and 6) returned data from end-point dilution series, produced using qualitative assays. These were analysed to determine the polymerase chain reaction (PCR)-detectable units/ml for each sample, using the statistical methods described in the publication of the original collaborative study to establish the 1st IS for HBV DNA [1].

The estimated IU/ml (log_{10}) from the quantitative assays and PCR-detectable units/ml (log_{10}) from the qualitative assays are shown in Tables 2 and 3, respectively. For both quantitative and qualitative assays, the results for Samples 1 and 2 are extremely close. For the quantitative assays, combining the results from laboratory 2 to give a single laboratory mean, the overall estimate for the 1st IS, Sample 1, is 6·00 log_{10} IU/ml, exactly the assigned unitage, and 5·93 log_{10} IU/ml for Sample 2. If the results of the assays from laboratory 2 are considered separately (2A and 2B), then the overall means are 6·02 and 5·94 log_{10} IU/ml for Samples 1 and 2, respectively. There is also very close agreement between the results from the individual laboratories. One set of results submitted by laboratory 3C was returned as crossing point (Ct) values; these were not included in the main analysis, as it was not possible to convert them to either IU or PCR-detectable units. However, these results were in line with all other assay methods (i.e. demonstrating equivalence of Samples 1 and 2). Calculating the pairwise difference in log_{10} estimates between Samples 1 and 2 for each laboratory that provided quantitative data, there was a small, but marginally significant (P = 0·044) difference of 0·08. When the results from laboratory 2 are combined to give a single laboratory mean, the difference between Samples 1 and 2 is similar (0·07), but no longer significant. Laboratory 4 also measured the Eurohep reference sample R1. Samples 1 and

Table 4 Estimated IU/ml (log_{10}) for accelerated degradation samples

Storage temperature	Sample	
	Sample 1	Sample 2
−20 °C	6·02	5·92
4 °C	5·92	5·91
20 °C	5·94	6·03

The accelerated thermal degradation samples were stored at 4 °C and 20 °C for a period of 51 and 56 months; these samples were compared to vials of 97/746 that were stored continuously at −20 °C. Four vials of each sample stored at 4 °C and 20 °C were analysed on four separate occasions, each sample extract was tested in triplicate on each occasion. The data were pooled for the two different storage times and mean values shown for the estimated IU/ml (log_{10}).

2 were originally prepared from R1 following a 1 in 500 dilution in human plasma. The titre of R1 was determined to be 8·73 log_{10} IU/ml, which is in very good agreement with the expected titre of 8·70 log_{10} IU/ml. The difference between Samples 1 and 2 was not significant when estimates from all laboratories were included. This was the case whether treating the different assay methods of laboratory 3 as three separate laboratories ($P = 0·099$) or combining their estimates into a single laboratory mean ($P = 0·124$).

Stability studies

A total of four separate assay runs were performed by a single laboratory. The overall mean estimated IU/ml (log_{10}) for the different samples and storage temperatures are shown in Table 4. From analysis of the raw data, no degradation was evident for any of the test samples when compared with baseline samples stored at −20 °C; as a consequence the results were combined for the samples stored for 51 months, and those stored for 56 months. The results summarized in Table 4 clearly demonstrate that no degradation has occurred. Performing a formal significance test, there was no significant difference in estimated IU/ml across the temperatures for either sample. It should be noted that the formal test allowed for any possible differences between the samples stored for 51 months and those stored for 56 months. It is not possible to obtain precise predictions of expected loss per year, because no observed degradation has taken place and, thus, it was not possible to apply the Arrhenius model of accelerated degradation [8,9]. However, if it were assumed that the degradation rate would double with every 10 °C increase in storage temperature, the lack of any detectable degradation at 20 °C for over 4 years would equate to no detectable degradation at −20 °C for 64 years. Real-time stability, of the 1st IS (Sample 1) and Sample 2, as effectively detemined in the present collaborative study, indicates no loss of potency of these two preparations since time of manufacture, as evidenced by the values reported by the participants.

Conclusions

The results of this collaborative study are in good agreement with the results of the original study [1]. Using only the results of the quantitative assays, which are expected to be more precise than the qualitative assays, there was a difference of around 0·07 to 0·08 log_{10} between the estimated IU/ml for the 1st IS and the candidate replacement, Sample 2. If assays from two differing methods used by laboratory 2 are treated as if from separate laboratories, this difference is marginally significant ($P = 0·044$). However, if the results for laboratory 2 are first combined, the difference is no longer significant. Including the results from all participants, using both quantitative and qualitative assays, there is no significant difference between the 1st IS and the candidate replacement, Sample 2. This lack of significant difference is in contrast to a recently completed study to establish the 3rd IS for hepatitis C virus (HCV) RNA [10]. Here two lyophilized preparations, derived from the same bulk, were evaluated by 33 laboratories that calibrated them against the 2nd HCV IS, using a wide range of commercial and in-house quantitative and qualitative assays. The relative potencies of the two new lyophilized HCV RNA preparations were 5·19 and 5·41 log_{10} IU/ml, while the unprocessed bulk material had a relative potency of 5·70 log_{10} IU/ml. These differences in relative potencies between the two lyophilized HCV RNA preparations were statistically significant ($P < 0·0001$), with a clear loss of potency on processing. This is in contrast to the HBV study presented here. From the original collaborative study and data from this new study, there is no significant difference between the potencies of the two HBV DNA Samples 1 and 2, nor was there any detectable loss of titre of the preparations following lyophilization [1].

The results of the accelerated degradation studies have also demonstrated that both Samples 1 and 2 are extremely stable and suitable for long-term use, with no detectable degradation for either preparation after storage at 20 °C for more than 4 years. This stability is in contrast to the 1st and 2nd IS for HCV RNA (96/790 and 96/798, respectively), which showed an average decrease of log_{10} 1·9 for samples stored at 20 °C for more than 5 years [11]. This difference in the observed stability may be due to the nature of the viral nucleic acid, which in the case of HBV is DNA, in contrast to the RNA genome of HCV, which is likely to be more unstable and susceptible to degradation. However, it is possible that further unknown factors influence the stability.

On the basis of this study, Sample 2 (97/750) was established as the 2nd IS for HBV DNA for NAT-based assays by the WHO

ECBS in October 2006. This preparation has a potency of 10^6 IU/ml. Each vial contains the equivalent of 0·5 ml of material, and the content of each vial is 5×10^5 IU per vial. Vials of 97/750 are available from NIBSC.

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別紙様式第2-1

No. 34

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 18	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	沼尾宏, 渡辺泰宏, 立花直樹. 第37回日本肝臓学会西部会; 2007 Dec 7-8; 長崎.	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○輸血によりHBs抗体エスケープ変異株に感染した一例
【症例】40歳代女性。平成16年8月より発熱あり、白血病の疑いにて平成16年10月21日入院。混合型急性白血病として10月25日より化学療法を施行。入院時HBsAg-、HCVAb-であった。10月末より11月にかけてALT387IU/Lまで上昇した。11月10日にはHBsAg-、HBsAb+、HbcAb+、HBeAg-、HBeAb-、HBV-DNAポリメラーゼ0CPM。一時肝機能は正常化したものの、平成18年2月中旬より再びトランスアミナーゼの上昇を軽度認めた。白血病が血液学的寛解となり4月17日退院。外来で化学療法を施行していたが、5月30日AST 947IU/L、ALT 1683IU/Lと上昇し再度入院。HBeAg+、HBeAb+、HBcAb·IgM+で、他の肝炎ウイルスマーカーが陰性であったためHBV感染を疑い、6月5日よりラミブジンを100mg/日で投与開始した。6月6日HBV-PCR 3.4LC/mLであった。その後ALT 2357IU/L、T-Bil 7.41mg/dlまで上昇し、肝炎の改善傾向はなかった。ラミブジンの継続と肝庇護療法にて肝機能は改善した。6月23日の肝生検では小葉内肝細胞壊死を伴った高度の炎症細胞浸潤を認め、急性肝炎の所見であった。AST17IU/L、ALT27IU/Lとなり7月22日退院。HBV-PCR陽性となるまでに患者に投与されたすべての血液製剤について個別HBV-NATを実施した結果、平成16年11月に輸血したFFPがHBV陽性であった。製剤と患者のHBVはいずれもGenotype C/Subtype ayrでS抗原のN末端から145番目のアミノ酸がGlyからSerに置換しており、エスケープミュータントであった。また、両者のα領域(PreS/S領域を含むP領域の前半部)の塩基配列は一部の塩基の共存を除き、完全に一致した。HBV-DNAはいずれも定量限界(100copies/mL)未満であった。患者はその後もラミブジンの投与を継続し、骨髄移植を行った。肝炎の再燃は認めなかったが、白血病のため平成18年10月永眠された。【考察】核酸増幅検査を含む献血者のスクリーニングを行っているにもかかわらず、本邦では年間10-20例のHBV感染が報告されている。その原因の一つがHBs抗体エスケープミュータントであるが、本症例のように献血者、受血者ともに塩基配列の解析を行い感染が証明された例はきわめて稀と考えられ報告する。

報告企業の意見	今後の対応
輸血によりHBs抗体エスケープ変異株に感染し、献血者、受血者の塩基配列の解析を行って感染が証明された症例の報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を順次進めている。NATの精度向上についても評価・検討している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

自発報告:2006年3月16日付1-05000059

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